Acta Morphologica

Academiae Scientiarum Hungaricae

ADIUVANTIBUS

J. BALÓ, P. ENDES, K. FARKAS, L. HARANGHY, B. KELLNER, I. KROMPECHER, GY. ROMHÁNYI, E. SOMOGYI, J. SZENTÁGOTHAI

REDIGIT

I. TÖRŐ

TOMUS XVI * FASCICULUS 1

Akadémiai Kiadó Budapest

1968

ACTA MORPH. HUNG.

ACTA MORPHOLOGICA

A MAGYAR TUDOMÁNYOS AKADÉMIA
ORVOSTUDOMÁNYI KÖZLEMÉNYEI

SZERKESZTŐSÉG ÉS KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY U. 21.

Technikai szerkesztő:
Dr. Somogyi Endre

Az Acta Morphologica német, angol, francia és orosz nyelven közöl értekezéseket a kísérletes orvostudomány tárgyköréből.

Az Acta Morphologica változó terjedelmű füzetekben jelenik meg. Több füzet alkot egy kötetet.

A közlésre szánt kéziratok a következő címre küldendők:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Ugyanerre a címre küldendő minden szerkesztőségi és kiadóhivatali levelezés.

Az Acta Morphologica előfizetési ára kötetenként belföldre 120, külföldre 165 Ft. Megrendelhető a belföld számára az Akadémiai Kiadónál (Budapest V., Alkotmány utca 21. Bankszámla 05-915-111-46), a külföld számára pedig a „Kultúra" Könyv- és Hírlap Külkereskedelmi Vállalatnál (Budapest I., Fő utca 32. Bankszámla: 43-790-057-181) vagy annak külföldi képviseleteinél és bizományosainál.

Die Acta Morphologica veröffentlichen Abhandlungen aus dem Bereiche der experimental-medizinischen Wissenschaften in deutscher, englischer, französischer und russischer Sprache.

Die Acta Morphologica erscheinen in Heften wechselnden Umfages. Mehrere Hefte bilden einen Band.

Die zur Veröffentlichung bestimmten Manuskripte sind an die folgende Adresse zu senden:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

An die gleiche Anschrift ist auch jede für die Schriftleitung und den Verlag bestimmte Korrespondenz zu richten.

Abonnementspreis pro Band: 165 Forint. Bestellbar bei dem Buch- und Zeitungs-Außenhandels-Unternehmen »Kultúra« (Budapest I., Fő utca 32. Bankkonto Nr. 43-790-057-181) oder bei seinen Auslandsvertretungen und Kommissionären.

Acta Morphologica

Academiae Scientiarum Hungaricae

Adiuvantibus

I. Baló, P. Endes, K. Farkas, L. Haranghy, B. Kellner,
I. Krompecher, Gy. Romhányi, E. Somogyi, J. Szentágothai

Redigit

I. Törő

Tomus XVI

Akadémiai Kiadó, Budapest
1968

ACTA MORPHOLOGICA

Tomus XVI

INDEX

INDEX AUTORUM

Acta morphologica Acad Sci. Hung. **16** *(1), pp. 1—5, 1968*

FIVE DECADES IN THE SERVICE OF SCIENCE 1917—1967

When in 1917 an ambitious medical student was appointed to an unsalaried post at the Institute of Pathological Anatomy of the University of Budapest, he would have not dared to think that once he will be elected to occupy the post of his learned master, Professor COLOMAN BUDAY. The student was JOSEPH BALÓ who then just had left the army after serving four years on the Russian and Italian theaters in World War I.

It was a fortunate term in the life of Joseph Baló that early in the course of his studies he became a member of an institute which brought forth a series of excellent experts of pathological anatomy. After taking his M.D. he devoted all his energy to his work at the Institute. This comprised post-mortem examinations of the material of the University Hospitals and in addition that of St. Stephen's Hospital. Here Baló joined an enthusiastic collective where all the members took an active part in medical education, in developing scientific work or public health.

It will be the task of the future to evaluate the eventful years between the two world wars with respect to medical science and public health, but it is certain that Coloman Buday's best pupils remained faithful and worthy of the example and teaching of their master. They occupied several chairs in the Hungarian universities and the former members of the staff of the Institute were engaged in teaching in different institutions and achieved considerable success in research. It will suffice to mention in this respect GEORGE GÖMÖRI, the pioneer in histochemistry, who had received his training at Buday's institute. One must also remember that Hungarian medical students learned for many decades from Buday's lucid textbook of pathological anatomy; previously they had been obliged to use foreign texts.

Besides the attractive personality of Coloman Buday the functional pathological conception of ALEXANDER KORÁNYI and his school, the impressive neurohistological lectures of CHARLES SCHAFFER and the material of the alert and noble minded professor of neurology of St. Stephen's Hospital ARTUR SARBÓ had a great influence on the further scientific career of Joseph Baló; these were attracting his attention to the diseases of the nervous system and to the importance of the functional view of pathological processes.

Baló's concept of pathology was greatly widened in the years 1923—1924 which he spent in the USA as a Rockefeller fellow. During this time he carried out studies in bacteriology, immunology and parasitology at Johns Hopkins University and became acquainted with diseases caused by filterable viruses and the methods of their investigation. Then his attention was directed to typhus and other rickettsial diseases in Wolbach's Institute in Boston. So when he in 1924 returned home he devoted himself to problems of epidemic encephalitis, prevailing then in Hungary, and the experimental herpes encephalitis.

In 1926, he went to the Research Institute of ADOLF LOEWY in Davos in order to acquaint himself with the effects of high altitude. This study trip opened new research possibilities to Joseph Baló. The further course of his career showed how the horizon of his activity became wider and wider starting from the dissecting table through the rigid structures of histological slides to experimental pathology, to biochemical and electronmicroscopic investigations.

A few additional data mark some stations of Baló's scientific career and a further increase of working possibilities:

In 1926 he became a Privatdozent.

In 1928 following the unanimous invitation of Szeged University to the chair of pathological anatomy he spent his next 17 years there.

In 1945 he accepted the equally unanimous invitation of Budapest University and became here professor of pathological anatomy and forensic medicine, as the successor of his highly esteemed beloved master, Coloman Buday. Since then more than 20 years have passed with successful work carried out with untiring endurance and zeal, inspiring many other workers.

As mentioned before, Baló's scientific interest was kept in the first period of his career by the diseases of the nervous system. Besides the problems of epidemic encephalitis and herpes encephalitis he studied the spontaneous encephalitis of rabbits then the nervous complications of periarteriitis nodosa. His observations concerning the encephalitis of dogs with Eck-fistula fed on meat raised much interest. These noteworthy experiments are still good models of encephalitis.

Among the diseases of the cerebral white matter, a peculiar condition was described first by Baló in 1927 under the term concentric periaxial encephalitis.

This process involving the destruction of medullated fibres in the white matter of the cerebral hemispheres occurs in such a particular manner that axis cylinders are spared while degeneration of myelin sheaths takes place in a peculiar way resembling the Liesegang rings, i.e. foci appear in which normal and demyelinated layers alternate covering one another like onion leaves when considered in the space. The disease brings about a swelling of the brain and may cause symptoms of a brain tumour. This may be considered as an

indication for brain surgery as it happened in Baló's case, the report of which caused such an echo that HALLERVORDEN and SPATZ asked for material to control the findings. Their results agreed in every respect with those of Baló. Since then the condition has been listed as Baló's disease or concentric sclerosis and its description can be found in every modern neurological textbook. With its deceiving course, its unknown etiology is one of the problems in neuropathology; in Baló's view some myelinolytic enzyme must be responsible for the manifestation of this process. He collected his rich experience on the demyelinating processes of the nervous system in a book published in German in 1940, then followed some careful works on diffuse sclerosis (1940, 1942) and on the endocrinological relations of tuberous sclerosis (1943, 1944). Well before that, in 1931 he edited a book on filterable viruses, containing much of the personal experience. No work summing up the results in this field had appeared in Hungarian up to that time and it must have been a novelty even abroad as in 1935 S. Karger of Berlin published the book in German.

Baló's observations on the cortico-visceral relations of peptic ulcer resulted in a convincing proof of this disease being due to an organic lesion of the central nervous system (1940), a problem which was the subject of his inaugural lecture at the Hungarian Academy of Sciences in 1941.

Though in the following decades, the diseases of arteries formed the chief interest of Joseph Baló, in 1954 he succeeded in showing that pemphigus is a neurotrophic disease of the skin brought about by inflammatory changes of the spinal ganglia.

In the late forties, Baló began to study experimentally the destruction of elastic fibres in the arteries and compared the findings with those in human arteriosclerosis. He concluded that the destruction of the arterial wall is due to a shift toward acid of the acid-base balance. Acidosis is then followed by the accumulation of fat and cholesterol in the blood, so that cholesterol will be deposited in the walls of arteries. Then Baló and his wife, ILONA BANGA, a pupil of ALBERT SZENT-GYÖRGYI, investigated the factor destroying the elastic fibres of arteries and succeeded in discovering an enzyme of the pancreas, elastase, which is able to digest elastic fibres and attributed to it an importance in the genesis of atherosclerosis (1949).

The discovery and isolation of elastase gained world-wide acceptance for the study of connective tissue elements. The Baló couple deserved well the Kossuth-prize of the Hungarian People's Republic in 1955 for their researches in this field.

Elastase consists of proteolytic and mucolytic components and exerts lipoprotein lipase activity as well. These important achievements have been proved by a number of authors and led to several morphological and biochemical results. In the opinion of the Balós, activation of elastase may occur in three ways, viz. by 1. acidification, 2. dialysis, 3. precipitation with ammonium

sulphate. They showed that in both pancreas and blood elastase is combined with an inhibitor and their separation happens in the mentioned three ways.

Administration of ammonium hydroxide to rabbits brings about acidosis in the course of which the elastic membranes of the arteries will be destroyed, the blood cholesterol level increases and the activity of the elastase inhibitor is reduced, so that the effect of elastase is enhanced and a destruction of the elastic fibres in the walls of the arteries takes place. In this way has been confirmed Baló's theory on the origin of atherosclerosis.

Another field of Baló's activity was oncological research, as he recognized the need for well-organized tumour-research in Hungary. One of his early achievements in this field was that together with Béla Korpássy he showed on the basis of 1800 postmortem examinations that individuals having senile warts become three times more frequently victims of cancer than those free of such warts (Warzen, Papillome und Krebs. Budapest—Leipzig 1936).

Investigating the tumour producing factors, Baló succeeded in inducing pulmonary adenoma in mice by the administration of urethane and examined with his collaborators the role of hypnotics in the production of tumours. He investigated the effect of cytostatic drugs to be applied in human treatment. He published his observations on human lung cancer and lung adenoma in 1957 in a book in German. The interest in the book was proved by the fact that a new edition was soon required (1959) and a Hungarian version was also published in 1960. An important contribution of Baló and his collaborators was the detection of the tumour producing activity of isoniazid.

This portrait of Baló would be incomplete if we would not characterize his skill as a teacher. He laid down his rich experience in the two volumes of his textbook of pathology (1948, 1952). This work edited again in 1961—1962 is not only a source of information offering a critical analysis of contemporary results, but an ample repertory of the intuition and observing mind of Joseph Baló.

Baló is honorary member of the Society of Pathologists of the Soviet Union, member of the International Academy of Pathology (Washington), the Gerontological Society (St. Louis), the Deutsche Gesellschaft für Pathologie, Deutsche Akademie der Naturforscher Leopoldina (Halle), an Affiliate of the Royal Society of Medicine (London), corresponding member of the Vereinigung Deutscher Neuropathologen und Neuroanatomen, of the Hungarian Academy of Sciences, president of the Hungarian Society of Pathology, member of the editorial board of several medical journals. He presented a paper by invitation at a special Symposium celebrating the Semicentennial of the foundation of the American Society of Experimental Pathology in Atlantic City (1963) and delivered the second Cesare Massari Lecture at the University Medical School in Perugia (1966).

RAMON Y CAJAL quotes in a place the words of CISNEROS: "Exemplary

conduct is the best preacher." It was by his creative work that Joseph Baló stimulated his collaborators' activity. The twenty-seven volumes where he collected his papers and those of his collaborators during almost four decades of his professorial activity are a convincing proof that he accomplished good work because he was able to transmit the passion for the recognition of truth to his pupils.

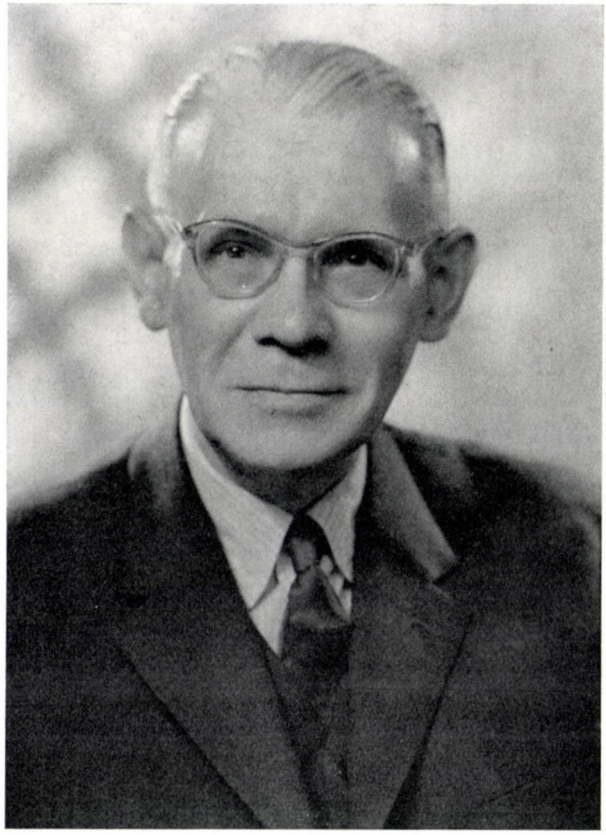
I had occasion to observe during a lifetime the ascending star of Joseph Baló in the field of scientific recognition, and was one of the beneficiaries just as all those near him in that atmosphere saturated with searching spirit. I still feel happy to have been a member of that community of thoughts of which the stimulating motive was the passion of man seeking after truth.

"This above all: to thine own self be true" I heard several times from Baló the words of Polonius. They are still ringing in my ears. And when hard times had come, when it was difficult to be true to one's own self, Joseph Baló remained true to himself and to the high ideas of humanity.

Prof. D. MISKOLCZY
Member of the Hungarian Academy
of Sciences

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 7—18, 1968*

VERZEICHNIS DER VERÖFFENTLICHUNGEN VON PROF. DR. JOSEF BALÓ



A) Monographien

1. Cukorbetegség és insulin. Mitarb.: R. Bálint, Z. Ernst und B. Purjesz. Dick Manó, Budapest, 18—50, 1927.
2. A láthatatlan kórokozók filtrálható vírusok. Magyar Orvosi Könyvkiadó Társulat, Budapest, 1—174, 1931.
3. Die unsichtbaren Krankheitserreger filtrierbare Vira. S. Karger, Berlin, 1—311, 1935.
4. Warzen, Papillome und Krebs. Acta litterarum ac scientiarum Reg. Universitatis Hung. Francisco-Josephinae, Sectio Medicorum. Band VII. J. A. Barth, Leipzig, 1—303, 1936. Mitarb.: B. Korpássy.
5. Die Erkrankungen der weissen Substanz des Gehirns und des Rückenmarkes. Acta Med. Szeged, Band X. Fasc. 1. J. A. Barth, Leipzig, 1—160, 1940.
6. Lungenkrebs und Lungenadenom. Verlag der Ungarischen Akademie der Wissenschaften, Budapest, 1—363, 1957.
7. Lungenkrebs und Lungenadenom. Zweite, erweiterte Auflage. Verlag der Ungarischen Akademie der Wissenschaften, Budapest, 1—379, 1959.
8. Tüdőrák és tüdőadenoma. Akadémiai Kiadó, Budapest, 1—374, 1960.

B) Lehrbücher

9. Kórbonctan I. Általános rész. Budapest, 1—441, 1948.
10. Kórbonctan II. Részletes rész. Egészségügyi Kiadó, Budapest, 1—592, 1952.
11. A rosszindulatú daganatok biopsiája. Onkologia I. Ált. rész. Red.: Béla Wald. Egészségügyi Kiadó, 117—137, 1952.
12. A részletes kórbonctan tankönyve. Medicina Könyvkiadó, Budapest, 1—610, 1961.
13. Az általános kórbonctan tankönyve. Medicina Könyvkiadó, Budapest, 1—488, 1962.

C) Zeitschriftenveröffentlichungen

14. Idegrendszeri elváltozások periarteriitis nodosa eseteiben. Orvosi Hetilap **65,** 223; 233; 241, 1921.
15. A vészes vérszegénységben előforduló idegrendszeri elváltozásokról, különös tekintettel a gerincvelő megbetegedéseire. Magyar Orvosi Archívum **20,** 131, 1922.
16. Eine interessante „Encephalitis-Epidemica" Endemie an einer Säuglingsabteilung. Jahrbuch für Kinderheilkunde. **99,** 209, 1922. Mitarb.: J. Duzár.
17. Kettős torzképződés két esete. Orvosi Hetilap **66,** 409; 421, 1922. Mitarb.: P. Markovits.
18. A hypophysis mellső lebenyének elhalásáról és ennek következményeiről. Orvosi Hetilap **67,** 453, 1923.
19. Adatok a perirenalis apoplexia kórképéhez. Magyar Orvosi Archívum **24,** 295, 1923.
20. Az encephalitis epidemicaról, különös tekintettel az agy és a belső szervek megbetegedésére. Orvosképzés **3—4,** 10, 1923.
21. Über Nekrosen des Hypophysenvorderlappens und ihre Folgen. Beitr. z. path. Anat. und z. allg. Pathologie **72,** 599, 1924.
22. Encephalitis epidemica und Gesamtorganismus, zugleich ein Bericht über solche Epidemien in Budapest. Frankfurter Zeitschrift für Pathologie **30,** 512, 1924.
23. Periarteriitis nodosa beim Hunde und vergleichende Untersuchungen über diese Erkrankungen bei Menschen und Hunden. Virchows Archiv f. pathol. Anat. u. Physiol. **248,** 337, 1924.
24. A járványos encephalitis alatt fellépő májmegbetegedésekről. Magyar Orvosi Archívum **26,** 97, 1925.
25. Über ein Aneurysma der Rückenmarksarterie, welches Tabes-dorsalisartige Symptome vortäuschte. Deutsche Zeitschrift für Nervenheilkunde **85,** 86, 1925.
26. Az ascaris lumbricoides vándorlásáról a gazda szervezetében és ennek kórtani jelentőségéről. Orvosi Hetilap **69,** 51, 1925.
27. A pneumococcus típusmeghatározásának jelentőségéről a pneumococcus fertőzések epidemiológiájában és gyógykezelésében. Orvosi Hetilap **69,** 615, 1925. Mitarb.: I. Lovrekovich.
28. Beiträge zum Krankheitsbilde der perirenalen Apoplexie. Beitr. path. Anat. u. z. allg. Pathologie **73,** 598, 1925.
29. A típusmeghatározás jelentőségéről a bakteriológiában és hazai pneumococcustípusok megállapításáról. Magyar Orvosi Archívum **26,** 284, 1925. Mitarb.: I. Lovrekovich.
30. A kísérleti tumorkutatásról W. E. Gye-nek a rosszindulatú daganatok ätiológiájáról végzett vizsgálataival kapcsolatban. Orvosi Hetilap **69,** 959, 1925.
31. A közönséges herpes és a járványos encephalitis között levő viszonyról, különös tekintettel az ätiológiára. Magyar Orvosi Archívum **26,** 441, 1925.
32. Über eine Häufung von Periarteriitis-nodosa-Fällen, nebst Beiträgen zur Polyneuritis infolge von Periarteriitis nodosa. Virchows Archiv f. path. Anat. u. Physiol. **259,** 773, 1926.
33. Über das Verhältnis des gewöhnlichen Herpes zur epidemischen Encephalitis, mit besonderer Rücksicht auf die Ätiologie. Monatschrift Ungarischer Mediziner **3,** 23, 1927.
34. Die Wirkung der Luftverdünnung auf das Blut und die blutbildenden Organe. Zeitschrift f. d. ges. exp. Med. **59,** 303, 1928.

35. A házinyulak spontán encephalitise és ennek jelentősége a járványos encephalitis kóroktani kutatásában, valamint egyéb házinyúl kísérleteiben. Orvosi Hetilap **71,** 723, 1927. Mitarb.: Ö. Gál.

36. Über die spontane Encephalitis der Kaninchen und deren Bedeutung in der ätiologischen Forschung der epidemischen Encephalitis, sowie in sonstigen Kaninchen-Versuchen. Virchows Archiv f. path. Anat. u. Physiol. **365,** 286, 1927. Mitarb.: Ö. Gál.

37. A leukoencephalitis periaxialis concentricaról. Magyar Orvosi Archívum **28,** 108, 1927.

38. A rákkutatás eredményeiről kórtani szempontból. Gyógyászat **22,** 1, 1927.

39. Pancreas-eredetű gerincvelő megbetegedés. Magyar Orvosi Archívum **28,** 448, 1927.

40. Encephalitis periaxialis concentrica. Archives of Neurology and Psychiatry **19,** 242—262, 1928.

41. Myelitis funicularis als Folgeerscheinung der Erkrankung des Pancreas. Deutsche Zeitschrift für Nervenheilkunde **102,** 275, 1928.

42. Die Wirkung der Gallensteine auf die Bauchspeicheldrüse. Monatschrift Ungarischer Mediziner **4,** 1, 1928.

43. Az 1928. évi internacionális rákkonferencia. Orvosi Hetilap **72,** 1197, 1928.

44. Die Lipomatose der Bauchspeicheldrüse und deren Bezug zur allgemeinen Fettsucht. Virchows Archiv **273,** 320, 1929.

45. A hasnyálmirigy lipomatosisa és annak viszonya az általános elkövéredéshez. Orvosi Hetilap **73,** 833, 1929.

46. Der Zusammenhang zwischen der Lipomatose des Pancreas und der allgemeinen Fettsucht. Verhandlungen der Deutschen Pathologischen Gesellschaft 343, 1929.

47. Metaplasia of Basal Cells in the Ducts of the Pancreas: Its Consequences. Collaborator: H. C. Ballon. Archives of Pathology **7,** 27, 1929.

48. Über die Periarteriitis nodosa auf Grund von 9 neueren Fällen. Mitarb.: Ö. Nachtnebel. Virchows Archiv **272,** 478, 1929.

49. Periarteriitis nodosa und innere Sekretion. Mitarb.: Ö. Nachtnebel. Endokrinologie **3,** 180, 1929.

50. Über die Virulenzänderung des Herpesvirus unter der Einwirkung verschiedener Bakterien. Zeitschrift f. Hygiene und Infektionskrankheit. **110,** 599, 1929.

51. A herpesvírus virulentiájának változása különböző bakteriumok hatására. Orvosi Hetilap **73,** 125, 1929.

52. Effects of Retention of Pancreatic Secretion. Collaborator: H. C. Ballon. Surgery, Gynecology and Obstetrics **48,** 1, 1929.

53. Pankreaslipase intravénás adagolásának befolyása a vérlipase szintjére. Mitarb.: I. Bach. Orvosi Hetilap **74,** 1231, 1930.

54. Der Einfluß intravenös verabreichter Pancreaslipase auf den Blutlipasespiegel. Zeitschrift d. ges. exp. Med. **75,** 583, 1931. Mitarb.: E. Bach.

55. Über die Komplikationen der Pockenschutzimpfung mit besonderer Rücksicht auf die Encephalitis. Jahrbuch f. Kinderheilkunde **131,** 106, 1931. Mitarb.: F. Lőrincz.

56. A himlőellenes védőoltás szövődményeiről, különös tekintettel az enkephalitisre. Orvosi Hetilap **74,** 1053, 1930. Mitarb.: F. Lőrincz.

57. Über die Ursache der im Verlaufe der Periarteriitis nodosa vorkommenden Polyneuritiden. Zeitschr. f. d. ges. Neurol. u. Psych. **134,** 71, 1931.

58. A vaccinatio után fellépő enkephalitisről. Budapesti Orvosi Újság **29,** 1, 1931.

59. The Encephalitis of Dogs with Eck Fistula Fed on Meat. Arch. of Pathology **13,** 80, 1932. Collaborator: B. Korpássy.

60. A hússal táplált Eck-fistulás kutyák enkephalitiséről. Orvosi Hetilap **76,** 181, 1932.. Mitarb.: B. Korpássy.

61. Die antagonistische Wirkung eines neuen Pancreasextractes auf die Thyroxinvergiftung. Arch. f. exp. Path. u. Pharm. **165,** 594, 1932. Mitarb.: L. Lovas, E. Bach und L. Neufeld

62. A pajzsmirigy és pancreas antagonismusáról. Orvosi Hetilap **76,** 261, 1932.

63. Über einen die Thyroxinvergiftung verhütenden Stoff des Pancreas. XIV. Congresso Internazionale di Fisiologia. Roma, 1932. Sunti Delle Communicazioni Scientifiche p. 22.

64. Zur Frage der Entstehungsursache der Pancreasnekrose. Beitr. pathol. Anat. **92,**14, 1933.

65. Über den Fermentgehalt der menschlichen Bauchspeicheldrüse. Mitarb.: L. Lovas. Virchows Archiv **288,** 326, 1933.

66. Az emberi pancreas fermenttartalmáról. Magyar Orvosi Archívum **34,** 179, 1933. Mitarb.: L. Lovas.

67. Die Verhinderung der Serumlipaseabnahme bei tuberkulösen Kaninchen. Beitr. z. Klinik der Tuberkulose. **82,** 164, 1933. Mitarb.: F. Gerlei und L. Lovas.

68. Gümőkóros házinyulak serum lipase csökkenésének meggátlásáról. Orvosi Hetilap **77,** 70, 1933. Mitarb.: F. Gerlei und L. Lovas.

69. A verrucák és rák gyakori együttes előfordulása. Orvosi Hetilap **77,** 797, 1933.

70. Pathologie des Fettstoffwechsels (Referat). Verhandlungen der Gesellschaft Ungarischer Pathologen **1,** 105, 1933.

71. Gleichzeitiges Vorkommen von Warzen und Oesophagus-Leukoplakie. Abhandlungen auf dem Gebiete der allg. Path. 1933. Mitarbeiter: B. Korpássy.

72. The Relation of Warts to Cancer. Congreso Internacional de Lucha Cientifica y Social contra el Cáncer. Madrid. Tomo II. Primera parte 35—38, 1933.

73. Über ein den die Thyroxinvergiftung paralysierenden Pankreasextrakt. Bericht der II. Internationalen Kropfkonferenz in Bern. 2, 1, 1933.

74. Über die Beziehung der Leukoplakie der Speiseröhre zu den senilen Warzen. Verhandlungen der Gesellschaft Ungarischer Pathologen **2,** 107, 1933. Mitarbeiter: B. Korpássy.

75. Die Gehirnabbaufähigkeit des menschlichen Blutserums. Archiv für Psychiatrie und Nervenkrankheiten **102,** 202—212, 1932.

76. Polypose des menschlichen Dickdarms und deren Beziehungen zum Krebs. Verhandlungen der Gesellschaft Ungarischer Pathologen, **3,** 119, 1934. Mitarb.: B. Korpássy.

77. Ein Beitrag zur Frage der Gehirnabbau-Tätigkeit des Blutserums. Verhandlungen der Gesellschaft Ungarischer Pathologen **3,** 94, 1934.

78. Warzen und Papillome bei Menschen und Tieren. Verhandlungen der Gesellschaft Ungarischer Pathologen **3,** 117, 1934.

79. Összehasonlító vizsgálatok emberek, sertések, szarvasmarhák adenomatosus bélpolypjairól. Állatorvosi Lapok **58,** 219, 1935. Mitarb.: B. Korpássy.

80. Über die Guanidin-Encephalitis. Verhandlungen der Gesellschaft Ungarischer Pathologen **4,** 121, 1935.

81. A röntgenártalmak különböző formái és azok törvényszéki orvostani vonatkozásai. Kenyeres-jubileumi kötet. Budapest, 1936.

82. Ein Beitrag zur Kenntnis des mit senilen Warzen behafteten Organismus. IX. Congressus Internationalis Dermatologorum, 1935.

83. Diskussionsbemerkung zum Referat von Prof. Kumer: Herpesforschung und Klinik. IX. Congressus Internationalis Dermatologorum, 1935.

84. Beitrag zur Frage der Lungenerkrankung der Paprikaspalter auf Grund pathologisch-anatomischer und histologischer Untersuchungen. Ärztliche Sachverständigen-Zeitung **42,** 304, 1936.

85. Ein Beitrag zur Pathologie der Zellteilung. Verhandlungen der Gesellschaft Ungarischer Pathologen **5,** 186, 1936.

86. A rák szövettana és a szövettani szerkezet gyakorlati vonatkozásai. A daganatok kór- és gyógytana című munkában. 1937. Kiadó: Manninger Vilmos.

87. Die Erkrankungen des Rückenmarks bei perniciöser Anämie. Comptes Rendus de la Troisième Conférence Internationale de Pathologie Géographique. Stockholm, 290, 1937.

88. A folliculushormon hatásáról. Orvosi Hetilap **81,** 644, 1937. Mitarb.: B. Purjesz.

89. Über die Wirkung des Follikelhormons. Klinische Wochenschrift **16,** 1150—1152, 1937.

90. A vészes vérszegénység májkezelésének néhány kérdése. Budapesti Orvosi Újság **35,** 61, 1937.

91. Azidose und Knochenmark. Verhandlungen der Gesellschaft Ungarischer Pathologen **6,** 106, 1937.

92. Die mit Ammoniumhydroxydvergiftung erzeugbare experimentelle Arteriosklerose. Frankfurter Zeitschrift f. Pathologie **52,** 205, 1938.

93. Die Änderung der Reaktion des Blutes in vitro. Pflügers Archiv f. d. ges. Physiol. des Menschen und der Tiere **239,** 726, 1938.
94. Der Einfluß der Ammoniavergiftung auf das Blut und auf die Blutbildung. Beitr. z. path. Anat. u. z. allg. Path. **101,** 66, 1938.
95. Sertésgondozók Bouchet-féle betegsége. Népegészségügy **21,** 3, 1938.
96. A vérsavanyodás in vitro. Ferenc József Tudományegyetem Baráti Egyes. Természettudományi Szakosztálya 1938. márc. 16.
97. A hypophysis cystáiról. Magy. Path. Társ. Nagygyűlésének munk. **3,** 1, 1938.
98. Die Wirkung des Thyroxins auf die Arterien. Beitr. z. path. Anat. und z. allg. Pathol. **102,** 341, 1939.
99. Thyroxin és arteriosclerosis. Orvosi Hetilap **83,** 609, 1939.
100. Multiple Hirnhernien als Folgeerscheinungen von Hirngeschwülsten. Arch. f. Psychiatrie und Nervenkrankheiten **110,** 445, 1939.
101. Többszörös agyhernia, mint agydaganatok következménye. Magyar Orvosi Archívum **40,** 247, 1939.
102. Sertések vastagbélpolypjainak aetiológiájáról. Állatorvosi Lapok **62,** 216, 1939. Mitarb.: B. Korpássy.
103. Sopra le cisti dell ipofisi. Archivio „De Vecchi" per l'Anatomia Patologica e la Medicina Clinica. **1,** 396, 1939.
104. Die Arteriosklerose (Referat), Verhandlungen der Gesellschaft Ungarischer Pathologen **8,** 133, 1939.
105. Problem of Virus Etiology of Benign Intestinal Tumours. Third International Congress for Microbiology. New York City. Sept. 2—9. 1939.
106. Multiple Adenomata of the Large Intestine in Men and Animals. Third International Cancer Congress. Atlantic City. Sept. 11—15. 1939.
107. Az érelmeszesedés megelőzése. 1939. okt. 1-én rektori székfoglaló a Szegedi Egyetemen.
108. A Betaxin gyógyhatásáról. A Szegedi Tud. Egyetem Baráti Egyesülete 1939. okt. 4.
109. Maladie de Kussmaul-Maier et sclérose diffuse. Journal Belge de Neurologie et de Psychiatrie **40,** 160—168, 1940.
110. Die neurogene Theorie des peptischen Magengeschwürs. Verhandlungen der Gesellschaft Ungarischer Pathologen **9,** 145, 1940.
111. Die neurogene Theorie des peptischen Magen- u. Duodenal-Geschwürs. Deutsche med. Wochenschrift **67,** 479, 1941.
112. Gehirnblutung u. peptisches Geschwür. Wiener klin. Wschr. **54,** 326, 1941.
113. Die Erkrankungen der weissen Substanz des Gehirns u. des Rückenmarks. Münch. med. Wschr. **88,** 443, 1941.
114. A gyomorfekély kórtana. Akadémiai lev. tag székfoglaló. Matematikai és Természettudományi Értesítő **60,** 530, 1941.
115. Az agy diffus sclerosisa. Orvostudományi Közlemények **3,** 545, 1942.
116. Über die Bedeutung der Corpora amylacea im Zentralnervensystem. Verhandlungen der Gesellschaft Ungarischer Pathologen **11,** 170, 1942.
117. Die Aetiologie der diffusen Hirnsclerose. Zschr. f. d. ges. Neurol. u. Psych. **176,** 80, 1943.
118. A Rocky Mountain spotted fever erdélyi előfordulása. Orvosi Hetilap **87,** 462, 1943.
119. A zsírmobilisatio mechanismusa. Magy. Orv. Archívum **44,** 3, 1943.
120. Sclerosis tuberosa és csontelváltozások. Orvostud. Közl. **4,** 345, 1943.
121. Az ammoniák acidosis. Június 24. Szeged 1943. A Magy. Élettani Társ. XIII. vándorgyűlése.
122. Néhány a hypophysis működési zavarából eredő megbetegedésről. Magy. Path. Társasága 1943.
123. Tuberöse Sclerose u. innere Secretion. Arch. f. Psych. **117,** 333, 1944.
124. Mecsnikov orosz természettudós. A Szovjetorosz Barátok Társaságában Szegeden 1945. június 3-án tartott előadás.
125. A kiütéses typhus kórbonctana. A szegedi orvosok szakszervezetében 1945. szept. 25-én tartott előadás.
126. Rákkutatás a Szovjetunióban. Orvosok Lapja **3,** 1209, 1947.

127. Der Resorptionsweg der im Gehirn entstehenden Degenerationsprodukte. Virchows Archiv **315,** 61, 1948.
128. Az érfal rugalmas rostjainak pusztulása. Orvosi Hetilap **89,** 465, 1948. Mitarb.: I. Banga.
129. Enzymic Activity of the Aorta. Adenylpyrophosphatase of the Aorta. Zeitschr. f. Vitamin, Hormon- und Fermentforschung **2,** 1, 1948. Collaborators: I. Banga and Gy. Josepovits.
130. Studies on the Properties of the Albuminoids of the Arterial Wall. Zeitschr. f. Vitamin, Hormon- und Fermentforschung **2,** 408, 1948. Collaborators: I. Banga and A. Nowotny.
131. La distruzione delle fibre elastiche dei vasi sanguigni. Società Italiana di Anatomia Patologica 1948.
132. Az érfal rugalmas rostjainak pusztulása. Centennaris Orvosi Nagyhét Budapest, 1948. szept. 6.
133. A pancreas kivonatok elasticus rostoldó hatását gátló anyag a vérsavóban. Orvosi Hetilap **90,** 45, 1949. Mitarb.: I. Banga.
134. Az agy vérkeringésének szabályozása. Orvosi Hetilap **90,** 321, 1949.
135. Specificity of the Elastolytic Enzyme. Zeitschr. f. Vitamin, Hormon- und Fermentforsch. **2,** 415, 1949. Collaborators: I. Banga and A. Nowotny.
136. Die Zerstörung der elastischen Fasern der Gefäßwand. Schweizerische Zeitschrift f. Path. u. Bakt. **12,** 350, 1949. Mitarb.: I. Banga.
137. Elastase and Elastase Inhibitor. Nature **164,** 491, 1949. Collaborator: I. Banga.
138. Az érelmeszesedés. A Magyar Tud. Akadémia IV. Osztályán rendes taggá választás alkalmával tartott székfoglaló 1949. május 2.
139. Az elastase inhibitor. Magyar Élettani Társaság 1949. évi vándorgyűlése.
140. Emberek és sertések poliomyelitise. Pathologus Szakcsoport Nagygyűlése 1949.
141. The Dural Venous Sinuses. The Anatomical Record **106,** 319, 1950.
142. The Elastolytic Activity of Pancreatic Extracts. The Biochemical Journal **46,** 384, 1950. Collaborator: I. Banga.
143. Az érpathológia újabb eredményei. Referátum a Path. Szakcsoport 1950. évi Nagygyűlésén.
144. Elastase és arteriosclerosis. Banga társszerzővel előadás a Magyar Élettani Társaság 1950. évi vándorgyűlésén.
145. Apoplexia és gyomorfekély. Orvosi Hetilap **91,** 801, 1950. Mitarb.: F. Freisinger und K. Lapis.
146. Az arteriák elváltozásai essentialis hypertoniában. Orvosi Hetilap **91,** 1215, 1950. Mitarb.: Gy. Róna und Z. Lábas.
147. Az elastase aktiválása. Kísérletes Orvostudomány **2,** 271, 1950. Mitarb.: I. Banga.
148. Az ulcus pepticum oka az idegrendszerben. A Magyar Tudományos Akadémia V. osztályán 1950. jún. 13-án tartott előadás.
149. Причина пептической язвы в нервной системе. Acta morphol. Acad. Sci. hung. **1,** 167, 1951.
150. Hydrochinon hatása az átoltható egérrák növekedésére. Kísérletes Orvostudomány **3,** 191, 1951. Mitarb.: F. Vándor und P. Reiman.
151. Az agy, mint a vér cholesterinjének forrása. Orvosi Hetilap **92,** 785, 1951. Mitarb.: J. László.
152. Malignus melanomák kóreredete. Orvosi Hetilap **92,** 525, 1951. Mitarb.: F. Vándor und P. Reiman.
153. A tüdőadenomák jelentőségéről. I. Magyarországi Onkológiai Nagygyűlés 1951. június 15.
154. Az intervertebralis dúcok vizsgálata pemphigus eseteiben. Pathologus Szakcsoport Nagygyűlése 1951. okt. 13. Mitarb.: F. Földvári.
155. Az intervertebralis dúcok vizsgálata pemphigus esetében. Magyar Dermatologiai Társ. Nagygyűlése 1951. dec. 15. Mitarb.: F. Földvári.
156. Veränderungen der Arterien bei essentieller Hypertonie. Acta morphol. Acad. Sci. hung **1,** 67, 1951. Mitarbeiter: G. Róna und Z. Lábas.
157. Az arteriák elváltozásai diffus glomerulonephritis következtében. Orvosi Hetilap **93,** 356, 1952. Mitarb.: Gy. Róna und Z. Lábas.

158. Die Veränderungen der Arterien infolge diffuser Glomerulonephritis. Acta morphologica Acad. Sci. hung. **1,** 399, 1951. Mitarbeiter: G. Róna und Z. Lábas.

159. L'examen des ganglions spinaux dans des cas de pemphigus. Annales de Dermatologie et de Syphiligraphie **79,** 626, 1952. En collaboration avec F. Földvári.

160. A spinalis ganglionok vizsgálata pemphigus esetekben. Bőrgyógy. és Venerológiai Szemle **6,** 75, 1952. Mitarb.: F. Földvári.

161. Az arteriosclerosis problémája. Magyar Tud. Akadémia Orvosi Osztályok Közleményei **3,** 545, 1952.

162. Diabeticusok glomerulosclerosisa (Kimmelstiel—Wilson syndroma). Orvosi Hetilap **93,** 1137, 1952. Mitarb.: Gy. Róna und Zs. Jakab.

163. Urethan okozta kísérletes tüdőadenomák. Kísérletes Orvostudomány **4,** 267, 1952. Mitarb.: J. Juhász und Gy. Varga.

164. Über durch Urethan verursachte experimentelle Lungenadenome. Acta morph. Acad. Sci. hung. **3,** 101, 1953. Mitarbeiter: J. Juhász und J. Varga.

165. Elastin and Elastase. Nature **171,** 44, 1953. Collaborator: I. Banga.

166. Change in the Elastase Content of the Human Pancreas in Relation to Arteriosclerosis. Acta physiologica Acad. Sci. hung. **4,** 187, 1953. Collaborator: I. Banga.

167. Das Problem der Arteriosklerose. Acta medica Acad. Sci. hung. **4,** 449, 1953.

168. Kísérleti glomerulosclerosis. Kísérletes Orvostudomány **5,** 196, 1953. Mitarb.: J. Juhász und G. Kendrey.

169. Über die geschwulsterzeugende Wirkung des Urethan in Schlafmitteln. Zeitschr. f. Krebsforsch. **59,** 561, 1953. Mitarbeiter: J. Juhász und G. Kendrey.

170. A Sevenal és Dial daganatkeltő hatásáról. Kísérletes Orvostudomány **5,** 301, 1953. Mitarb.: J. Juhász und G. Kendrey.

171. A pneumocystis carinii előfordulása Magyarországon és szerepe újszülöttek plasmasejtes pneumoniájának keletkezésében. Orvosi Hetilap **94,** 966, 1953. Mitarb.: D. Schuler.

172. Diabetesesek hyalinos arteriosclerosisa. Orvosi Hetilap **94,** 1273, 1953. Mitarb.: Gy. Róna und E. Kerekes.

173. A Gasser-dúc elváltozásai szájpemphigus esetekben. Bőrgyógy. és Venerológiai Szemle **7,** 135, 1953. Mitarb.: F. Földvári.

174. Pemphigus esetekben a spinalis ganglionokban észlelt elváltozások kóreredete. Bőrgyógy. és Venerol. Szemle **7,** 175, 1953. Mitarb.: L. Liszkai.

175. The Effect of Substances Influencing Oxido-reduction on the Growth of Mouse Cancer. Acta physiologica Acad. Sci. hung. **5,** 273, 1954. Collaborators: I. Banga and P. Reiman.

176. Diabetic Angiopathy and Glomerulosclerosis. Acta morph. Acad. Sci. hung. **4,** 51, 1954. Collaborators: G. Róna, S. Jakab and E. Kerekes.

177. Das Vorkommen von Pneumocystis carinii in Ungarn und ihre Rolle bei der interstitiellen plasmazellulären Pneumonie der Neugeborenen. Acta morphologica Acad. Sci. hung. **4,** 1, 1954. Mitarb.: D. Schuler.

178. Vergleichende Untersuchungen über die Elastolyse der Gefäßwand und des Lig. nuchae in histologischen Schnitten. Acta morphologica Acad. Sci. hung. **4,** 141, 1954. Mitarbeiter: I. Banga und D. Schuler.

179. Contraction and Relaxation of Collagen Fibres. Nature **174,** 788, 1954. Collaborators: I. Banga and D. Szabó.

180. Studies of the Elastolysis of Elastin and of Collagen. Acta physiologica Acad. Sci. hung. **6,** 235, 1954. Collaborator: I. Banga.

181. Les altérations du ganglion de Gasser dans des cas de pemphigue de la bouche. Annales de Dermatologie et de Syphiligraphie. **81,** 507, 1954. En collaboration avec F. Földvári.

182. A pemphigusról általában. Katamnesztikus vizsgálatok. Bőrgyógy. és Venerol. Szemle **8,** 42, 1954. Mitarb.: F. Földvári und K. Márton.

183. A mellékvese működésének idegrendszeri szabályozása. Orvosi Hetilap **95,** 1296, 1954.

184. Die Neuroregulation der Nebennierenfunktion. Acta medica Acad. Sci. hung. **6,** Suppl. 1, 126, 1954.

185. Újszülöttek sejtzárlatos betegsége (Cytomegalia infantum). Orvosi Hetilap **95,** 1230, 1954. Mitarb.: J. László und G. Kendrey.

186. Les adénomes bronchiques et leur importance. Bulletin de l'Association Française pour l'Étude du Cancer. **42,** 76, 1955. En collaboration avec D. Kassay et A. Bikfalvi.
187. Die Bronchialadenome und ihre Bedeutung. Acta morphologica Acad. Sci. hung. **5,** 71, 1955. Mitarbeiter: D. Kassay und A. Bikfalvi.
188. Bronchialadenome. Thoraxchirurgie **3,** 24, 1955. Mitarbeiter: D. Kassay und A. Bikfalvi.
189. Ein neuer experimenteller Geschwulststamm: das Amytal-Ascites Sarkom. Acta morphologica Acad. Sci. hung. **5,** 243, 1955. Mitarbeiter: J. Juhász und G. Kendrey.
190. Új kísérletes daganattörzs: Az amytal-ascites sarcoma. Kísérletes Orvostudomány **7,** 186, 1955. Mitarb.: J. Juhász und G. Kendrey.
191. Tüdőtuberculosis és tüdőrák. Tuberculosis Kérdései **8,** 4, 1955. Mitarb.: J. Juhász und G. Kendrey.
192. A pemphigus gamma-globulin kezelése. Bőrgyógyászati és Venerológiai Szemle **9,** 70, 1955. Mitarb.: F. Földvári.
193. Change in Velocity of Elastolysis of Bloodvessels due to Arteriosclerosis. 3ème Congrès International de Biochimie Bruxelles 1955. Collaborator: I. Banga.
194. A hörgőadenomák és jelentőségük. Orvosi Hetilap **96,** 1317, 1955. Mitarb.: D. Kassay und A. Bikfalvi.
195. Az emberi multiplex tüdőadenomák kérdése. Magyar Radiológia **7,** 198, 1955. Mitarb.: Leszler.
196. El origen multifocal del cáncer pulmonar. Folia Clínica Internacional **6,** 3, 1956.
197. Infarkt und Lungenkrebs. Acta morphologica Acad. Sci. hung. **6,** 367, 1956. Mitarbeiter: J. Juhász und J. Temes.
198. Pulmonary Infarcts and Pulmonary Carcinoma. Cancer (Philadelphia) **9,** 918, 1956. Collaborators: J. Juhász and J. Temes.
199. Elastomucoproteinase and Collagenmucoproteinase, the Mucolytic Enzymes of the Pancreas. Nature (London) **178,** 310, 1956. Collaborator: I. Banga.
200. Metacollagen as the Apparent Elastin. Journal of Gerontology **11,** 242, 1956. Collaborators: I. Banga and D. Szabó.
201. The Procollagen, as a Component of Collagen Fibres. Acta Physiologica Acad. Sci. hung. **9,** 61, 1956. Collaborators: I. Banga and D. Szabó.
202. Submicroscopic Structure of Collagen Fibres: their Contraction and Relaxation. Acta morphologica Acad. Sci. hung. **6,** 391, 1956. Collaborators: I. Banga and D. Szabó.
203. A kollagen rostok szerkezetének vizsgálata. Magyar Tud. Akadémia Biol. és Orvosi Tud. Osztályának Közleményei **7,** 385, 1956. Mitarbeiter: I. Banga und D. Szabó.
204. The Structure, Ageing and Rejuvenation of Collagen Fibres. Experientia Supplementum **4,** 28, 1956. Collaborators: I. Banga and D. Szabó.
205. Pemphigusban elhalt 62 beteg kórbonctani vonatkozásai. Bőrgyógy. és Venerológiai Szemle **10,** 97, 1956. Mitarb.: F. Földvári und K. Márton.
206. The Structure and Chemical Composition of Connective Tissue. Connective Tissue Symposium London, July 22—26, 1956. Blackwell P. 254. C.I.O.M.S. Collaborator: I. Banga.
207. Lungenadenomatose des Menschen. Zentralblatt f. Pathologie **97,** 242, 1957. Mitarb.: G. Karády.
208. Effect of Metal Complexes upon Experimental Carcinoma. Acta Unio International Contra Cancrum **13,** 463, 1957. Collaborator: I. Banga.
209. Der Alveolarzellkrebs der Lunge. Frankf. Ztschr. f. Path. **68,** 530, 1957.
210. Über die geschwulsterzeugende Wirkung der Isonikotinsäurehydrazid (INH). Zschr. f. Krebsforsch. **62,** 188, 1957. Mitarbeiter: J. Juhász und G. Kendrey.
211. On Elastase. Ciba Foundation Colloquia on Ageing Vol. 3. Churchill. London 1957, p. 101—103.
212. A Na-Amytal daganatkeltő hatása. Kísérletes Orvostudomány **9,** 537, 1957. Mitarb.: J. Juhász und G. Kendrey.
213. Az Androfort (Testosteron-acetat) daganatkeltő hatása. Kísérletes Orvostudomány **9,** 531, 1957. Mitarb.: G. Kendrey und J. Juhász.
214. Effect of ATP and Acid Swelling of Collagen. Biochimiya **22**/1—2, 60, 1957. Collaborator: I. Banga.

215. Effect of Intraperitoneal Administration of Elastase on White Rats. Gerontologia **1,** 315, 1957. Collaborators: I. Banga, J. Juhász, D. Szabó and E. Szalay.

216. Metacollagen, mint „látszólagos" elastin. Magyar Tud. Akadémia Biol. és Orvosi Tud. Oszt. Közleményei **8,** 231, 1957. Mitarb.: I. Banga und D. Szabó.

217. A spinalis ganglionok tömlős megbetegedéséről. Ideggyógyászati Szemle **10,** 142, 1957.

218. Vírus hepatitis szerepe a cirrhosis keletkezésében. Orvosi Hetilap **98,** 1290, 1957. Mitarb.: I. Besznyák und G. Kendrey.

219. Az izonikotinsavhydrazid (INH) daganatkeltő hatásának kísérletes vizsgálata. Tuberkulozis **10,** 49, 1957. Mitarb.: J. Juhász und G. Kendrey.

220. Investigations Concerning the Genesis of Pemphigus. Acta dermat. venerol. Proc. 11th Internat. Congr. Dermat. **3,** 372, 1957. Collaborator: F. Földvári.

221. Über die geschwulsterzeugende Wirkung des Glandubolins. Acta morph. Acad. Sci. hung. **8,** 95, 1958. Mitarbeiter: G. Kendrey und J. Juhász.

222. A kollagen duzzadása és annak meggátlása ATP-vel. A Magyar Tud. Akadémia Biol. és Orvosi Tud. Oszt. Közleményei **9,** 75, 1958. Mitarb.: I. Banga.

223. Elastase és atherosclerosis. A Magyar Tud. Akadémia Biol. és Orvosi Tud. Oszt. Közleményei **9,** 425, 1958. Akadémiai lev. tag. székfoglaló.

224. Die Rolle der Virushepatitis für die Entstehung der Leberzirrhose. Schweiz. Ztschr. f. allg. Path. Bakt. **21,** 1064, 1958. Mitarbeiter: I. Besznyák und G. Kendrey.

225. System nerveux et ulcère peptique. Schweiz. Ztschr. f. allg. Path. Bact. **21,** 561, 1958.

226. History and Pathogenesis of Atherosclerosis. Sectio XX. Excerpta Medica, Gerontology and Geriatrics **2,** 279, 1959.

227. Nephelometric Determination of Elastase Activity and Method for Elastolytic Measurements. The Biochemical Journal **71,** 544, 1959. Collaborators: I. Banga and M. Horváth.

228. Differences between Metacollagen and Collastromin. Acta physiol. Acad. Sci. hung. **15,** 127, 1959. Collaborator: I. Banga.

229. Az elasztolitikus enzim kémiai tulajdonsága és biológiai hatása. Magyar Kémiai Folyóirat **65,** 127, 1959. Mitarb.: I. Banga und D. Bagdy.

230. Effect of 1,6-Di-(2-Bromoethylamino)-1,6-Dideoxy-D-Mannitol Dihydrobromide on Tumours of Laboratory Animals. Nature **183,** 395, 1959. Collaborators: G. Kendrey, J. Juhász and I. Besznyák.

231. Filtrálható vírus szerepe a daganatok előidézésében. Orvosi Hetilap **100,** 491, 1959.

232. Tumor Inhibitory Action of 1,6-Di-(2-Bromaethyl-Amino)-1,6-Dideoxy-D-Mannitol Dihydrobromide (DBM) R_{13}. Brit. J. Cancer **13,** 634, 1959. Collaborators: G. Kendrey, J. Juhász and I. Besznyák.

233. Инфаркт и рак легкого. Труды III Всесоюзного Съезда Патологоанатомов. Харков 1961 стр. 62.

234. Über die Wirkung der Kollagenmukoproteinase auf die neutralen Mukopolysaccharide der kollagenen Fasern. Acta histochemica **9,** 69, 1960. Mitarb.: D. Szabó und I. Banga.

235. The Elasticity-increasing Property of Elastomucoproteinase. Biochimica et Biophysica Acta **40,** 367, 1960. Collaborator: I. Banga.

236. Isolation of Neutral Heteropolysaccharide Containing Mucoprotein from Bovine Achilles Tendon with the Aid of Collagenmucoproteinase. Biochem. J. **74,** 388, 1960. Collaborator: I. Banga.

237. Manifestaciones secundarias de los Quistes Hipofisarios. Folia Clín. Intern. **10,** 296, 1960.

238. A rákkutatás fejlődése. Orvosképzés **35,** 67, 1960.

239. Elastase és atherosclerosis. Orvostudományi Szemle **5,** 62, 1960.

240. Az elasztáz komplex természete és az elasztáz termelés helye. Orvostud. Szemle **5,** 70, 1960.

241. A zsírmobilisatio kérdése. Magyar Tud. Akad. Biol. és Orvosi Tud. Oszt. Közleményei **11,** 361, 1960.

242. Cholesterin adagolás házinyulaknak vizes suspensio alakjában. Kísérletes Orvostudomány **12,** 630, 1960. Mitarb.: E. Szalay.

243. Anatomía pat. e histopatología de las enfermedades alérgicas. Tratado de Alergía 1960. Barcelona. P. 237—266. Editorial Científico Médica. Barcelona—Madrid—Lisboa—Rio de Janeiro—Montevideo. Dir. Dr. F. Arasa.

244. Geschwulsterzeugende Wirkung von Arzneimitteln. Berliner Symposion über Fragen der Carcinogenese. Akad. Verlag, Berlin S. 208. 1960.

245. Kleine Tumoren (Tumourlets) der Lunge. Arch. De Vecchi **31,** 21, 1960.

246. Kísérletes vizsgálatok a DBM (R_{13}) (1,6-bis)-2-bromaethylamino (1,6-desoxy-D-mannit dibromhydrat) daganatnövekedést gátló hatásáról. Orvosi Hetilap **101,** 157, 1960. Mitarb.: G. Kendrey, J. Juhász, I. Besznyák.

247. Превращение коллагеновых волокон в эластические. Симпозиум по соединительной ткани. Медгиз, Москва, 1960 стр. 30.

248. Az elastase és kollagenmukoproteinase kutatás területén elért eredmények. Orvosképzés **36,** 161, 1961. Mitarb.: I. Banga.

249. Collagen Mucoproteinase and other Collagenases. Acta physiol. Acad. Sci. hung. **19,** 19. 1961. Collaborators: I. Banga and D. Szabó.

250. L'importanza dell'elastasi. Minerva Medica **52,** 3918, 1961.

251. Über die Ergebnisse der Elastase- und Kollagenmukoproteinase-Forschung. Ztschr. Vitamin, Hormon- und Fermentforsch. **12,** 59, 1961/62. Mitarbeiter: I. Banga.

252. Az emberi sperma leukaemogen hatása. Magyar Onkológia **5,** 199, 1961. Mitarb.: J. Juhász und G. Kendrey.

253. On the Leukaemogenic Effect of Human Sperm. Collaborators: J. Juhász and G. Kendrey. The Morphological Precursors of Cancer. Edited by Lucio Severi Perugia, 1963. p. 279.

254. Кормление кроликов водной взвесью холестерина. Атеросклероз, Сборник посвящен 75-летию дня рождения и 55-летию научной, педагогической и общественной деятельности академика Николая Николаевича Аничкова. Медгиз, Ленинградское отделение 1961 стр. 98.

255. Chemotherapie des Krebses. Vortrag in dem Jenaer Institut für Mikrobiologie und Experimentelle Therapie am 28. November 1961.

256. Elasticity of the Vascular Wall I. The Elastic Tensibility of the Human Carotid as a Function of Age and Arteriosclerosis. Acta physiol. Acad. Sci. hung. **20,** 237, 1961. Collaborator: I. Banga.

257. Elasticity of the Vascular Wall II. Effect of Elastolytic Enzymes on the Elasticity of the Human Carotid in vitro. Acta physiol. Acad. Sci. hung. **20,** 250, 1961. Collaborator: I. Banga.

258. Zur Frage der Fettmobilisierung. Acta morph. Acad. Sci. hung. **10,** 313, 1961.

259. Experimentelle Untersuchung neuer Stoffe mit zytostatischer Wirkung. V. Ungarische Krebstagung 1961 in Budapest, Akad. Kiadó P. 145. Mitarbeiter: G. Kendrey, J. Juhász, G. Gyenes und M. Sellyei.

260. Újabb cytostatikus hatású anyagok kísérletes vizsgálata. Orvosi Hetilap **103,** 257, 1962. Mitarb.: G. Kendrey, J. Juhász, G. Gyenes und M. Sellyei.

261. Aetiology of Lung Cancer. VIII. International Cancer Congress Moscow, Abstract of Papers, Medgiz 282, 1962.

262. Experimental Investigations into the Antitumoral Activity of 1,2,5,6-Tetramethane-Sulphanyl-D-Mannitol (TSM) VIII. International Cancer Congress Moscow, Abstracts of Papers, Medgiz 311, 1962. Collaborators: G. Kendrey, J. Juhász, G. Gyenes and M. Sellyei.

263. Korpássy Béla emlékezete. Morph. és Ig. Orv. Szemle **2,** 81, 1962.

264. International Academy of Pathology, Zürich July 8—13, 1962. On conditions proceeding lung carcinoma.

265. Zur Frage der Karzinogenese. München. med. Wschr. **104,** 1424, 1962.

266. Über den fermentativen Abbau des Nervengewebes. IV. Internat. Kongreß für Neuropathologie 4—8. September 1961. Vol. I. 63, 1962.

267. The Role of Elastase in Atherosclerosis. Medical and Clinical Aspects of Aging. Columbia University Press, New York and London 1962, 357—373 p. Fifth Congress of the International Association of Gerontology. Ed. Hermann T. Blumenthal. Collaborator: I. Banga.

268. Les adénomes multiples du rein. Bulletin du Cancer **49,** 196, 1962. En collaboration avec Arlette Masset.

269. Multiplex veseadenomák. Magyar Onkológia **7,** 81, 1962. Mitarb.: Arlette Masset.

270. The Enzymatic Degradation of Collagen Fibers. Collagen, Proceedings of a Symposium

sponsored by the Central Leather Research Institute, Council of Scientific and Industrial Research, Madras, India, held on November 29—30, 1960. Ed. by N. Ramanathan P. 305—309. Interscience Publishers New York—London 1962. Collaborators: I. Banga and D. Szabó.

271. Elasztáz és elasztomukoproteináz hatásmechanismusa közötti különbség vizsgálata. Kísérletes Orvostudomány **14,** 628, 1962.

272. Neue experimentelle Angaben zur geschwulsterzeugenden Wirkung des Isonicotinsäurehydrazid (INH). Z. Krebsforsch. **65,** 434, 1963. Mitarbeiter: J. Juhász und B. Szende.

273. Connective Tissue Changes in Atherosclerosis. International Review of Connective Tissue Research, Vol. **1,** 241—306, 1963. Edited by David A. Hall, Academic Press, New York and London.

274. Process of Fat Mobilization. Semicentennial of the founding of the American Society for Experimental Pathology Atlantic City, April 18, 1963. Federation Proceeding **22/6** Part I., 1380, 1963.

275. Role of the Central Nervous System in Peptic Ulcer Development. Pathophysiology of Peptic Ulcer. Stanley C. Skoryna, Editor. McGill University Press, Montreal 1963. p. 389.

276. Lipoproteinek és atherosclerosis. Magy. Tud. Akadémia V. Orvosi Tud. Oszt. Közleményei **15,** 59, 1964.

277. Experimental Investigation into the Antitumoral Activity of Tetrahydromethanesulphonyl-D-Mannitol (TSM). Acta Union Internat. Contre le Cancer **20,** 141, 1964. Collaborators: G. Kendrey, J. Juhász, G. Gyenes and M. Sellyei.

278. Újabb kísérletes adatok az isonikotinsavhydrazid (INH) daganatkeltő hatásáról. Magyar Onkologia **8,** 193, 1963. Mitarb.: J. Juhász und B. Szende.

279. Pathologic Changes in the Pacinian Corpuscles around the Pancreas. Acta morphol. Acad. Sci. hung. **13,** 75, 1964. Collaborator: M. Sellyei.

280. Az elastase kutatás eredményei. Orvosi Hetilap **106,** 49—54, 1965.

281. A kristályos amyloid. MTA. V. Oszt. Közl. **16,** 97—104, 1965.

282. Rozwój nauczania medycznego na Wegrzech. Miedzynarodowa Sesja Naukowa Kraków. Mitarb.: J. Kovácsics 121—125, 1965.

283. Histopathological Findings in Animal Experiments on the Aetiology of Viral Hepatitis. Acta microbiologica Acad. Sci. hung. **12,** 357, 1965/66. Collaborators: G. Kendrey, M. Sellyei and A. Bajtai.

284. Correlation between Crosslinkages, Steric Structure and Digestibility of Collagen. Biochemische Zeitschrift **342,** 330, 1965. Collaborator: I. Banga.

285. Chronicus ólomphosphat kezeléssel létrehozott kísérletes veseadenomák. Magyar Onkologia **9,** 144, 1965. Mitarb.: A. Bajtai und B. Szende.

286. Az agy és vér lipoidjainak viszonya. Ideggyógyászati Szemle **18,** 137—143, 1965.

287. Über kristallinisches Amyloid. Beitr. path. Anat. **132,** 376, 1965.

288. Egyes modern gyógyeljárások igazságügyi orvostani vonatkozásai. Morphologiai és Igazságügyi Orvosi Szemle **5,** 246—247, 1965.

289. La relation entre les lipides du cerveau et du sang. Acta med. Acad. Sci. hung. **21,** 411—421, 1965.

290. Атеросклероз и мобилизация жира. IV Всесоюзный Съезд Патологоанатомов Кишинев 1965. стр. 13.

291. Über die Wirkung der Bleiphosphatbehandlung auf die Nierenepithelzellen von Albinoratten. Acta morphol. Acad. Sci. hung. **14,** 187—195, 1966. Mitarbeiter: B. Szende und A. Bajtai.

292. Effect of Enzymes on Collagen. Proceedings 7th International Congress of Gerontology 149—151, 1966. Collaborator: I. Banga.

293. Determination of Elastolytic Activity and the Importance of Elastase. Meth. Achievm. exp. Path. **1,** 96—123, 1966. S. Karger, Basel/New York. Edited by Bajusz and Jasmin, Collaborator: I. Banga.

294. Kórszövettani vizsgálatok a vírus-hepatitis kóroktanára vonatkozó állatkísérletekben. Orvosi Hetilap **107,** 443—445, 1966. Mitarb.: G. Kendrey, M. Sellyei und A. Bajtai.

295. Kórbonctani és kórszövettani elváltozások a laser sugarak hatására. Orvosi Hetilap **107,** 1016—1018, 1966.

296. Lung Tumours in Laboratory Animals Indicating the Tumour-Provoking Property of Some Drugs. Perugia Quadrennial International Conferences on Cancer. Vol. 3. Lung Tumours in Animals. 623—636, 1966. Edited by Lucio Severi.
297. Comparison of Lung Tumours in Animals and Men. Perugia Quadrennial Internationa l Conferences on Cancer. Vol. 3. Lung Tumours in Animals. 127—139, 1966. Edited by Lucio Severi.
298. Pathogenesis and Significance of Renal Adenomas (The Cesare Massari Lecture for 1966, Accademia Anatomico-Chirurgica in Perugia). Lavori Dell'Istituto Di Anatomia e Istologia Patologica, Università Degli Studi Perugia **26,** 89, 1966.
299. Tumour-inducing Effect of Hydrazine in Mice. Nature **210,** 1377, 1966. Collaborators: J. Juhász and B. Szende.
300. Emberek és állatok tüdődaganatai. Magyar Onkológia **10,** 129, 1966.
301. A hydrazin daganatkeltő hatása. Magyar Onkológia **11,** 31, 1967. Mitarb.: J. Juhász und B. Szende.
302. Die Ätiologie des Pemphigus. Pemphigus Occupational Dermatoses due to Chemical Sensitization. Congress of the Hungarian Dermatological Society, 1965, pp. 374. Published in 1967.
303. Über die geschwulsterzeugende Wirkung des Hydrazins. Z. Krebsforsch. **70,** 150, 1967. Mitarb.: J. Juhász und B. Szende.
304. Some Problems of Chemical Carcinogenesis (Co-reference) Abstracts, European Cancer Meeting, Vienna, July 4, 1967, pp. 37—38.
305. Az atherosclerosis kórbonctana és kóreredete. A Korányi Sándor Társaságnak és a Magyar Pathológusok Társaságának együttes ülésén tartott referátum 1967. október 25-én.
306. Az elastasekutatás eredményei. A Budapesti Orvostudományi Egyetem Semmelweis előadása 1967. november 24-én.
307. Aethionin okozta pancreas elváltozások. Morph. Ig. Orv. Szemle **8,** 34, 1968. Mitarb.: Sz. Varga.

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 19—22, 1968*

ERINNERUNG AN BÉLA KORPÁSSY
1907—1961

Professor KORPÁSSY war einer der ältesten und besten Schüler von Professor BALÓ. Sein früher Tod bedeutete einen schweren Schlag für seinen Meister, der ihn wegen seiner menschlichen Qualitäten und wissenschaftlichen Ergebnisse hochgeschätzt hat. Deshalb glauben wir, Korpássy würde sich seines Meisters anläßlich dieser Gelegenheit freudigst gedenken, wenn er noch lebte.

Die ungarischen Pathologen erlitten einen großen Verlust als Béla Korpássy am 27. November 1961 im Alter von 55 Jahren, am Gipfel seiner schaffenden Tätigkeit plötzlich verschied.

Korpássy wurde am 12. Januar 1907 in Szeged geboren, wo sein Vater Richter war. In der damals neu instituierten Universität dieser Stadt studierte Korpássy Medizin, und hier wurde er 1931 zum Doktor promoviert.

Seit 1929 arbeitete Korpássy im Pathologisch-Anatomischen Institut der Universität. Seine erste wissenschaftliche Arbeit (mit A. KÁLLÓ) beschäftigte sich mit der Enzephalitis der mit Fleisch ernährten Hunde mit Eck-Fistel; mit intrazerebral injizierter Gehirnemulsion konnte nachgewiesen werden, daß die Zeichen der Enzephalitis nicht infolge eines angenommenen Enzephalitisvirus zustande kommen, sondern die Ursache des Todes in den auf eine Gehirnverletzung zurückzuführenden blutenden Erosionen des Magens zu suchen sei. Eine weitere Untersuchung hat Korpássy mit E. BACH »Über den Katalase- und Glutathiongehalt der roten Blutkörperchen bei experimentellen Anämien« ausgeführt.

Als Stipendiat verbrachte Korpássy dann zwei Semester in Wien, wo er die Instituten und Kliniken kennen lernte und unter Leitung von CHIARI im Pathologischen Institut der Universität arbeitete. In der Vereinigung der Pathologischen Anatomen hielt er Januar 1937 einen Vortrag über seine Untersuchungen über die Entwicklung des Brustkrebses, mit Rücksicht auf das Alter und die präkanzerösen Zustände. Ein anderes Thema mit dem er sich in Wien befaßte, war die Basalzellenmetaplasie der Ausführungsgänge des Pankreas; er führte diese Veränderung sowie auch die Epithelveränderungen in der Brustdrüse auf die im fortgeschrittenen Alter auftretende Störung des hormonalen Gleichgewichts zurück.

Nach seiner Rückkehr nach Szeged arbeitete Korpássy mit Baló über

Warzen, Papillome und Krebs. Sie sammelten ein großes Material von solchen Gebilden des Menschen und der Tiere, und konnten in dem Dickdarm von aus Herden stammenden Schweinen häufiger Dickdarmpolypen finden als bei Schweinen aus Kleinbesitz. Sie führten sogar Übertragungsversuche mit Darmpolypen aus.

Das höchste Interesse Korpássys galt den onkologischen Problemen, und er habilitierte 1939 aus der Pathologie der Geschwülste.

1939 heiratete Korpássy Piroska Hajnal; aus der Ehe sind zwei Söhne und ein Mädchen geboren, die alle drei den medizinischen Beruf wählten.

In demselben Jahre wurde Korpássy Oberarzt im Spital von Ungvár. Hier hatte er Gelegenheit, viele Fälle von Verbrennung sezieren zu können. Er konnte feststellen, daß an den Organen von Menschen die mit Tannin behandelt wurden, schwere toxische Veränderungen vorkamen, die bei den nicht mit Tannin behandelten nicht zu finden waren.

1944 wurde Korpássy zum Militärdienst einberufen und mit der Leitung des Laboratoriums des dortigen Militärspitals betraut. Zur Zeit der Verschleppung des Militärspitals nach dem Westen blieb Korpássy in Budapest. 1945 war er eine Weile Prosektor einer Irrenanstalt in Budapest und 1946 wurde er zum Pathologen des Spitals in Szombathely ernannt. Kurz nachher wurde er zum Professor der Pathologie in Szeged berufen. Zweimal hat er die Würde des Dekans der medizinischen Fakultät bekleidet. Er erhielt die Kandidatur und das Doktorat der medizinischen Wissenschaften und wurde mit dem Titel »hervorragender Arbeiter des Unterrichtswesens« ausgezeichnet.

1956 hat er in Mailand am Symposium Internazionale sul Diencefalo und am Internationalen Neurovegetativen Symposium in Genf teilgenommen. Im selben Jahre hat er einen Vortrag über den Mechanismus der Entstehung von Geschwulstmetastasen in der Accademia Medico-Fisica Fiorentina gehalten. 1957 wohnte er dem Internationalen Neurovegetativen Kongreß bei. 1958 hielt er einen Vortrag an dem VII. Internationalen Krebskongreß in London. 1959 war er 8 Monate lang Stipendiat des Centre National de la Recherche Scientifique in Paris und arbeitete als Gast im Histophysiologischen Institut von Benoit. Er hielt Vorträge im Collège de France, und als er visiting guest im Chester Beatty Institut in London war, auch in London, Glasgow und Strasbourg. 1960 erhielt er eine Einladung zur Gordon Cancer Conference in New London, und besuchte Krebsinstitute in Boston, Chicago, Washington, Bethesda und New York. Im selben Jahr hat er die Ungarische Akademie der Wissenschaften an der Konferenz der Medizinischen Akademie der Sowjetunion über die Struktur und Funktion des zentralen Nervensystems, und im nächsten Jahr die Szegediner Universität an der internationalen Konferenz aus Anlaß des hundertjährigen Jubileums des Lehrstuhls für pathologische Anatomie der Universität von Perugia vertreten. Im November 1961 feierte man in Padova die zweihundertjährige Erinnerung an das Erscheinen von

Morgagnis De sedibus et causis morborum. Ein Vortrag von Korpássy stand auf dem Programm, er konnte aber nicht mehr hinreisen, da er krank war und bald danach starb.

Ein Teil der wissenschaftlichen Tätigkeit von Korpássy richtete sich auf die Geschwulstforschung. Schon 1943—44 unternahm er Versuche über die leberschädigende Wirkung der Gerbsäure. Nach Beendigung des zweiten Weltkrieges hat er diese Untersuchungen wieder aufgenommen. Er untersuchte an Hunden, Kaninchen und Ratten die Wirkung des Tannins. Nach großen Dosen verendeten die Tiere rasch, kleinere Dosen überlebten sie 10—15 Tage und es entstanden in der Leber azinozentrale Nekrosen. 1949 berichtete Korpássy mit seinem Schüler K. Kovács, daß nach chronischer subkutaner Tanninbehandlung eine Zirrhose vom Typ Laennec in weißen Ratten hervorgerufen werden kann. In einer weiteren Arbeit wurde festgestellt, daß die in entsprechender Dosis peroral verabreichte Gerbsäure in der Leber weißer Ratten, Kaninchen und Hunde ebenfalls azinozentrale Nekrosen verursachen kann. Es wurde auf diese Weise erwiesen, daß die Gerbsäure im Verdauungskanal resorbiert wird. Die nächste Feststellung war, daß in der zirrhotischen Leber mit Gerbsäure chronisch behandelten weißer Ratten Hepatome und Cholangiome entstehen. Korpássy faßte 1961 seine Feststellungen über die hepatokarzinogene Wirkung des Tannins zusammen. Auch sind die Arbeiten von Korpássy über die Metastasenbildung beachtenswert. Wie bekannt, sind Metastasen in der Milz sehr selten. Korpássy konnte experimentell nachweisen, daß die Milz die Geschwulstzellen nicht zurückhält, doch wird diese translienale Passage durch nervöse und humorale Faktoren aufgehoben. Solche Faktoren dürften auch bei der transpulmonalen Passage mitspielen. Wertvoll sind auch Korpássys Arbeiten über die verschiedenen Formen der Präblastomatosen.

Die zweite Hauptrichtung in der Forschungsarbeit Korpássys bildete das Verhältnis zwischen Hypophyse und Hypothalamus. Er und seine Mitarbeiter haben bewiesen, daß im Hypothalamus von mit Pikrotoxin behandelten Hunden das Sekret der Ganglienzellen sich vermehrt. Es stellte sich heraus, daß es in dem Hypothalamus-Hypophysen-System dreierlei Kolloide gibt. Das Kolloid des Hypothalamus kann von dem des Hypophysenvorderlappens und Mittellappens unterschieden werden. Auf Behandlung mit hypertonischer Kochsalzlösung nimmt der Kolloidgehalt in den hypothalamischen Ganglienzellen zu, aber nach 6 Stunden vermindert er sich und häuft sich in der Neurohypophyse zu. Demgemäß strömt das Kolloid aus dem Hypothalamus der Neurohypophyse zu. Zwei Schüler von Korpássy, namentlich Kovács und Bachrach identifizierten das antidiuretische Hormon des Hypothalamus mit dem der Neurohypophyse. Mehrere Arbeiten von Korpássy und seiner Schüler behandeln die Rolle des Hypothalamus im Salz- und Wasser-Metabolismus, die traumatische Oligurie und den Diabetes insipidus.

Korpássy kann als Wissenschaftler durch eine vollkommene Orientiertheit charakterisiert werden. Er war über alle neuen Entdeckungen und Feststellungen der Medizin informiert. Von seinen Lektüren, Beobachtungen und Experimenten gestaltete er seine wissenschaftlichen Pläne mit Intuition und erzielte bedeutende Resultate. Er konnte sich glänzend an alle Einzelheiten erinnern und somit allen gute Ratschläge geben. Mit der Erziehung vieler Fachmänner und Wissenschaftler hat er sich besonders hervorgetan.

Er besaß ein besonderes Talent für fremde Sprachen. Sein Freund, Prof. Mario Raso schrieb in seinem Nachruf, daß Korpássy seine Vorträge immer in der Sprache des Landes gehalten hat, wohin er eingeladen war, da er fast alle europäischen Sprachen wie seine Muttersprache beherrschte. Als Pathologe bewunderte er MORGAGNI. Er war ein Mann von hoher allgemeiner Kultur. Italien bewunderte er wegen seiner natürlichen Schönheit, Kunstdenkmäler und wegen der Errungenschaften, mit denen es die menschliche Zivilisation gefördert hat. Die Pathologen haben in ihm einen zuverlässigen Kollegen und die Wissenschaft einen enthusiastischen Forscher verloren.

DIE REDAKTION

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 23—33, 1968*

First Department of Pathological Anatomy and Experimental Cancer Research (Director: Prof. J. Baló), University Medical School, Budapest

ROLE OF FLUORESCENT SUBSTANCES IN SENILE VASCULAR LESIONS

Ilona Banga

It may be stated that research at the molecular level disclosing cross linkages of vascular scleroproteins appears to be of a great value in the better understanding of the senile and pathologic lesions of the vessel walls.

The development of all experimental sciences, including biology, has its objective reasons. Molecular biology has become the central subject of modern biological research by having elucidated the biological role of nucleic acids. In these days, however, any research that examines phenomena at the molecular level is duly considered molecular biology. Thus, the study concerned with the steric arrangement and inter-peptidic linkages of the elastin's polypeptide chains in senile vascular lesions is as much molecular biology as has been the study of DNA structure and its relations to protein synthesis.

During the last 20 years, much effort has been made to study the changes taking place with age or atherosclerosis in the vascular scleroproteins, particularly in elastin present in the elastic arteries. Research at the molecular biological level has started only some years ago, when data had become available to permit the negative statement that the classical method of study of macroscopically visible and histologically examined fat deposits in altered vessel walls, particularly in the aorta's intima, was inadequate for solving the problem of the primary lesion in the vessel wall in connection with ageing or atherosclerosis. Various theories have been proposed like that of mucopolysaccharide (MPS) accumulation; MPS phanerosis, correlation between the altered serum proteins and the formation of precipitates in the vessel wall; dystrophy of lipid metabolism; etc. None of these theories have, however, led to results which would satisfactorily explain the primary factors in producing lesions and being involved in the essence of the phenomenon.

The new approach, molecular biology, has unintentionally initiated, as an objective factor of development, the study of substances responsible for the cross linkages in elastin. For this laboratory, that approach has been of particular importance, appearing to support our 10—14 years old hypothesis that both the vascular elastin and the collagen have a complex structure.

In the present paper, while giving details on the fluorescent substances

and on the components involved in cross linkages of elastin of the vessel wall, a number of unsolved problems will be raised related to molecular biology as applied to the vessel wall.

Relation of fluorescent substances to senile yellow pigment

The so-called senile pigment is one of the longest known coloured subtsances accumulating in tissues. In his survey of intracellular lipids, DEANE (1958) characterized this substance as follows. It is sudanophilic, basophilic, fluorescent and Schiff-positive. These characters suggest the senile pigment to be a lipid-like acid material with fluorescence and reducing aldehyde content. All these characters are demonstrable in purified elastin from the aorta as well as from other tissues, thus elastin appears to contain the yellow senile pigment (LABELLA, 1961—1962).

A considerable confusion has, however, arisen as to the problem whether senile pigment was the only pigment in the vessel wall and whether the measured fluorescence and its increase with age had any quantitative relationship with the amount of pigment present. According to LABELLA (1962) and PARTRIDGE (1962), yellow pigment is coupled through strong links to fluorescent peptides. In such compounds the senile pigment exhibits peak fluorescence at 340 mμ following activation at 290 mμ (LABELLA 1962). In isolated elastins, however, three additional fluorescence maxima were detected. In Table 1 are presented the activation and fluorescence maxima of the different fluorescent substances found in the vessel wall.

Table 1

Activation (A) and fluorescence (F) maxima in the vessel wall

No	Activation mμ	Fluorescence mμ	Relative intensity	A/F
I	290	340	8	Senile pigment
II	330—340	360—405	37	340/400
III	380—385	460—470	34	385/460
IV	410—430	460—470	26	410/470

In our studies (BANGA, MAYLÁTH-PALÁGYI, 1966), from elastolysates of human aorta specimens, yellow-coloured polypeptides could be isolated by Sephadex G 25, chromatography. These polypeptides showed peak fluorescence at 405 and 460 mμ. The component containing the yellow pigment could be separated as a non-fluorescent substance by simple freezing and fractional

centrifugation. Consequently, the yellow pigment is non-fluorescent and can be separated from the fluorescent material by appropriate techniques. LABELLA (1962) precipitated with alkali the yellow pigment from acid hydrolysates of elastin and demonstrated its non-fluorescence and its absorption maximum at 330 mμ. Also, by column chromatography of partially hydrolysed elastin, he could separate the yellow pigment and the fluorescent peptides. None of these experiments could, however, exclude the possible involvement of certain pigments in the intensive fluorescence of the vessel wall. The fluorescent substance to be discussed below and appearing parallel with atherosclerosis, may not be related to the yellow pigment. The former is namely completely hydrolysed by acid which leaves the yellow pigment intact.

Cross-linking agents of elastin and the fluorescence of the vessel wall

Desmosines and substance X[4]

PARTRIDGE (1962), PARTRIDGE *et al.* (1963) and THOMAS *et al.* (1963) have shown in the polypeptide chains of elastin, in addition to the usual hydrogen and other electrostatic bonds, the presence of unknown amino acid involved in the formation of specific cross links in elastin. The new amino acid occurs in two isomeric forms designated by THOMAS *et al.* (1963) as desmosine and isodesmosine. The substance turned out to be tetracarboxy-tetraamino

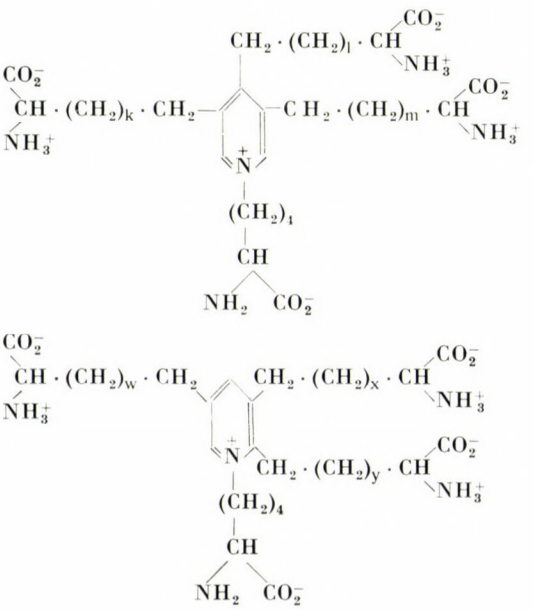


Fig. 1

acid, containing an N-substituted pyridine ring in its molecule. Desmosine appears to be an 1, 3, 4, 5 tetrasubstituted, while isodesmosine an 1, 2, 3, 5 tetrasubstituted, pyridine salt, both having the empirical formula $C_{24}H_{40}N_5O_6Cl$ (the hydrochloride salt, Fig. 1; PARTRIDGE *et al.* 1966). Considering the chemical structure of both substances, the development of cross links between peptide chains by participation of desmosines can only be supposed if 4 lysine radicals pre-existing in the proelastin form a ring structure during biosynthesis (PARTRIDGE *et al.* 1964). To check this supposition, they gave $U^{14}C$-lysine to 25-day old rats and examined the lysine, desmosine and isodesmosine contents of the elastin obtained from the aortas at various points of time. Following a certain lag period (17 days), formation of one-desmosine or isodesmosine molecule, each from 4 lysine molecules, was demonstrable. According to PARTRIDGE *et al.* (1966), the incorporation of 4 lysine molecules into one desmosine or isodesmosine molecule, and the low speed of cyclization (in rats 17 days) would explain the differences in the physical characteristics of elastin in different phases of growth.

SINEX's group (FRANZBLAU *et al.* 1965, 1965a) demonstrated the participation in the cross link formation of primary polypeptide chains of elastin beside desmosines also of lysine residues involved in the formation of X^4 amino acid, the lysinonorleucine: N^{ε}(5-amino, 5-carboxypentanyl)-lysine.

The desmosines were isolated by PARTRIDGE *et al.* (1963) from a fluorescent peptide of 1000 molecular weight obtained from the acid hydrolysate of elastin. As the decrease of elasticity of elastic fibres, going parallel with age and the degree of atherosclerosis is supposed to result from the increase in the number of cross links, it seemed to be of interest to examine whether the accumulation of fluorescent peptides runs parallel also to the increase of desmosines. On the other hand, it seemed questionable whether the increase of fluorescence with age (LABELLA and LINDSAY 1963) and with the gravity of atherosclerosis (BANGA, MAYLÁTH-PALÁGYI and JOBBÁGY 1966) could be brought into correlation with the desmosines. To elucidate this problem, we examined the desmosine content and the four types of fluorescence (Table 1) in elastolysates of normal and sclerotic human aortas, using an Opton M_4 QM spectrophotofluorimeter. Elastolysates of normal and sclerotic aortas were purified by filtration through a Sephadex G 25 column with and without previous hydrolysis. In the collected fractions, desmosines were estimated by measuring the absorption maxima in ultraviolet light of 275 mμ at pH 8.6 on the assumption that the desmosines will be represented by that particular fluorescence where absorption in alkaline solution and fluorescence maxima will coincide in the gel-filtered samples obtained both before and after hydrolysis. PARTRIDGE (personal communication) believes that the desmosines exhibit fluorescence only after exposure to air, as in freshly isolated condition they fail to do so. This possibility was also studied in our experiments. The specia-

absorption prior to and after hydrolysis of readily dissolving elastin (extract 1) and the 13 gel separated fractions from normal aortas are shown in Fig. 2. In filtrates 1—4, no peptide was present, thus they were not included into the figure. In the lower part of Fig. 2 are shown the specific fluorescence values of the same samples, both prior to and after hydrolysis; this fluorescence maximum corresponded best to the absorption maximum of the desmosines.

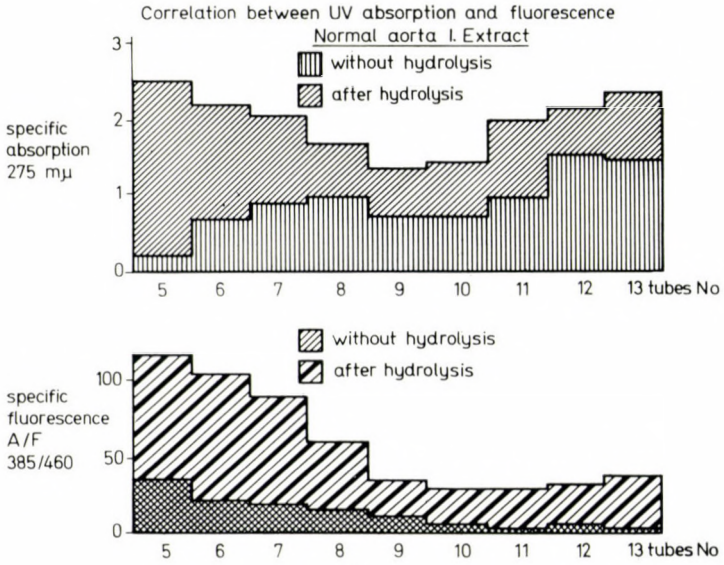


Fig. 2

The other fluorescences obtained did not appear to be related to desmosines. Therefore, if desmosines or desmosine peptides exhibit fluorescence, this is what could be correlated with the specific absorption by desmosines in the vessel wall. In Fig. 3, a similar experiment is demonstrated, but in this case the innermost and least soluble regions (extract III) of normal aortas were examined in respect of the specific UV absorption of desmosines and of the A/F = 385/460 fluorescence. Interestingly, in this region all desmosines were present in the form of peptides and exhibited the typical 275 mμ UV absorption only after acid hydrolysis. Similarly, the A/F = 385/460 specific fluorescence was very low prior to hydrolysis. Theoretically, the activation maximum of the fluorescence of desmosines should have been obtained at 275 mμ; the fact that the measured value was about 100 mμ higher, was either a measurement error or the result of the desmonsines' oxidation or other quenching effect.

In sclerotic aortas, the amount of desmosines was about double that found in normal ones. While in normal aortas most of the desmosines are bound to peptides (see Fig. 4, upper part), in sclerotic aortas they occur also in free

form. The hatching with double lines in the lower part of Fig. 4 indicates that the specific absorption of desmosines estimated at 275 mμ was measurable also without hydrolysis, since this amount did not change on hydrolysis. Thus, apparently, in the majority of gel filtrated samples of extract I obtained

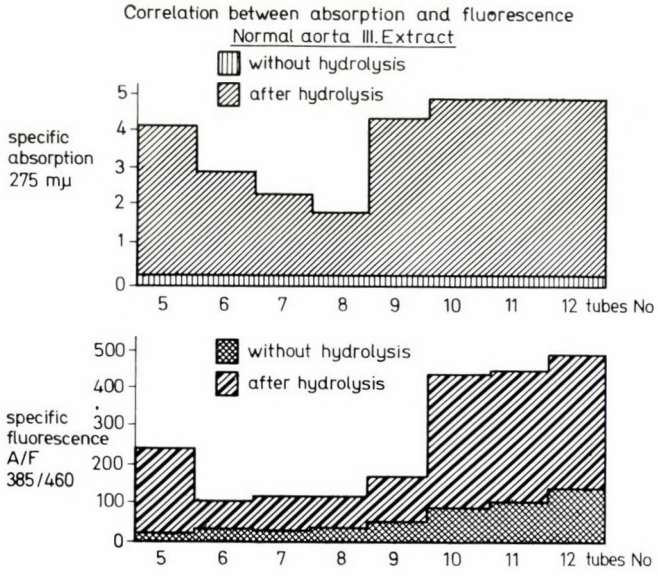


Fig. 3

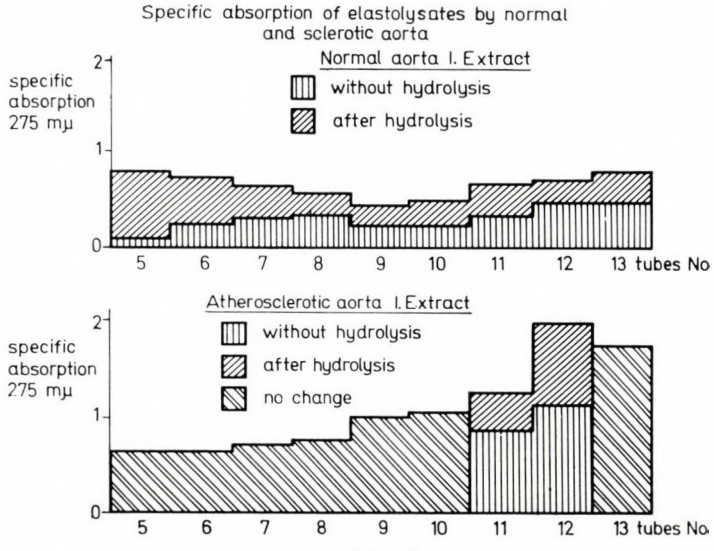


Fig. 4

by elastase treatment, the desmosines were released from their linkages by the enzyme. In samples 11 and 12, high values were obtained after hydrolysis, nevertheless in sample 13, desmosines were present only after hydrolysis.

Fluorescent materials and atherosclerosis

Certain fluorescent substances are accumulating in human aortas with the progress of age (LaBella and Lindsay 1963). It seemed to be of interest to examine whether the appearance of any fluorescence maximum could be correlated with the degree of atherosclerosis depending on, or independently of, the actual age. It has been shown sclerotic aortas exhibit increased fluorescence,

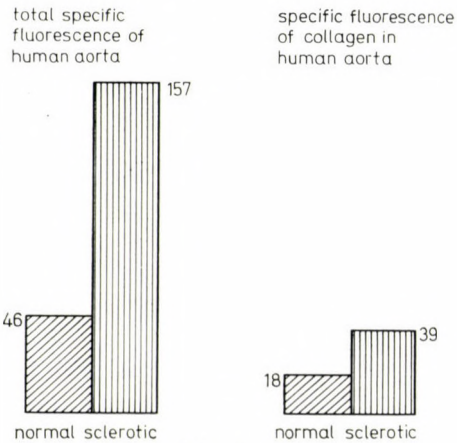


Fig. 5

and that that fraction increases mostly which has its activation maximum at 340—350 mμ and its peak fluorescence at 385—405 mμ. This fraction which was designated A/F = 385/400 (Table 1), was, however, destroyed during acid hydrolysis. Therefore, it could not have originated from the desmosines, nor from tyrosine or its oxidation products. LaBella and Paul (1965), examining the fluorescence and tyrosine content of collagen, concluded that in the cross linkages of collagen in the course of ageing, oxidation products of tyrosine with reactive quinoid structure take a certain part and might link the neighbouring polypeptide chains with covalent bonds. In our experiments (Banga, Mayláth-Palágyi and Jobbágy 1966), the specific fluorescence (A/F = 385/400) of the collagen and elastin fractions as well as of the total protein fractions from 6 normal and 19 sclerotic aortas was examined. The fractions of elastin readily (30 minutes) solubilized with elastase were examined separately from the slowly (24 hours) solubilized fraction. In Fig. 5 are shown the average specific fluorescences of normal and of sclerotic aortas. The differ-

ence between the two was about threefold. The difference referred to the separated collagen fractions and elastin fractions was twofold and fourfold between normal and sclerotic aortas, respectively (Fig. 6). The greatest difference was observed in the readily soluble elastin fractions of normal and sclerotic aortas. LABELLA and PAUL (1965) measured a fluorescence maximum at

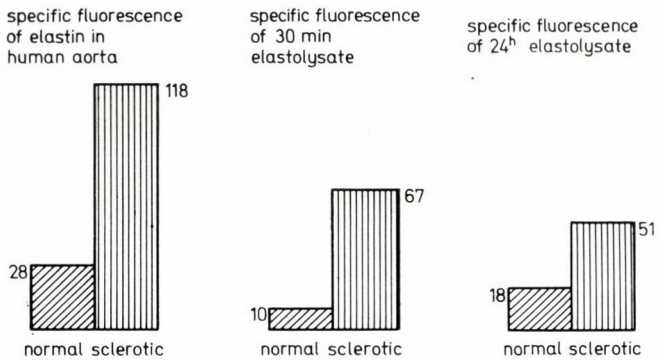


Fig. 6

405 mμ in human Achilles tendons and observed significant increases with age. Nevertheless, we think that the elastin-linked fluorescent substance in sclerotic aortas is not identical with the fluorescing oxidation products of tyrosine, as has been supposed by the above authors. Our opinion is supported by the fact that in the course of sclerosis the amount of the fluorescent substance bound to collagen increased less than did the elastin-bound fraction. The most serious argument, however, is that 90% of the fluorescence observed by us was destroyed on acid hydrolysis, while tyrosine quinones are stable under similar conditions. We assume that the increase of fluorescence with advancing sclerosis originates from lipoproteins, since splitting of the lipid-protein link causes reduction of fluorescence. Long-chain alcohols (butanol, propanol) are known to destroy lipid-protein linkages and have been shown considerably to reduce the fluorescence in sclerotic arteries. SINEX and FARIS (1962) described a substance in elastin that exhibits activation and fluorescence maxima at 310 mμ and 390 mμ, respectively. They think that this substance resembles the aqueous extracts of auto-oxidized lipids. The yellow chromogen and the fluorescence at 460 mμ probably also originate from auto-oxidized lipids. In sclerotic as well as in normal arteries, all fluorescences shown in Table 1 were demonstrable, but in sclerotic aortas at least two additional fluorescences were present which were not observed in the normal aorta.

Correlation between elasticity and fluorescence of the vessel wall

It appeared to be of particular interest to examine the correlation between the amount of fluorescent material and the severity of atherosclerosis. Results

presented in Fig. 7 show that the fluorescent substance exhibited a sudden increase at the appearance of atherosclerosis, while it failed to increase parallel with the progress of atherosclerosis. In Fig. 7, the percentage of atherosclerosis is plotted according to the WHO standard against the increase of specific fluorescence in an arbitrary scale referred to the normal values. The individual curves from A to E show the fluorescence of the individual fractions of the sclerotic aortas as related to the severity of sclerosis.

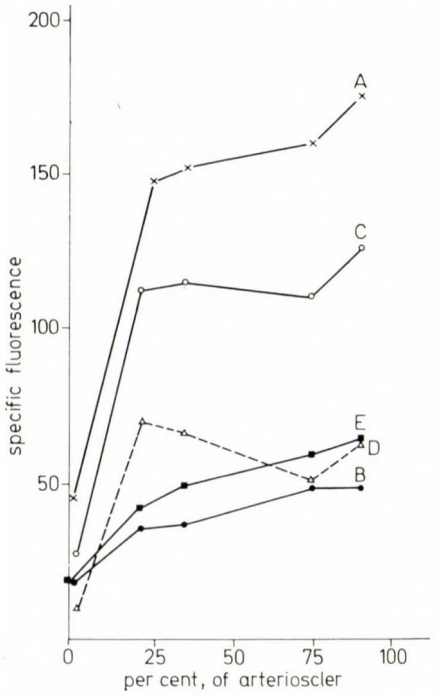


Fig. 7

A = total spec. fluoresc. of human aorta
B = spec. fluoresc. of collagen in human aorta
C = spec. fluoresc. of elastin in human aorta
D = spec. fluoresc. of 30 min. elastolysate
E = spec. fluoresc. of 24^{h} elastolysate

It was supposed that if fluorescence does not increase parallel with the severity of atherosclerosis, it might be elicited by some initial lesion developing with progressing age. This supposition is supported by the results in Table 2, where the total fluorescence of the aortas is shown for 4 different age groups. No increase in fluorescence was demonstrable from infancy up to the 40th year of age. A sudden threefold increase was observed in the age group of 40—70 years, while very little if any further increase was demonstrable in the age group of 70—90 years. Very similar results were obtained concerning the elasticity of human carotid stripes as compared to age and the degree of

atherosclerosis. As shown in Table 3, in the age group 14—47 years in atherosclerosis-free (group I) vessels the elasticity modulus did not change up to 35 years. On 70% stretching, a sudden increase of the value was observed at 47 years of age. In group II, the elasticity modulus was examined in cases

Table 2

Age-dependent changes in A/F = 340/400 fluorescent substance of aortic wall

Years mean							
0—10		20—40		40—70		70—90	
40		46		163		143	
35	45	51	48	191	154	193	173
53		47		141		167	
51				161		172	
				111		217	
				190		141	
				141		143	
				138		157	
				150		211	

Table 3

Elasticity modulus, age, and degree of atherosclerosis
Elasticity modulus g/sq.mm at 40% and 70% stretching

Carotid Prot. No.	Group	Age, Years	Elasticity modulus g/sq.mm		Degree of athero-sclerosis
			40%	70%	per cent
56		14	2.8	11.6	0
67		17	2.9	11.6	0
74	I	21	2.9	11.6	0
73		35	2.8	16.5	0
71		47	2.8	25.0	0
77		28	3.1	10.3	20
52	II	30	4.0	10.0	25
75		43	4.0	17.5	20
72		61	5.5	22.0	20
81		61	6.1	23.0	75
66	III	70	7.0	24.0	75
60		80	7.2	25.5	85
63		82	7.6	30.0	90

with 20% atherosclerosis as referred to age. In these cases, on 40% stretching a sudden increase of the elasticity modulus was observed at the age of 30—40 years. In group III which comprised cases of severe atherosclerosis, the increase of elasticity modulus and the severity of the involvement were not correlated. These results suggested that the elasticity of the vessel wall and the accumulation of fluorescent material were correlated at the beginning of atherosclerosis. As to the chemical characterization of the substance, further chemical studies are required.

REFERENCES

1. Banga, I., Mayláth-Palágyi, J.: (1966) Acta biochim. biophys. Acad. Sci. hung. **1,** 345—354. — 2. Banga, I., Mayláth-Palágyi, J., Jobbágy, A.: (1966) Acta physiol. Acad. Sci. hung. **30,** 79—89. — 3. Deane, H. W.: (1958) Intracellular Lipids: Their Detection and Significance. In: Frontiers in Cytology, S. L. Palay ed. Yale University Press, New Haven, pp. 227—263. — 4. Franzblau, C., Sinex, F. M., Faris, B.: (1965) Nature (Lond.) **205,** 802—803. — 5. Franzblau, C., Sinex, F. M., Faris, B., Lampidis, R.: (1965a) Biochem. Biophys. Res. Com. **21,** 575—581. — 6. LaBella, F. S.: (1961) Arch. Biochem. **93.** 72—79. — 7. LaBella, F. S.: (1962) J. Geront. **17,** 8—13. — 8. LaBella, F. S., Lindsay, W. G.: (1963) J. Geront. **18,** 111—118. — 9. LaBella, F. S., Paul, G.: (1965) J. Geront. **20,** 54—59. — 10. Partridge, S. M.: (1962) Advanc. Protein Chem. **17,** 227—302. — 11. Partridge, S. M., Elsden, D. F., Thomas, J.: (1963) Nature (Lond.) **197,** 1297—1298. — 12. Partridge, S. M., Elsden, D. F., Thomas, J., Dorfman, A., Telser, A. Ho, P—L.: (1964) Biochem. J. **93,** 30c—33c. — 13. Partridge, S. M., Elsden, D. F., Thomas, J.: (1966) Nature (Lond.) **209,** 399—400. — 14. Sinex, F. M., Faris, B.: Fed. Proc. (1962) **21,** No 2, 408. — 15. Thomas, J., Elsden, D. F., Partridge, S. M.: (1963) Nature (Lond.) **200,** 651—652.

DIE ROLLE FLUORESZIERENDER STOFFE IN DEN GEFÄßWANDVERÄNDERUNGEN IM HOHEN ALTER

ILONA BANGA

Die zur Klärung der Kreuzbindungen der Skleroproteine der Gefäßwand auf molekularer Ebene geführten Forschungen können uns dem Verständnis der altersbedingten und pathologischen Gefäßwandveränderungen näherbringen.

РОЛЬ ФЛЮОРЕСЦИРУЮЩИХ ВЕЩЕСТВ ПРИ ИЗМЕНЕНИЯХ СТЕНКИ СОСУДОВ, НАБЛЮДАЕМЫХ В ПРЕКЛОННОМ ВОЗРАСТЕ

И. БАНГА

Исследования, проводимые в области молекул в целях выяснения перекрестных связей склеропротеиной стенки сосудов, могут способствовать пониманию патологических и старческих изменений стенки сосудов.

Dr. Ilona Banga; Budapest VIII. Üllői út 26. Hungary

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 35—40, 1968*

Department of Pathology, Englewood Hospital, Englewood, New Jersey, U.S.A.

ADENOMYOSIS OF THE UTERUS: SOME OBSERVATIONS IN THE MECHANISM OF ITS PRODUCTION

I. A. GÁSPÁR

1. A brief review of the history and pathology of adenomyosis uteri was presented.

2. The various theories, which were developed through the years, concerning the origin of adenomyosis were briefly outlined.

3. A study of 70 cases of adenomyosis uteri observed at Englewood Hospital, Englewood, New Jersey, U.S.A. was submitted and searched for an answer concerning the histologic mechanism of the disease.

4. The intravascular invasion route and the embolic process in the production of adenomyosis uteri was demonstrated in 61.4% of the cases.

5. Sampson's vascular transmission theory was confirmed.

6. Factual observations, not theories, were presented, which seemed to give an answer to the "how" of the adenomyotic process, but the "why" of adenomyosis uteri remains still unanswered.

Adenomyosis of the uterus is a benign, non-neoplastic disease consisting of endometrium extending into the myometrium at least to the accepted minimal depth of one high power field's width, or to varying depths beyond and there is an overgrowth of the myometrium in the vicinity.

This disease is known for over 100 years and it was first described by ROKITANSKY [17] in 1860, in Wien, Austria. Contributions were made to the subject by a number of authors, among them BABES [1] in 1881 from Budapest, Hungary, VON RECKLINGHAUSEN [16] in 1896 from Germany, CULLEN [6, 7] in 1896 and 1908 from Baltimore, Maryland, U.S.A., SAMPSON [18, 19] in 1918 and 1927 from Albany, New York, U.S.A., VON BURG [3] in 1927 from Budapest, Hungary, FRANKL [9, 10] in 1932, BRINES and BLAIN [2] in 1943 from Detroit, Michigan, U.S.A. and JAVERT [12] in 1951 from New York, New York, U.S.A., etc.

In earlier publications, adenomyosis of the uterus was described under a varied terminology, such as adenomyosis, adenomyoma, von Recklinghausen's disease, adenometritis, adenomyositis, adenomyomatosis and internal and external uterine endometriosis. In the present day literature, adenomyosis is the accepted term whether it is of a diffuse or a more circumscribed variety of the disease.

The gross appearance of the uterus with adenomyosis has been well described by various authors and in textbooks. The adenomyomatous uterus

is generally an enlarged uterus, with slight to moderate enlargement of the uterine body. Occasionally, a very marked enlargement may be produced up to 16 cm diameter or more. However, such enlargements caused solely by the adenomyosis are rare and great enlargements are usually caused by coexisting myomata. If there are no coexisting fibroids the enlargement of the uterine body may be symmetrical or asymmetrical. In the former instance, both the anterior and posterior walls show the same degree of enlargement, while in the latter either the anterior or posterior wall is thicker than the other with a slight preponderance of a thicker posterior uterine wall. The hypertrophy of the myometrium around the invading endometrium can be seen most advantageously on a sagittal cut section of an unfixed uterus. The hypertrophied muscle bundles will project on the cut surface and within this bulging area of the myometrium there are many small retracted spots of soft, pale grey tissue representing areas of invading endometrium. Infrequently there are small chocolate colored cysts caused by retained menstrual hemorrhage into endometrial islands or there may be minute cysts without retained hemorrhage. The adenomyosis area is firm and pale pinkish-yellow, while the remaining normal subserosal myometrium is soft, retracted and it also has a darker bluish-pink color.

The serosa of the uterus is smooth but in some of the cases there are adhesions to the surrounding tissues and organs. Such adhesions may be caused by the extension of the adenomyosis to the serosal layer or by pelvic endometriosis. The latter is frequently associated with adenomyosis. NOVAK and DE LIMA [14] feel that adenomyosis and endometriosis coexist in about 25 to 40 per cent of cases. In my series of 70 cases of adenomyosis, 17 were associated with endometriosis, a 24.3 per cent of the total, confirming NOVAK's feeling. The percentage of association may be higher, indeed, considering the fact that many of the examined uteri did not include the adnexa. Thus, the tubes and ovaries were not studied in such cases and a possible endometriosis remained undiscovered.

The microscopic picture of adenomyosis is characterized by endometrial glands and stroma being scattered in a hypertrophied myometrium. The inner one-third or half of the uterine wall is the usually involved area, but invasion may be throughout the uterine wall and beyond. It is generally accepted that the invading endometrium has to invade at least one high power field's depth to allow a diagnosis of adenomyosis. The endometrial islands and strands are usually perpendicular to the endometrial surface and some may follow the blood vessels. The hormonal response is similar to that occurring in the basal endometrium. There is response to estrogenic stimulation but decidual reaction and secretory activity develop only under the influence of excessive progesterone, thus bleeding occurs rather rarely into the gland lumina.

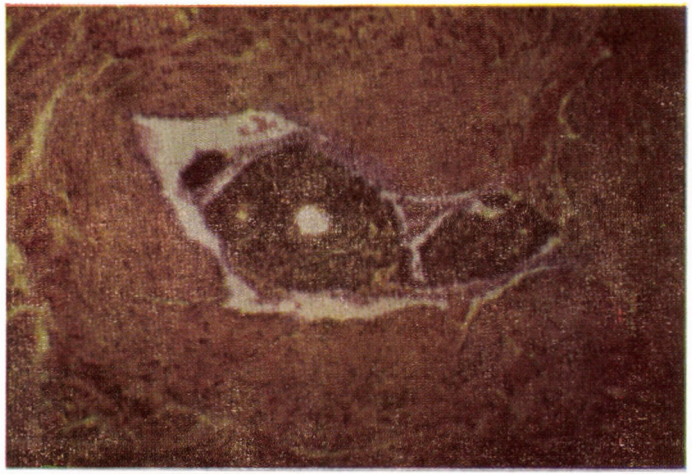


Fig. 5. Endometrial embolus in a larger vein in a case of adenomyosis uteri

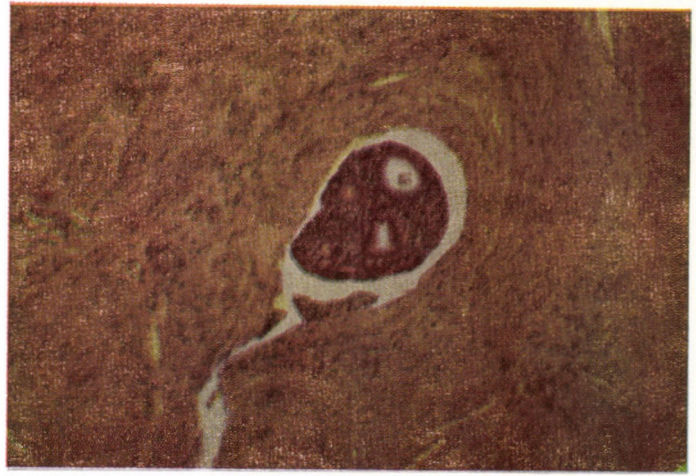


Fig. 6. Endometrial embolus in a larger vein, very similar to Fig. 5, but in a different patient having adenomyosis uteri

Fig. 7. Stromal embolus in a receiving sinus of the myometrium in adenomyosis uteri

Fig. 8. Stromal embolus in adenomyosis uteri in a venous sinus close to the serosal surface of the uterine body

Fig. 1. Endometrium bulging into a receiving sinus in polyplike fashion almost at the level of the base of the endometrium

Fig. 2. Endometrium growing intravascularly into the myometrium at a level just one high power field distance from the base of the endometrium. The endometrium is partially adherent to the endothelium of the venous sinus

Fig. 3. Polyp-like intravascular growth of endometrium in adenomyosis uteri

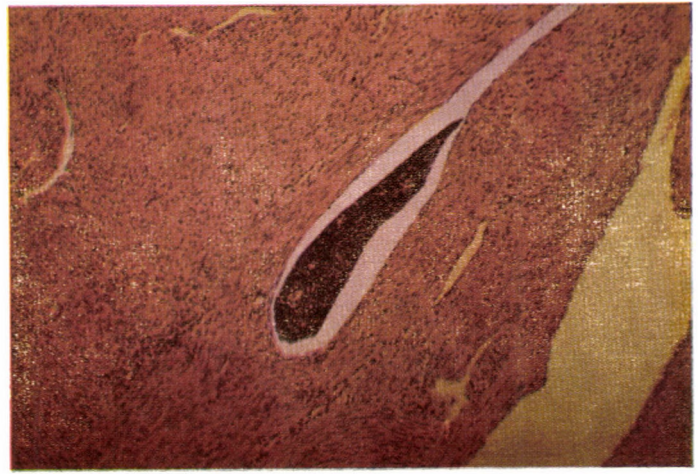


Fig. 4. Stromal embolus in the myometrium in a case of adenomyosis uteri

Theories of origin of adenomyosis

During the 100 years since the first detailed description of adenomyosis was reported by ROKITANSKY [17] a number of theories evolved to explain its origin. Many of these theories are only of historical interest to us at the present time. In the latter part of the 19th century it was assumed that adenomyosis was an embryonic error in Müllerian cell distribution. Several authors also favored the idiopathic stromal hyperplasia origin. VON RECKLINGHAUSEN [16] theorized that Wolffian duct elements mesonephric tissue, gave origin to adenomyosis.

CHIARI's [5] theory of invasion of the myometrium by gland alveoli during inflammation was short-lived. MEYER's [13] theory of invasive endometrial basal hyperplasia, a direct invasion of myometrium by endometrium was strongly supported by CULLEN [7] and FRANKL [9, 10]. The hormonal factor, hyperestrinism theory, as primary motivating factor was introduced in 1923. Today, the most widely accepted concept of adenomyosis is the invasive endometrial hyperplasia theory aided by hyperestrinism. However, EMGE [8] states that in his extensive experience with animal experiments in white rats maintained on high estrogen he never observed invasive endometrial hyperplasia.

SAMPSON [18, 19] in 1918 proposed the vascular transmission theory and developed it further in 1927. HALBAN [11] supported it in 1924, JAVERT [12] in 1951 and CANDREVIOTIS [4] in 1953, yet the general opinion is that the production of adenomyosis by metastatic transmission is a rare occurrence.

BRINES and BLAIN [2] in 1943, introduced the theory of spontaneous generation of endometrial stroma by a process of dedifferentiation of myofibrils under the influence of unopposed estrogen stimulation. Their theory seems to be very questionable, particularly in regard to adenomyosis and up to this moment I am unaware of any publications confirming their theory.

The above theories indicate that even today we do not have a complete unanimity in the understanding of the actual mechanism in the production of adenomyosis and we do not know and cannot point out the actual initiating factor or factors responsible for the production of adenomyosis. It seems that further studies are indicated and any pertinent observations in the clarification of some points in the mechanism of this condition may be worth while.

Material studied with observations concerning the histologic mechanism of adenomyosis uteri

My own special interest in the subject of adenomyosis dates back to 1951, when during the routine study of an adenomyomatous uterus removed from a 41 year old white female at the Englewood Hospital, Englewood, New

Jersey, U.S.A. I observed that one or two of the veins of the myometrium contained fairly large pieces of endometrium, namely endometrial glands with surrounding stroma (Fig. 5). I thought that this was rather unusual, which was worthy of notice. From this time on with a searching mind, I was collecting material for further histologic observations. A total of 70 cases were collected including some in 1966 and the histologic findings in these cases form the basis of this report.

The age distribution of the patients in this study of adenomyosis uteri showed that 63 patients, 90 per cent of the total, were between 36 and 55 years of age. Furthermore, an almost identical number of cases was found for each 5 year group of patients. This age distribution is in agreement with the figures given in various reports on this subject. In the technical preparation of the material, the uteri were formalin fixed at first and subsequently multiple blocks were cut out with perpendicular sections through the mucosa and uterine walls. Only a routine hematoxylin-eosin staining was used in all microsections. In a few of the cases, the entire uterine body was cut into blocks and from each block either a single or multiple microsections were made.

Out of the 70 cases under study 43 uteri, or 61.4 per cent of the total, presented significant histologic findings which indicated the essential intravascularly invading nature of this disease process. A study of the endometrial base line and of the inner myometrium showed the numerous venous capillaries and sinuses radiating from the endometrium into the myometrium. Some of the venous sinuses, which SAMPSON [19] called radiating or receiving sinuses, showed endometrium bulging into the venous sinus in a polyp-like fashion (Fig. 1). Again, in other capillary veins there was a string-like intravascular downgrowth of stroma which could be followed to a certain distance downward until the venous capillary changed its course and disappeared from the section. Occasionally, endometrial glands alone without demonstrable stromal attachment were extending deeply into the myometrium by such intravascular proliferation. When larger endometrial masses streamed downward from the endometrial base line, this phenomenon could not be demonstrated. Frequently there was intravascular growth of endometrium just a short distance from the baseline of the endometrium (Fig. 2). As seen in this microphotograph a portion of the intravascularly growing endometrium is partly adherent to the endothelium of the venous sinus, on the other hand, a large part of it is still free and the lumen of the sinus is visible on both sides of the invading endometrium. Such intravascular growth in venous capillaries and sinuses was demonstrated many times in my material in different parts of the adenomyosis area, however, they were most frequent at the proliferating edges of the adenomyosis process. In SAMPSON's [19] 1927 article his Fig. 39 is almost identical with my Fig. 2. His interpretation was that endometrial tissue invaded the sinus in a retro-endothelial course and distorted its lumen. He also considered

the possibility of a "canalized endometrial thrombus". On the basis of my study of adenomyosis, such formations are not retro-endothelial, but definitely intravascular growth processes. In the larger sinuses the invading endometrium has plenty of space to grow and becomes a large mass of varying size, filling the sinuses. At first there may be only a few adhesions between the endometrial masses and the sinus walls, but as the process becomes older complete adhesions occur. Thus, the venous sinuses become entirely obliterated and the intravascular nature of the process is obscured. In the extending parts of the process, usually in the peripheral areas, polypoid intravascular proliferations were demonstrable (Fig. 3). Such polypoid extensions could be demonstrated even in older but still active cases of adenomyosis, particularly if many sections were made from multiple blocks.

In addition to the slow evolving adenomyosis process produced by the intravascularly invading endometrium, I have definitely found that extension of adenomyosis by embolization also occurs. The embolization can be produced either by endometrial stroma mass (Figs 4, 7 and 8) or by varying sized endometrial tissue masses, that is tissue pieces in which both endometrial glands and stroma can be demonstrated in the same microsection (Figs 5 and 6). The emboli can be within the adenomyosis area of the uterus, but they can be seen also at a distance from the main adenomyomatous process. When this occurs the emboli may be in a myometrial layer which shows no evidence of hypertrophy. It is not very rare to find stromal or endometrial emboli in venous sinuses just beneath the serosal surface of the uterus (Fig. 8). I have observed one case with endometrial embolus in a subserosal sinus. Serial sections demonstrated that this embolic endometrium broke through the serosa of the uterine body and produced an area of endometriosis on the serosal surface of the uterus. However, I do not want to elaborate on this point because it is beyond the scope of this study.

Distant emboli within the uterus could be seen not only in a well advanced case of adenomyosis, but also in cases in which the process involved only a rather shallow myometrial layer beneath the endometrium. Again, it has to be emphasized that the demonstration of stromal and endometrial emboli, intravascular growths and polypoid intravascular endometrial proliferations require a thorough study of cases in which histologic activity of the disease can be suspected. In one of the cases included in this study, multiple sections were prepared from 35 different blocks of the uterus and only 2 of the blocks were revealing.

REFERENCES

1. Babes, V.: (1881) Orv. Hetil. **30,** 1205. — 2. Brines, O. A., Blain, J. H.: (1943) Surg. Gynec. Obstet. **76,** 197. — 3. Burg, E, von: (1927) Zbl. Gynäk. **51,** 886. — 4. Candreviotis, N.: (1953) Frankf. Z. Path. **64,** 1. — 5. Chiari, H.: (1887) Prager Z. Heilk. **8,** 457. —

6. CULLEN, T. S.: (1896) Bull. Johns Hopkins Hosp. **7,** 112. (1897) **8,** 133. — 7. CULLEN, T. S. (1908) Adenomyoma of the Uterus. Saunders, Philadelphia. — 8. EMGE, L. A.: (1962) Amer. J. Obst. and Gynec. **83,** 1541. — 9. FRANKL, O.: (1925) Amer. J. Obst. and Gynec. **10,** 680. — 10. FRANKL, O.: (1932) Zbl. Gynäk. **56,** 902. — 11. HALBAN, J.: (1924) Wien. klin. Wschr. **37,** 1205. — 12. JAVERT, C. T.: (1951) Amer. J. Obst. and Gynec. **62,** 477. — 13. MEYER, R.: In: Handbuch der pathologischen Anatomie, ed. Henke and Lubarsch, 1930. Berlin, Springer, Vol. 7. P. 249. — 14. NOVAK, E. R., DELIMA, O. A.: (1948) Amer. J. Obst. and Gynec. **56,** 634. — 15. NOVAK, E. R., WOODRUFF, J. D.: (1962) Gynecologic and Obstetric Pathology. Saunders, Philadelphia. — 16. RECKLINGHAUSEN, E. VON: Die Adenomyome der Uterus und Tubenwandung. Hirschwald, Berlin. 1896. — 17. ROKITANSKY, K. F. VON: (1860) Z. Ges. Aerzte, Wien. **16,** 577. — 18. SAMPSON, J. A.: (1918) Amer. J. Obst. **78,** 161. — 19. SAMPSON, J. A.: (1927) Amer. J. Path. **3,** 93.

ADENOMYOSE DER GEBÄRMUTTER BEITRÄGE ZU IHREM ENTSTEHUNGSMECHANISMUS

I. A. GÁSPÁR

1. Geschichte und Pathologie der Adenomyose werden kurz geschildert.

2. Die verschiedenen Entstehungstheorien werden besprochen.

3. An 70 Adenomyose-Kranken wurde der histologische Mechanismus der Erkrankung untersucht.

4. In 61,4% der Fälle wurde bei der Entstehung der Adenomyose ein intravaskulärer Invasionsweg und ein embolischer Prozeß nachgewiesen.

5. Die vaskuläre Verschleppungstheorie von Samson wurde bestätigt.

6. Die Beobachtungen scheinen das »Wie« des adenomyotischen Prozesses geklärt zu haben, die Frage nach dem »Warum« der Entstehung blieb jedoch offen.

АДЕНОМИОЗ МАТКИ. ДАННЫЕ К МЕХАНИЗМУ ЕГО ВОЗНИКНОВЕНИЯ

И. А. ГАШПАР

1. Дается краткий обзор истории и патологии аденомиоза матки.

2. Описаны различные теории о происхождении аденомиоза.

3. 70 случаев аденомиоза матки были исследованы с точки зрения гистопатологического механизма поражения.

4. При возникновении аденомиоза в 61,4% случаев были выявлены внутрисосудистая инвазия и эмболический процесс.

5. Теория Самсона о васкулярном переносе болезни была подтверждена.

6. Наблюдения авторов, повидимому, выяснили «способ» развития аденомиотического процесса, однако вопрос о «причине возникновения» аденомиоза остается открытым.

Dr. I. A. GÁSPÁR; Dept. of Path., Englewood Hospital,
Englewood, New Jersey, U.S.A.

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 41—52, 1968*

First Department of Pathological Anatomy and Experimental Cancer Research, (Direktor: Prof. J. Baló), University Medical School, Budapest

THE ROLE OF VASCULAR LESIONS IN THE MALIGNANT TRANSFORMATION OF CHRONIC PEPTIC ULCER

J. Juhász

In our autopsy material 11.5% of all gastric cancer cases showed malignant transformation of chronic peptic ulcer. Carcinomatous transformation starts at the edge of the ulcer, multiple foci arising from the regenerating gastric mucosa. In the scar of the ulcer and in the surrounding areas hypoxia prevails as a result of vascular occlusion, accounting for atypical regeneration of the mucosa. The cancer arising from chronic peptic ulcer is a typical scar carcinoma; in its genesis, the abnormal cell metabolism due to hypoxia seems to play an important part. According to Baló, a close study of the epithelial proliferations arising from hypoxic areas may cast light on the interrelationship of vascular lesions and carcinogenesis. Hypoxia is assumed to play a primary role in the malignant transformation of chronic callous ulcer.

Visceral cancers often arise from the central parts or the edge of fibrotic and cicatrized areas. A thorough study of this type of carcinogenesis is possible only if such tumours are recognized early, when their surgical treatment is still successful. Histological study of the growth in the early phase of its development will reveal the regenerating tendency of the epithelial cells in or around the scar, their atypical growth and atypical epithelial proliferation showing all the characteristics of malignancy. Destruction of the parenchyma and the formation of a mesenchymal scar is most frequently the result of some chronic inflammatory process, nevertheless, scars often arise after some injury, vascular necrosis or other disturbances. Diffuse scars of considerable size may be formed in place of an atrophized tissue, e.g. a contracted kidney due to successive occlusion of the supplying vessels. The malignant tumour arises usually from the atypical regenerating tissue; less frequently, first a benign neoplasm is formed, which then undergoes malignant transformation.

The relationship between scars and carcinoma has been disclosed mainly by studies of pulmonary carcinoma. It is particularly the peripheral pulmonary cancers which tend to arise from scars. The scars are usually tuberculous in origin: since the progress of chemotherapy, the number of patients bearing scars in their lungs as a result of healed tuberculous processes is growing continuously. In addition, cancer often arises from scars of chronic pulmonary abscesses, chronic bronchiectasis, infarction, or from diffuse cicatrization due to scleroderma. In his monograph on pulmonary carcinoma, Baló [4] gives many examples of carcinoma arising from scars of different origin.

Malignant tumours in the genesis of which a scar had an important part may occur in the skin, urinary tract, and other organs (BRACK [10]). Sarcomas arising from a scar have been observed far less frequently (EBERT [13]; BETZLER and LEONHARDT [7]).

BALÓ [3] stressed the importance of hypoxia in the genesis of malignant tumours; in his opinion, hypoxia affects cell metabolism and this disturbance finally results in a malignant transformation of the cells. All pathological processes associated with disturbances of tissue oxygenation as a result of a partial or complete occlusion of the supplying vessels and leading to necrosis and/or the formation of scar should be regarded as leading to malignancy. This is clearly indicated by the epithelial proliferation frequently observed in the marginal areas of pulmonary infarction (BALÓ, JUHÁSZ and TEMES [5]) from which cancer may arise without any carcinogenic impact. Pulmonary cancer arising from epithelial proliferation around an infarction or from scars formed in the place of an infarction has been first reported by BALÓ [4], and later by RAEBURN and SPENCER [23], BERKHEISER [6], YOKOO and SUCKOW [36], and others. By analogy, it seemed interesting to study the eventual relationship between vascular lesions and carcinogenesis in other organs where epithelial proliferation is frequently observed in the margin of fibrotic or necrotic tissue.

Materials and methods

On the basis of the above considerations, the relationship between vascular lesions and the induction of tumours has been studied in cases of carcinoma arising from gastric ulcer. This kind of tumour is known to arise from the atypical proliferation of the regenerating columnar epithelium that covers a circumscribed scar.

A total of 443 surgically removed gastric carcinomas was recorded and verified histologically in the course of 16 years (from January 1st, 1950, until December 31st, 1965). In 51 cases (11.5%) it could be satisfactorily proved that the carcinoma had arisen from chronic gastric ulcer. Gastric carcinomas revealed at autopsy were not considered, since it is often impossible, or at least uncertain, whether some ulceration had played a role in their genesis.

Particular importance has been attached to the thorough study of callous ulcers. According to the size of the ulcer, 3 to 8 tissue samples were cut out of the edge, thus, the whole marginal portion of the ulcer could be examined histologically. By this means it was possible to reveal any malignant proliferation in the earliest phase of its development. In a great number of cases, ulcer carcinoma could be detected by microscopic investigation although the gross examination of the gastric residue failed to disclose any apparent change (Figs 1 and 2). In our opinion, this is the only means by which in the regenerating mucosa an early change can be detected, and the morphogenetic aspects of the ulcer carcinoma revealed. A brief summary of the findings has already been published [16]. The present paper deals only with the role of vascular lesions in the genesis of ulcer carcinoma. The great number of cases, and the uniform preparation and treatment of the material for many years allowed several conclusions concerning the relationship between vascular lesions, tissue hypoxia, and carcinogenesis. The comparative study comprises not only those 443 cases in which gastric carcinoma could be verified, but also a great number of peptic ulcer cases in which histological examination did not reveal malignant transformation.

The morphology of the blood vessels in and around a peptic ulcer has been dealt with by many investigators. It should be stressed that arteriosclerosis leading to significant narrowing or complete obstruction of the vessels supplying the stomach is infrequent. Earlier data in this field have been surveyed by BUDAY [11]. Sclerosis of the gastric arteries gives rise to abdominal angina, a condition characterized by mucosal erosions and bleeding, the latter

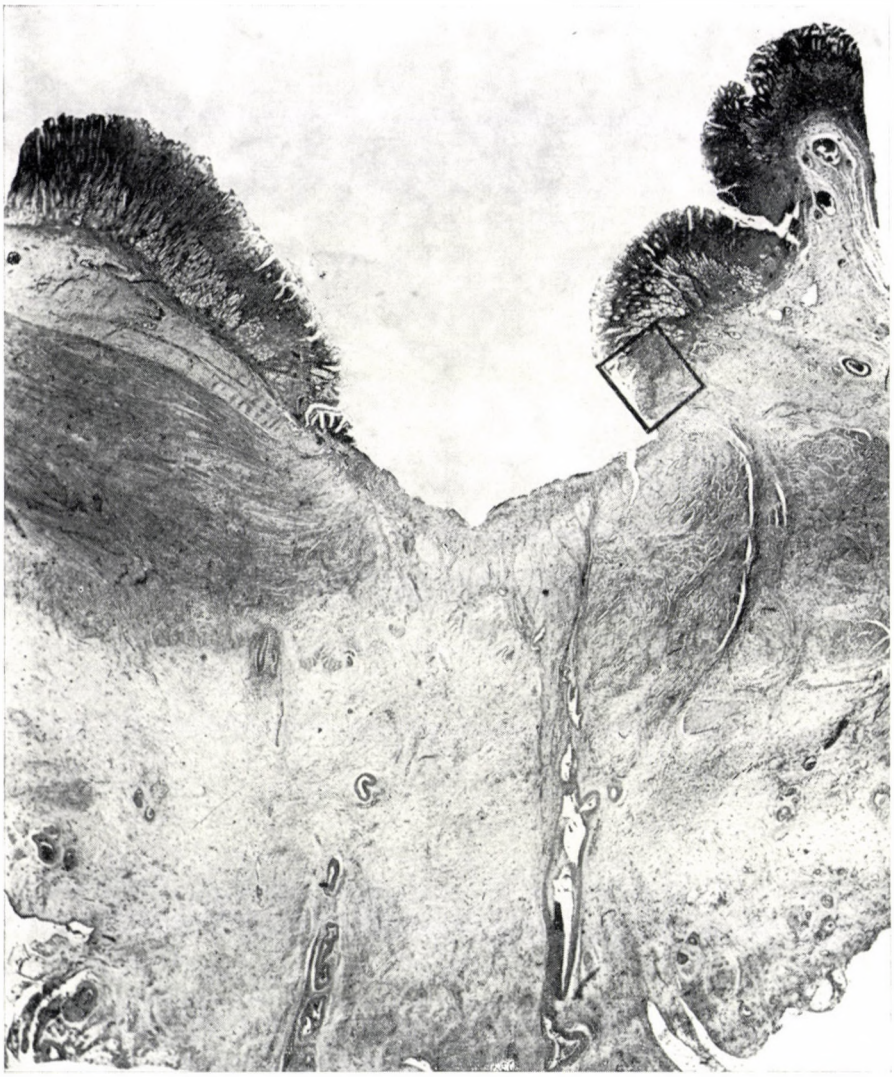


Fig. 1. Early ulcer carcinoma (low power). On the surface of the thick scar (left) the mucosa shows a regenerative tendency (papillary proliferation). In the framed area, neoplastic proliferation consisting of solid cell groups not demarcated from the adjacent structures (ulcer carcinoma)

often being the cause of lethal haematemesis (ANTONIE [2]). This is a disease, however, which is rarely encountered in practice; PALMER and BOYCE [22] could collect not more than 29 cases from the literature until 1964. There is apparently no relationship between these vascular changes and those observed in association with cicatrized peptic ulcer.

Most authors consider the vascular changes of chronic ulcer to be obliterating endarteriitis which is a result of the progression of the inflammatory process from the ulceration to the vascular wall (WANKE [26]); the process involves arteries as well as veins. The most frequent change in the veins is fibrosis, hyaline degeneration, or elastosis of the vascular wall, with gross narrowing of the lumen (MEYER [21]). Inflammatory changes of the arteries are usually

Fig. 2. Ulcer carcinoma shown in Fig. 1 under high power

seen in the areas near to the surface of the ulcer. The intimal proliferation which causes the vessel to narrow usually begins with an oedematous swelling of the intima (serous endarteriitis). According to ROTTER [24], this is a result of diminished oxygen supply to the vessel wall. In this stage of the disease, acid mucopolysaccharides tend to accumulate between the intima and the media, particularly along the internal elastic membrane; LINDNER [20] suggested the formation of reticular, collagenous, and elastic fibres which later occlude the lumen of the vessel. Others, like STEHBENS [25], believe that the process is not an obliterating endarteriitis, for the vascular changes in the region of the ulcer are first of all of a degenerative character, rather showing the characteristics of diffuse hyperplastic sclerosis. The arteriolar changes and the deposition of lipoids under the intima make the process very similar to atherosclerosis.

In the following, a survey will be given of the sequence of events in the gastric vessels during the development of callous ulcer and ulcer carcinoma.

Results

On the luminal side of the chronic peptic ulcer, immediately under the necrotic and fibrinous layer covering the ulcer, there is a newly formed tissue rich in capillaries and fibroblasts. In the arterioles, as a sign of inflammatory reaction, the endothelial cells are swollen, and there is a cellular infiltration of the wall, significant arrosions being seen in many of the vessels; there is often a thrombus in the vascular lumen. Advancing towards the serosal surface of the stomach, the scar is more and more devoid of capillaries and connective tissue cells.

Changes different in character can be observed in the arteries lying under the serosa and in the scar replacing the necrotized muscular layer of the stom-

ach. The muscular layer of these arteries is apparently intact, without cellular infiltration. The internal elastic membrane is also intact. Within the latter, however, intimal hyperplasia is seen, with a remarkably large amount of

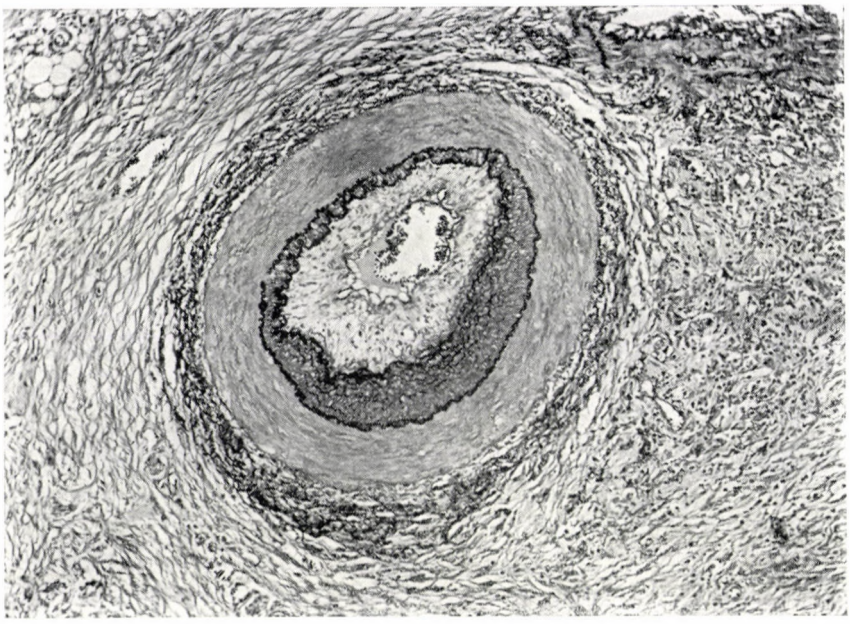


Fig. 3. Connective tissue hyperplasia consisting of elastic and reticular fibres, which considerably narrows an artery lying deep in the scar

elastic fibres which are arranged concentrically, but occasionally excentrically. This layer is sharply demarcated from the intimal proliferation made up of loose reticular tissue and devoid of elastic fibres. In the centre of this, the narrowed vascular lumen is seen, lined with endothelium (Fig. 3). Inflammatory cells are absent from the subintimal tissue excess. Thrombosis is infrequent in the residual lumen; if a thrombus is present, the intimal hyperplasia lying under it displays no inflammatory reaction (Fig. 4), in sharp contrast to the vessels running immediately under the ulcer surface, for in the latter thrombosis occurs as a result of panarteriitis or arrosion of the vessel.

The increased number of elastic fibres under the intima cannot be regarded as inflammatory in origin, but seems rather to be due to increased blood pressure. The rise in intravascular pressure can be accounted for by at least two mechanisms. First, the formation of a thick and massive scar leads to the obliteration of the capillaries arising from the artery. On the other hand, the vessel wall itself undergoes deep morphologic changes, and the initially elastic arteries moving freely in the loose connective tissue become rigid and

immobile. The latter circumstance seems to be responsible for the excentric localization of the intimal elastic hyperplasia at sites where the artery is curved. Thus, the changes shown by the arteries in more remote parts of the scar and in subserosal scars are due to the altered functional and mechanical factors.

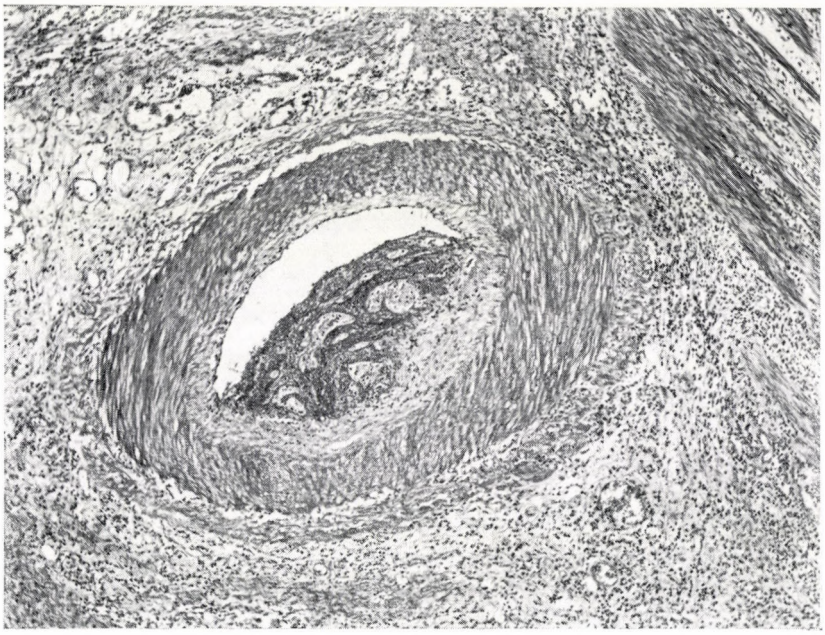


Fig. 4. Thrombus formed on the surface of excentric intimal hyperplasia, with early signs of fibrous transformation

In our cases of early ulcer carcinoma the vascular lesions in the scar showed the following characteristics. Under the ulcer surface, the number of capillaries running perpendicularly to the surface are significantly decreased in number. The proliferating epithelium near to the edge of the ulcer consists of columnar epithelium of one or two layers, no acini being formed at all. Owing to the continuous mechanical and chemical irritation, this weak epithelial layer is soon destroyed, nevertheless, the tendency to regenerate persists. Regeneration requires adequate vascular supply, which is certainly not secured on the surface of the scar. From the continuously formed, destroyed, and newly formed epithelial proliferation then arises the malignant tumour. This displays invasive properties already in the early stage of its development. In the deeper layers of the scar, and also under the serosa, there is a great number of arteries showing narrowing or complete obstruction. The intimal proliferation obstructing the vessel is made up almost exclusively of elastic fibres (Fig. 5). In the windings of the arteries, there is always an excentric intimal hyperplasia, the

remaining part of the lumen being obstructed by the fibroblastic intimal proliferation (Fig. 6). Thus, the vascular changes seen in callous ulcer and scar carcinoma differ only quantitatively. A thorough examination of a great number of scar cancers revealed that there is practically no proliferation of capillaries on the base of the transformed ulcer, and complete obliteration

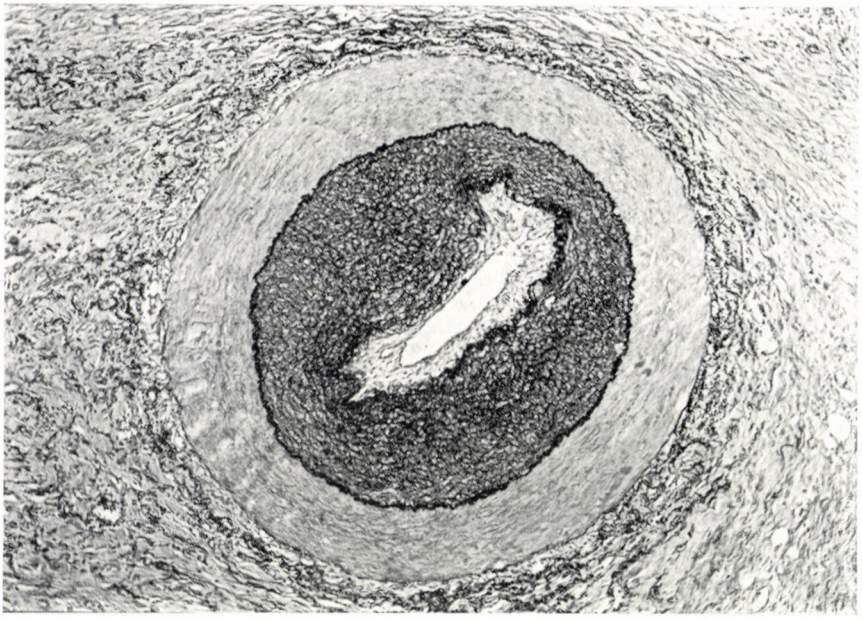


Fig. 5. Extreme narrowing of vessel in the scar of ulcer carcinoma. Thickening of intima is mainly due to the large number of elastic fibres

occurs in a significantly larger number of arteries than in simple ulcer. Complete obstruction of the arteries is apparent also in section of the vessels lying outside the scar, indicating that the branches lying in the scar have been completely obliterated and, consequently, circulation has ceased also in the portions outside the scar. Thus, the ischaemic-hypoxic zone is much larger than the scar itself.

Angiography revealed that the chronic peptic ulcer is surrounded by a zone whose blood supply is significantly impaired, and this ischaemia is responsible for the absence of the healing process in the callous ulcer (KEY [18], ANFOSSI [1]). Although new vessels and capillaries are abundantly formed around the scar (VICZIÁN and SZABÓ [27]), they are insufficient for securing an adequate blood supply to the callous ulcer. On the margin of the ulcer, regeneration occurs continuously, while on the surface of the scar the blood supply is never sufficient to secure conditions for further proliferation and the formation of acini. Regeneration is further impaired by mechanical and chem-

ical irritation due to ingested food, thus, the newly formed mucosal layers are continuously torn off from the periphery of the ulcer.

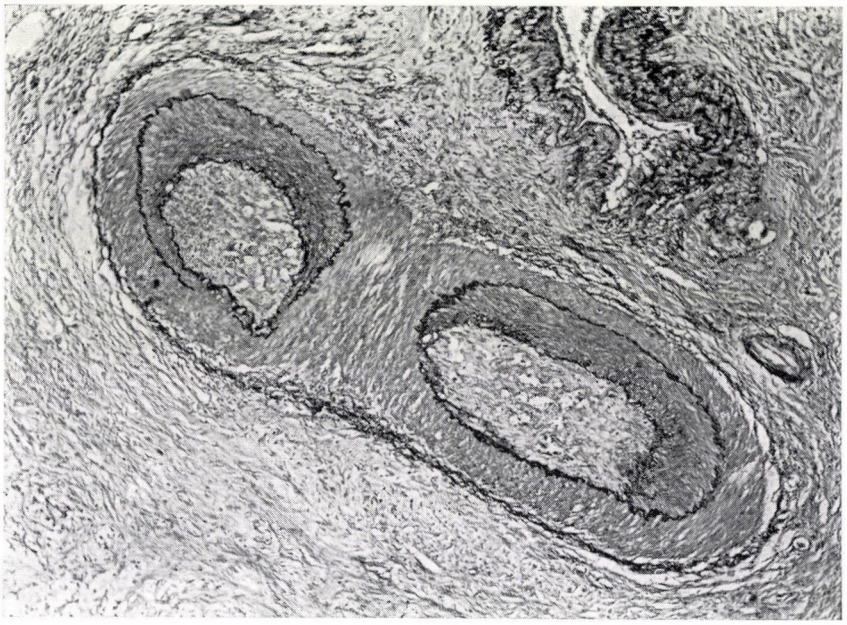


Fig. 6. Obliteration of the windings of an artery lying close to the serosal surface of the stomach. Excentric elastic hyperplasia is apparent; the lumen is completely obliterated by intimal proliferation

Discussion

It is remarkable that of the many studies dealing with the morphology and incidence of ulcer carcinoma so few should have discussed the problem of aetiology, attributing an almost exclusive role to the mechanical and chemical irritation caused by the ingested food. Chronic peptic ulcer, chronic gastritis, and gastric polyposis are known to be precarcinomatous conditions. The exact cause of gastric carcinoma, however, is still obscure.

The study of scar carcinomas has revealed certain facts pointing to the significance of vascular lesions. The interrelationship between scar formation and vascular lesions has two different aspects: a scar may arise in consequence of vascular obliteration, or, else a scar induced by some inflammatory process, injury, etc. leads secondarily to vascular obliteration. A common feature of scar formation, whether or not vascular in origin, is parenchymal atrophy or necrosis, which is followed by regeneration of the epithelial cells on the margin of the scar or in the substance of the fibrotic tissue. The poor vascularization in and around the scar and the subsequent hypoxia are the reason why epithe-

lial regeneration both qualitatively and quantitatively insufficient, and the altered metabolism of these cells may induce malignant transformation.

Hypoxia is invariably present in scars, irrespective of their origin, owing to the inadequate vascular supply. Other factors, too, may have a role in the malignant transformation. The regenerating epithelium is exposed to carcinogenic impacts which may act directly on the surface of the freshly formed cell layer, or carcinogenic agents may accumulate owing to the impaired regional flow of blood. The observation of BALÓ *et al.* [5] of epithelial proliferation arising on the marginal zone of pulmonary infarction points to the primary role of hypoxia in the induction of cancer, as an impaired oxygen supply alone, without the additional action of chemical or other carcinogenic impacts, may induce a malignant transformation of cells.

The relationship between hypoxia and cell metabolism has been thoroughly studied by WARBURG *et al.* [29—34]. In the absence of oxygen, normal tissues tend to cover their energy requirement by anaerobic glycolysis, which is an irreversible characteristic common to all malignant cells. According to WARBURG [28], a metabolism altered in the above sense is not only a characteristic feature of malignant cells, but it constitutes the primary cause of malignant transformation, and carcinogenic agents exert their action by irreversibly damaging cell metabolism. In BICKNELL's [8] view a pathologic alteration of cell metabolism should be considered a precarcinomatous state, and all cells which, owing to a deficient oxygen supply, cover their energy requirement by glycolysis, may be regarded as tumour cells. There is some relationship between the degree of malignancy and the rate of anaerobic glycolysis (BURK [12]). The fundamental investigations of WARBURG into the metabolism of normal and neoplastic cells have been criticized by WEINHOUSE [35] and LEPAGE [19]. The observations, however, which have definitely shown the malignant transformation of hypoxic cells in vitro (GOLDBLATT and CAMERON [14]; BURK [12]) provide sufficient evidence of the importance of hypoxia in carcinogenesis.

According to BALÓ [3], pulmonary carcinoma arising from infarction has opened new possibilities for studying the effect of similar vascular lesions in the induction of cancer in other parts of the organism. Tissue hypoxia undoubtedly plays a significant role in atypical, neoplastic proliferation of regenerating epithelia. In view of the more recent investigations, hypoxia, in addition to its role played in atrophic, dystrophic, and other pathologic changes, is an important pathogenetic factor in carcinogenesis. Moreover, hypoxia must be considered a decisive factor giving rise to reversible and irreversible changes in cell metabolism. Details of this mechanism should be disclosed by further morphological, particularly ultrastructural and biochemical studies. The close relationship between vascular changes, hypoxia, and tumour induction is clearly demonstrated by the fact that carcinoma often arises from chronic peptic ulcer.

Gastric cancer developing from a callous ulcer shows all the characteristics of a scar carcinoma: the growth starts from the regenerating mucosal cells lying at the margin of the poorly vascularized scar. On the surface of the scar, regeneration is always incomplete. With old ulcers the scar becomes increasingly thick and massive, successively reducing the chance for healing. The obliteration of vessels results in tissue hypoxia. It has been shown that the degree of arterial obliteration is significantly more severe in ulcer carcinoma than in simple ulcerations without malignant transformation. If healing of the ulcer occurs, the scars are star shaped, small and involve circumscribed areas of the gastric wall. The probability that carcinoma arises from the scar of such healed ulcers is practically nil (BORRMANN [9]; HAUSER [15]). This observation again supports the view that mucosal regeneration may be complete and malignant transformation does not occur whenever little scar tissue is formed and, consequently, vascular changes are insignificant. Thus, whether a peptic ulcer heals or undergoes malignant transformation is greatly influenced by scar formation and the vascular changes occurring in it. For obvious reasons, this assumption bears some clinical importance, for the indication of surgical or conservative treatment should always be considered on the basis of the presence or absence of the above factors.

REFERENCES

1. ANFOSSI, C.: (1955) Vascolarizzazione dello stomaco ulceroso. Ulcera peptica e carcinomatosa. Arch. Sci. med. **99,** 297—319. — 2. ANTONIE, TH.: (1961) Arteriosclerosis of the Arteries of the Stomach. Med. J. Aust. **48,** 209—210. — 3. BALÓ, J.: (1962) Zur Frage der Karzinogenese. Münch. med. Wschr. **104,** 1424—1428. — 4. BALÓ, J.: (1959) Lungenkarzinom und Lungenadenom. Akademie Verlag, Budapest. — 5. BALÓ, J., JUHÁSZ, J., TEMES, J.: (1956) Pulmonary Infarcts and Pulmonary Carcinoma. Cancer **9,** 918—922. — 6. BERKHEISER, S. W.: (1965) Pulmonary Infarction Associated with Lung Cancer. Dis. Chest **47,** 36—41. — 7. BETZLER, H. J., LEONHARDT, J.: (1959) Sarkom in einer Laparotomienarbe. Z. Krebsforsch. **63,** 118—121. — 8. BICKNELL, E. A.: (1957) Cancer and Anoxia. A Further Evaluation of Clinical and Experimental Trends. J. Mich. med. Soc. **56,** 456—460. — 9. BORRMANN, R.: (1926) Geschwülste des Magens und Duodenums. In: HENKE, F., LUBARSCH, O.: Handbuch der Speziellen Pathologischen Anatomie und Histologie. Bd. IV/1., S. 812—1054. Springer, Berlin. — 10. BRACK, K.: (1957) Zur Frage der Narbenkarzinome der Harnwege. Z. Urol. **50,** 310—327. — 11. BUDAY, K.: (1908) Über die Sklerose der Magenarterien. Beitr. path. Anat. **44,** 327—348. — 12. BURK, D.: cit. WARBURG, O.: (1956) On Respiratory Impairment in Cancer Cells. Science **124,** 269—270. — 13. EBERT, G.: (1954) Sarkomentstehung nach Verwundung. Langenbecks Arch. klin. Chir. **278,** 218—288. — 14. GOLDBLATT, H., CAMERON, G.: (1953) Induced Malignancy in Cells from Rat Myocardium Subjected to Intermittent Anaerobiosis during Long Propagation in vitro. J. exp. Med. **97,** 525—552. — 15. HAUSER, G.: (1926) Die krebsige Entartung des chronischen Magen- und Duodenalgeschwüres. In: HENKE, F., LUBARSCH, O.: Handbuch der Speziellen Pathologischen Anatomie und Histologie. Bd. IV/1. S. 497—518. Springer, Berlin. — 16. JUHÁSZ, J.: (1962) Das Anfangsstadium der krebsigen Umwandlung bei chronischem Magenulcus. In: V. Ungarische Krebstagung 1961. S. 67—71. Akademie Verlag, Budapest. — 17. KELLNER, B.: (1962) A gyomorrák pathologiájának néhány kérdése. Orv. Hetil. **93,** 1413—1418. — 18. KEY, J. A.: (1950) Blood Vessels of a Gastric Ulcer. Brit. med. J. **2,** 1464—1465. — 19. LE PAGE, G. A.: (1950) A Comparison of Tumour and Normal Tissue with Respect to Factors Affecting the Rate of Anaerobic Glycolysis. Cancer Res. **10,** 77—88. — 20. LINDNER, J.: (1961) Histochemie der Arteriosklerose. In: SCHETTLER, G.: Arteriosklerose. S. 51—87. G. Thieme, Stuttgart. — 21. MEYER, W. W.: (1953) Histologisches

zum Verhalten des Mesenchyms bei fortschreitenden und abheilenden Magengeschwüren. Virchows Arch. path. Anat. **323,** 402—441. — 22. PALMER, E. D., BOYCE, H. W. JR.: (1964) Sclerotic Submucosal Gastric Artery: A Source of Hemorrhage. Amer. Surg. **30,** 83—87. — 23. RAEBURN, C., SPENCER, H.: (1957) Lung Scar Cancers. Brit. J. Tuberc. **51,** 237—245. — 24. ROTTER, W.: (1949) Über die Bedeutung der Ernährungsstörung, insbesondere des Sauerstoffmangels für die Pathogenese der Gefäßwandveränderungen mit besonderer Berücksichtigung der „Endarteriitis obliterans" und der „Arteriosklerose". Zugleich ein Beitrag zum Entzündungsproblem. Beitr. path. Anat. **110,** 46—102. — 25. STEHBENS, W. E.: (1964) Vascular Changes in Chronic Peptic Ulcer. Arch. Path. **78,** 584—590. — 26. WANKE, M.: (1964) Primäre Arteriitis obliterans und Ulcus ventriculi. Zugleich ein Beitrag zur Frage der radikulären-intramuralen Sklerose der Magenarterien. Langenbecks Arch. klin. Chir. **305,** 174—190. — 27. VICZIÁN, A., SZABÓ, J.: (1958) Adatok a gyomorfekély körüli elzáródások kérdéséhez. Orv. Hetil. **99,** 19—22. — 28. WARBURG, O.: (1955) Über die Entstehung der Krebszellen. Naturwissenschaften **42,** 401—406. — 29. WARBURG, O.: (1956) On the Origin of Cancer Cells. Science **123,** 309—314. — 30. WARBURG, O., GAWEHN, K., GEISSLER, A. W., KAYSER, D., LORENZ, S.: (1965) Experimente zur Anaerobiose der Krebszellen. Klin. Wschr. **43,** 289—293. — 31. WARBURG, O., GAWEHN, K., GEISSLER, A. W., LORENZ, S.: (1962) Über den Einfluß des Sauerstoffdruckes auf die Umwandlung des embryonalen Stoffwechsels in Krebsstoffwechsel. Z. Naturforsch. 17/b. 772—774. — 32. WARBURG, O., GAWEHN, K., GEISSLER, A. W., LORENZ, S.: (1960) Über die Umwandlung des embryonalen Stoffwechsels in Krebsstoffwechsel. Hoppe-Seylers Z. physiol. Chem. **321,** 252—257. — 33. WARBURG, O., GEISSLER, A. W., LORENZ, S.: (1962) Atmung und Wachstum von Krebszellen bei niedrigen Sauerstoffdrucken. Z. Naturforsch. 17/b. 758—761. — 34. WARBURG, O., GEISSLER, A. W., LORENZ, S.: (1965) Messung der Sauerstoffdrucke beim Umschlag des embryonalen Stoffwechsels in Krebs-Stoffwechsel. Z. Naturforsch. **20/b,** 1070—1073. — 35. WEINHOUSE, S.: (1955) Oxidative Metabolism of Neoplastic Tissue. Advanc. Cancer Res. **3,** 269—325. — 36. YOKOO, H., SUCKOW, E. E.: (1961) Peripheral Lung Cancers Arising in Scars. Cancer **14,** 1205—1215.

DIE BEDEUTUNG DER GEFÄSSVERÄNDERUNGEN IN DER KANZERÖSEN ENTARTUNG DER CHRONISCHEN MAGENGESCHWÜRE

J. JUHÁSZ

Die geschwülstige Entartung chronischer peptischer Magengeschwüre kam im Kranken gut des Verfassers bei 11,5% der zur Untersuchung gelangenden Magenkarzinomfälle vor Die krebsige Entartung setzt multizentrisch am Rande des Geschwürs in der regenerierenden Schleimhaut ein. In den Ulkuskarzinomen entsteht im Narbengewebe des Geschwürs und in dessen Umgebung eine durch Gefäßverschluß bewirkte Gewebshypoxie, die den atypischen Verlauf der Schleimhautregeneration herbeiführt. Das aus dem chronischen peptischen Magengeschwür entstehende Karzinom entspricht einem Narbenkarzinom. An seiner Entstehung ist die durch die Hypoxie bewirkte pathologische Veränderung des Zellstoffwechsels beteiligt. BALÓ meint, daß die Untersuchung der in hypoxischen Bezirken entstehenden Epithelwucherungen wichtige Beiträge zur Aufklärung der Zusammenhänge zwischen den Gefäßveränderungen und der Tumorentstehung liefern kann. Auch in der kanzerösen Entartung des Ulcus callosum kann die primäre Rolle der Hypoxydose angenommen werden.

ЗНАЧЕНИЕ ИЗМЕНЕНИЙ СТЕНКИ СОСУДОВ ПРИ РАКОВОМ ПЕРЕРОЖДЕНИИ ХРОНИЧЕСКОЙ ЯЗВЫ ЖЕЛУДКА

Е. ЮХАС

В материале автора опухолевое перерождение хронической пептической язвы желудка встречалось у 11,5% больных раком желудка. Раковое перерождение начинается с края язвы мультицентрично в регенерирующейся слизистой оболочке. У больных раком на почве язвы в результате закупорки сосудов в рубцовой ткани язвы и в ее окрестности создается тканевая гипоксия, вследствие чего регенерация слизистой принимает нетипичную форму. Рак на почве хронической пептической язвы соответствует

рубцовому раку и в его развитии участвует патологическое изменение клеточного метаболизма, вызванное гипоксией. Согласно *Бало* изучение разрастания эпителия в гипоксических областях может предоставлять важные данные к выяснению связей между сосудистыми изменениями и карциногенезом. При раковом перерождении хронической мозолистой язвы также можно предполагать первичную роль гипоксидоза.

Dr. Jenő Juhász; Budapest VIII. Üllői út 26. Hungary

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 53—63, 1968*

First Department of Pathological Anatomy and Experimental Cancer Research
(Director: Prof. J. Baló), Medical University, Budapest

STRUCTURE AND IMPORTANCE OF INTRANUCLEAR INCLUSIONS IN THIOACETAMIDE HEPATOCARCINOGENESIS

G. Kendrey

Male white rats fed a standard diet were treated for long periods with thioacetamide. After 3 weeks, in the nuclei of hepatic cells large numbers of pyroninophilic inclusions, each surrounded by a membrane, were seen under the light microscope. Electronmicroscopy revealed that the inclusions were bordered by double-contoured nuclear membrane with pores in it. Except mitochondria, all kinds of cytoplasmic organelles were present in the inclusions, in amounts and composition not occurring in the cytoplasm of hepatic cells. The bodies, which seemed to be intranuclear inclusions under the light microscope, were in reality cytoplasmic elements invaginating into the nuclei. Some of the elements developed into real intranuclear inclusion bodies, others showed signs of regressive processes. It is concluded, that the "intranuclear" inclusions frequently observed in thioacetamide carcinogenesis are indicators of the increased synthetic activity of hepatic cells.

The hepatocellular toxicity of thioacetamide (TAA) recommended for the preservation of orange (Childs and Siegler [9]) was demonstrated in white rats by Fitzhugh and Nelson [13]. Characteristic cytological changes of liver cells, hepatic cirrhosis — and carcinoma of the rat induced by TAA have been studied by a number of authors [3, 4, 7, 14, 15, 18, 21—25, 33, 36—39, 41, 44].

Our own light and electronmicroscopic studies of TAA-hepatocarcinogenesis demonstrated large numbers of intranuclear inclusions in all stages of the treatment. The present work deals with the development, light and electronmicroscopic structure and importance of these formations.

Material and method

In 6 experiments a total of 138 male, 3- to 4-month-old, white rats weighing 120 to 150 g were used. The animals were fed a standard synthetic diet. A total of 35 white male rats of the same age and weight served as controls; they were given the same diet. TAA (obtained from VEB Berlin-Chemie, Berlin, Adlersdorf; and Merck, Darmstadt) was administered intraperitoneally to 103 animals in doses of 25 mg/kg (one experiment) or 50 mg/kg (five experiments daily), for various periods, the longest being 6 months. Some animals were observed for 1 year after discontinuation of the treatment. The rats were sacrificed by decapitation between the 1st and 196th day of treatment always 24 hours after the last injection, and in the morning hours. Liver fragments were removed within one minute after killing. Blocks of liver tissue for light microscopy were fixed in 8 per cent formalin, Carnoy's solution and embedded in paraffin. Sections were stained with hematoxylin-eosin, van Gieson, Feulgen, methyl-green-pyronine, PAS, Best-carmine and Gomori's silver impregnation.

Tissue for electron microscopic studies was fixed for 1 hour in 1 per cent OsO_4 buffered according to MILLONIG [30], dehydrated in a graded alcohol series, immersed in 70 per cent alcohol saturated with uranyl acetate for 20 minutes and embedded in araldite. Polymerization took place during an incubation at 56 °C for 24 hours. Ultrathin sections were cut with glass knives in a Reichert ultramicrotome, and stained with lead citrate [34] or lead hydroxide [20]. Electron micrographs were taken in a SEM 3 electron microscope at 60 kV.

Results

After three weeks of TAA treatment, at first in the pericentral area then at the periphery of the hepatic lobules, extremely large liver cells began to appear among the somewhat enlarged parenchymal cells (Fig. 1). The nuclei

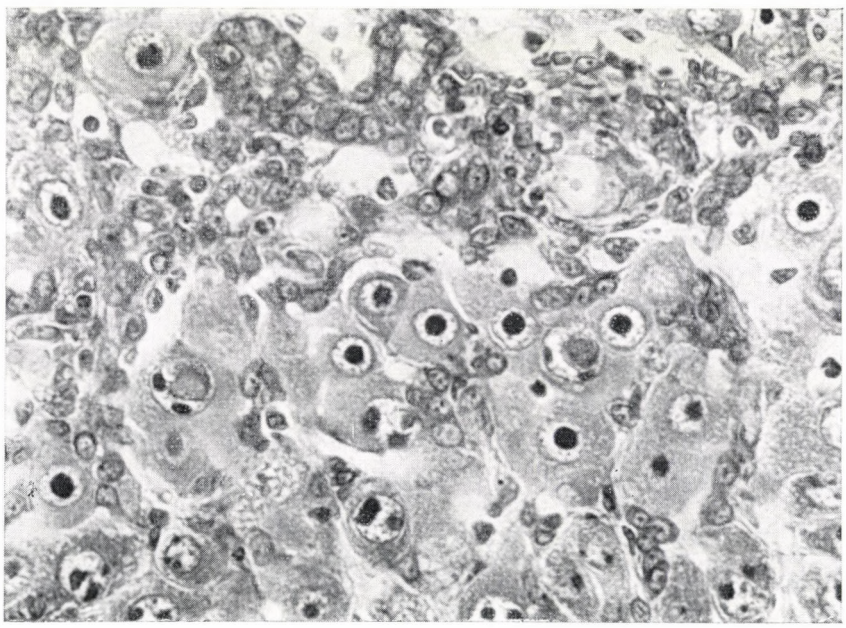


Fig. 1. Inclusion bodies in the nuclei of considerably enlarged hepatic cells are pressing the nucleoli sideways. Giant nucleoli in liver cells free from inclusions (Methylgreen-pyronine). ×400

of the large cells were considerably swollen, the nuclear membrane was conspicuous and the chromatin content decreased. The giant nucleoli were generally compact in structure; only some of them contained vacuoles. In the nucleus of some hepatic cells one, sometimes more, generally round bodies surrounded by a membrane were shown. The bodies varied considerably in size; some inclusions were larger than the nucleoli and pressed them sideways. In methylgreen-pyronine preparations the intranuclear bodies were pyroninophilic, but stained much less definitely than the nucleoli.

Electron microscopic examination frequently revealed multiple cytoplasmic indentations of the nuclear membrane in the enormously enlarged liver cells

(Fig. 2). Many of the considerably enlarged nuclei contained inclusion bodies varying in structure. Most of these were demarcated from the nucleoplasm by the nuclear membrane but intranuclear lipid droplets surrounded by a single membrane were also observed (Fig. 3). The content of the inclusions varied considerably. Where cytoplasmic invaginations were bordered by a double-

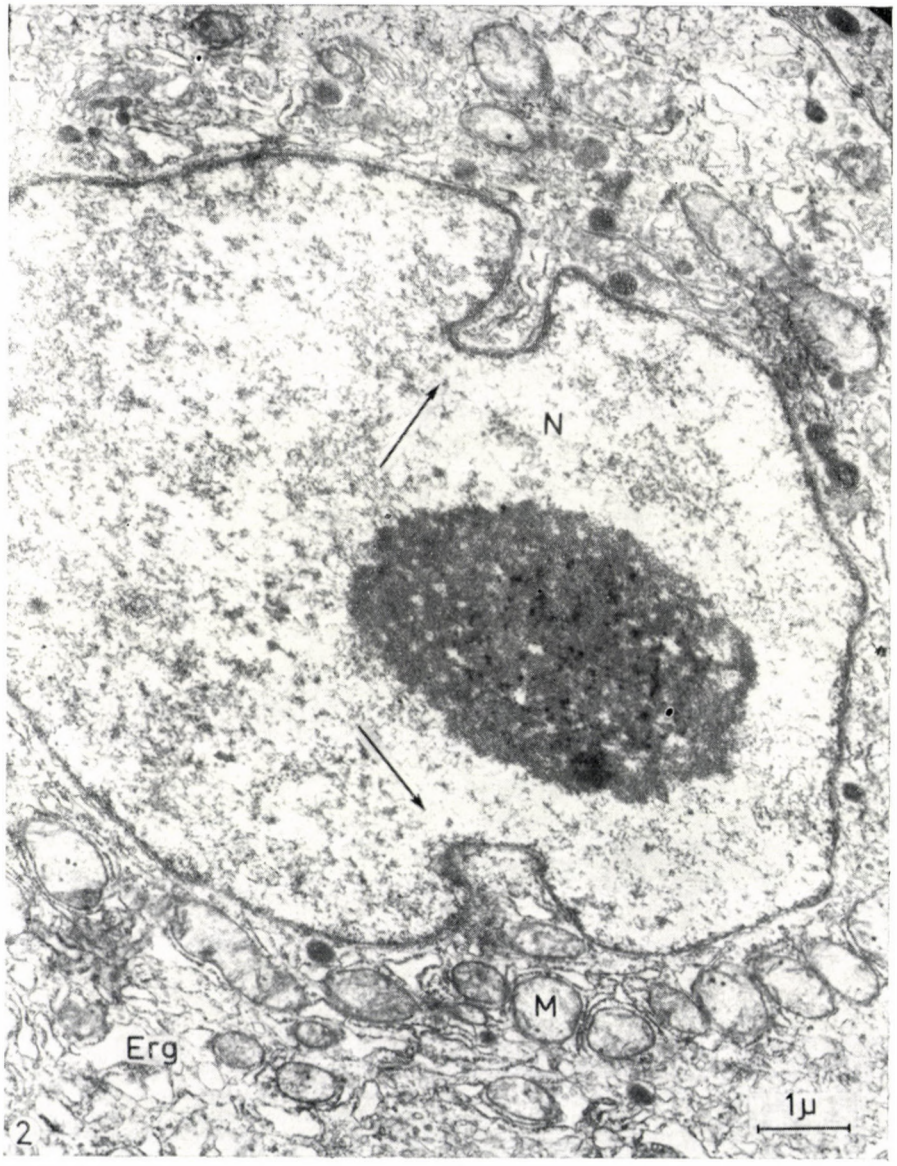


Fig. 2. Large nucleus (N) with greatly enlarged nucleolus in it. Nuclear membrane with two cytoplasmic indentations (arrows) opposite each other. (M = mitochondrium, Erg = rough endoplasmic reticulum). ×12 000

contoured nuclear membrane with pores in it, only hyaloplasm was seen, either with free ribosomes and rough endoplasmic reticulum, or with smooth endoplasmic reticulum and large lipid droplets as well as many glycogen spherules (Figs 4 and 5). On rare occasions, solitary bodies enveloped by a single or double membrane were observed in the nucleoplasm, in the vicinity of the invaginating portion of the cytoplasm (Fig. 6).

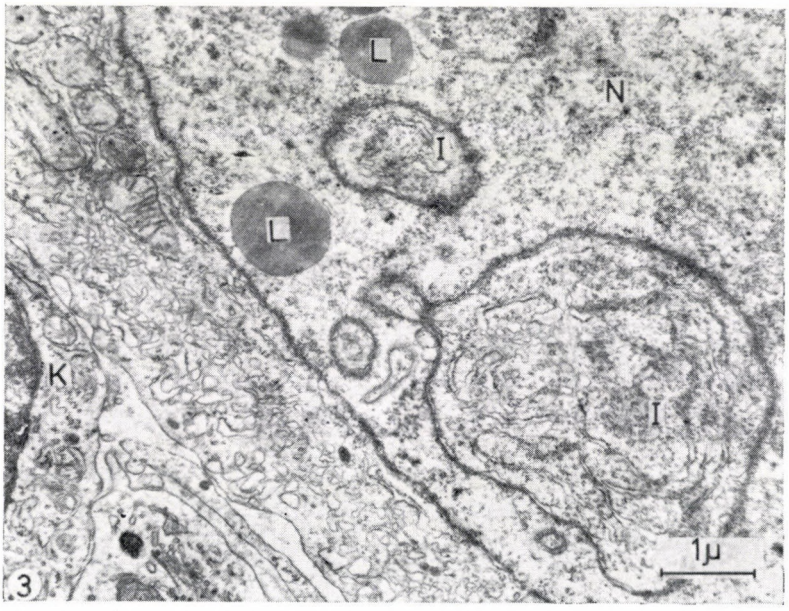


Fig. 3. Part of hepatic cell with enlarged nucleus. In the nucleus (N) there are inclusions (I) surrounded by double membrane and containing mainly rough endoplasmic reticulum (Erg) and lipid droplets (L); the latter are bordered by a single membrane (K = Kupffer cell) ×12 000

The above-described intranuclear changes occurred only in the liver of treated animals; similar alterations were never demonstrated in control rats.

It is apparent from these studies that most of the inclusions giving the impression of being intranuclear light microscopically, are not true inclusions; they arise in the portion of the cytoplasm that invaginates the nucleus.

Discussion

Intranuclear inclusion bodies have infrequently been shown in the liver of normal laboratory rats and mice. KLEINFELD and KOULISH [26] demonstrated such formations in the liver of C_3H mice. Intranuclear bodies were shown in the hepatic cells of mice treated with colchicine (WESSEL [45], ALTMANN

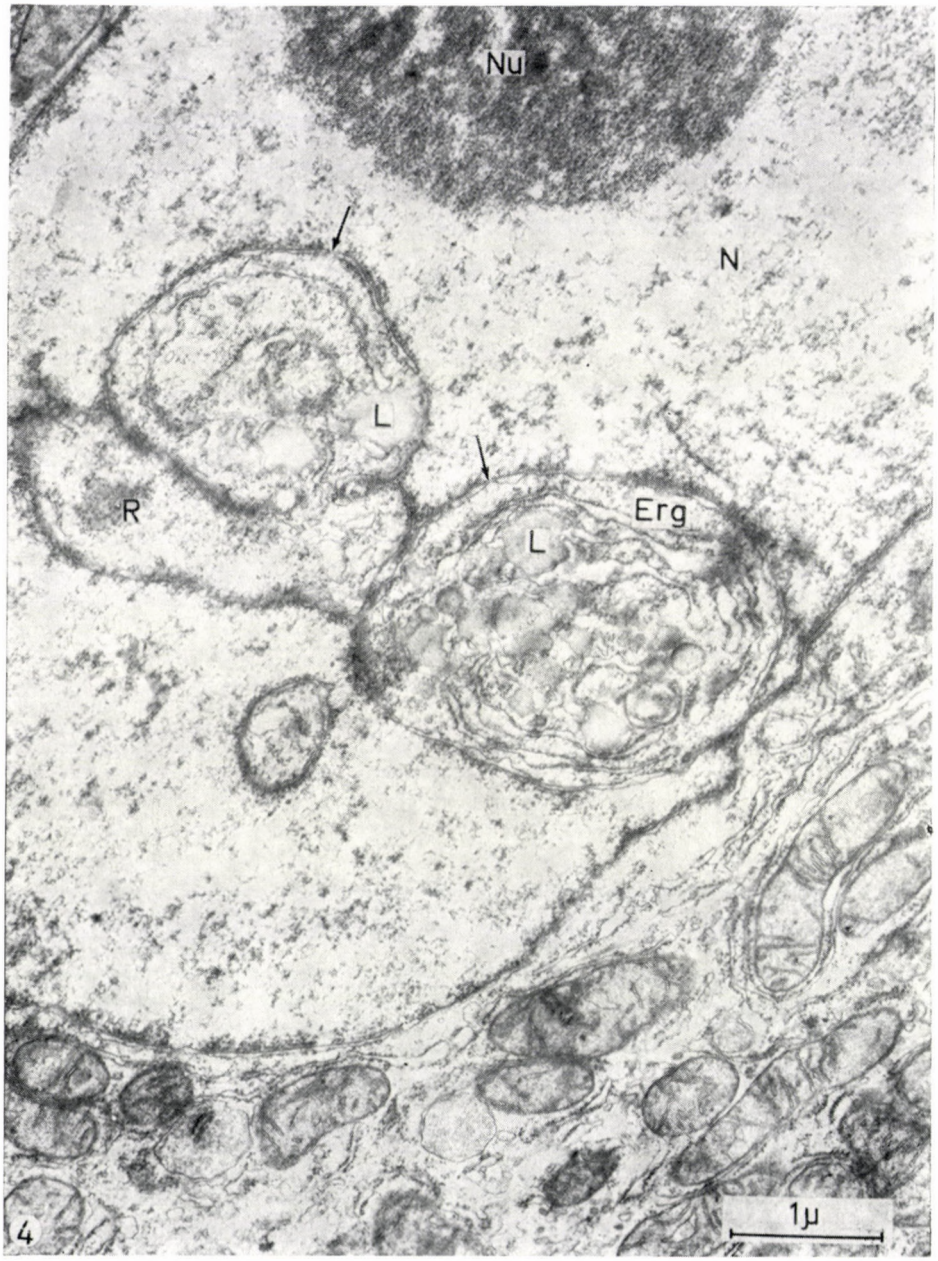


Fig. 4. Sharply outlined, compact nucleolus (Nu) in considerably enlarged nucleus (N) which contains multiple cytoplasmic invaginations with many lipid droplets (L) and hyaloplasm containing rough endoplasmic reticulum (Erg) and free ribosomes (R). The inclusions are surrounded by the double nuclear membrane with pores in it (arrows). ×18 000

and HAUBRICH [2]), in starving mice (DAVID [12]), in transplantable mouse-hepatoma and mouse hepatic cells (LEDUC and WILSON [29]), and in ethionin-induced hepatic carcinoma (GRISHAM [16]).

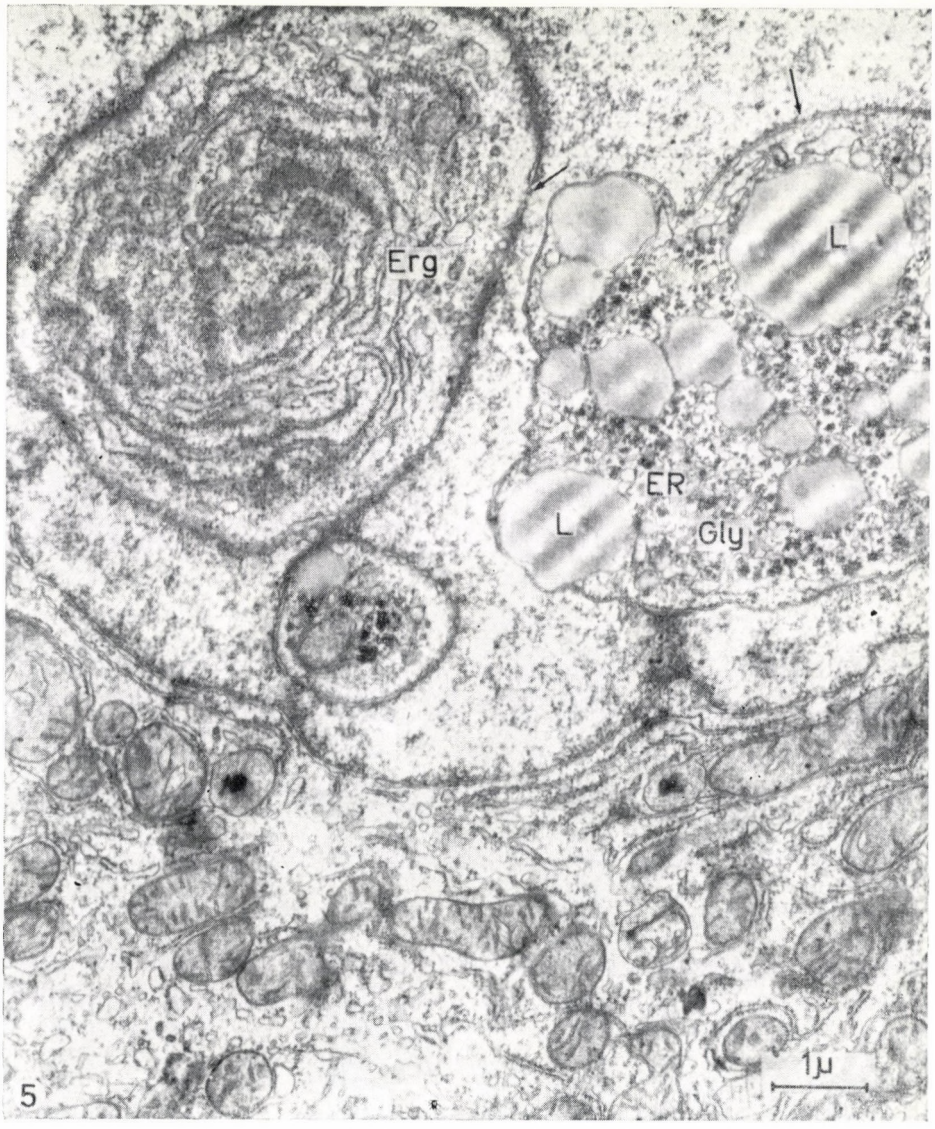


Fig. 5. Three inclusion bodies in a nucleus. The one on the left shows concentric whorls of rough endoplasmic reticulum (Erg) and hyaloplasm containing free ribosomes (R); the other two show a number of lipid droplets (L) and glycogen granules (Gly) with vesicular smooth endoplasmic reticulum (ER) near them. Arrows point to nuclear pores. ×12 000

Light and electron microscopic observations showed similar bodies in human viral hepatitis (NICOLAU [31], BALÓ [5], COSSEL [10]); COSSEL [11] described their presence in the liver of healthy humans.

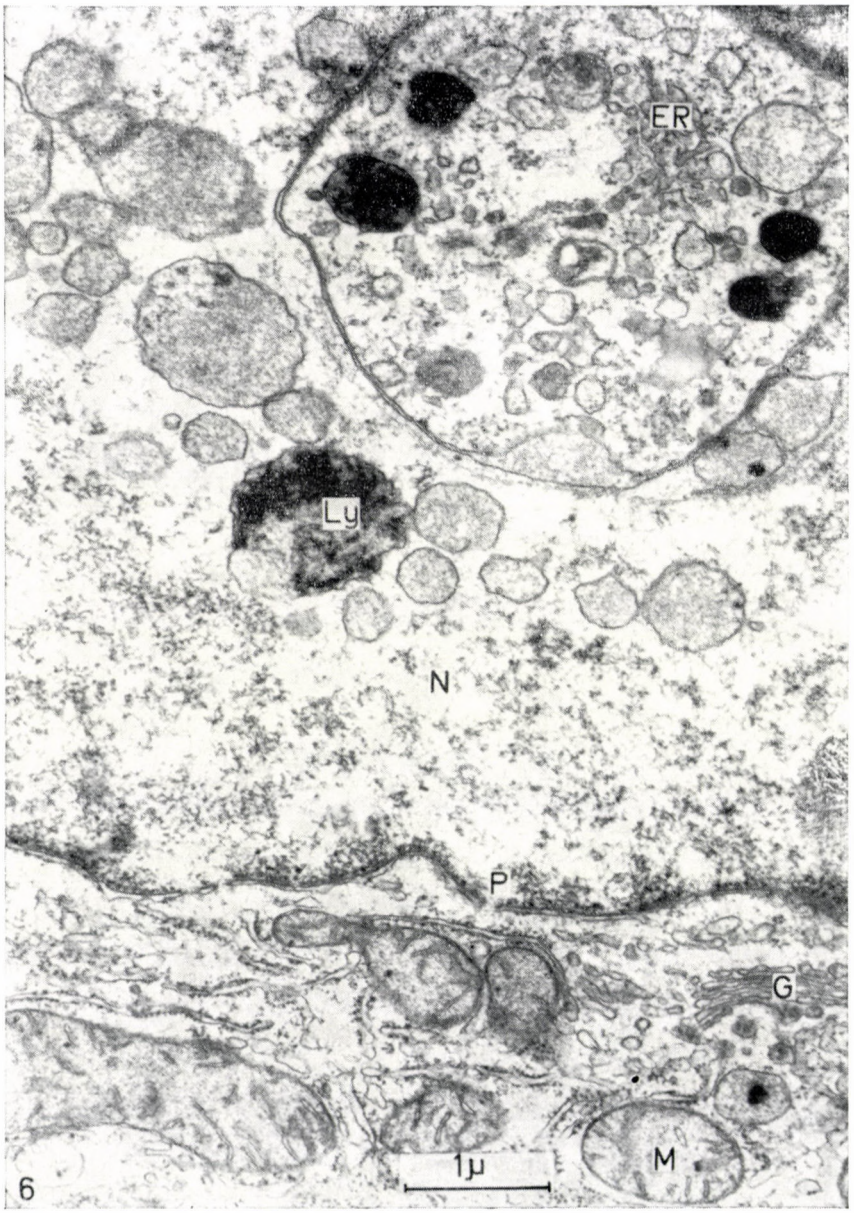


Fig. 6. Cytoplasmic invagination of a large nucleus (N). Other bodies varying in content and enveloped by a single membrane, lie free in the nucleoplasm ER = smooth endoplasmic reticulum, Ly = lysosome, P = nuclear pore, G = Golgi apparatus, M = mitochondrium). ×20 000

In the liver of rats treated with TAA KLEINFELD [25] was the first to demonstrate the presence of intranuclear bodies which, in her opinion, contained carbohydrate and lipid substances. RONDEZ *et al.* [36] and SALOMON *et al.* [40] presented electron micrographs of lipid-containing inclusions produced during TAA treatment. Recently, THOENES [43] has demonstrated lipid substances in the nucleoli of hepatic cells of rats treated with TAA. According to the above authors, the bodies corresponded to cytoplasmic invaginations, but they failed to deal with the origin, structure and importance of these formations.

As such "intranuclear" inclusion bodies are frequently observed in almost every stage of TAA hepatocarcinogenesis, it seems advisable to discuss briefly the described alterations and relevant problems to their origin.

It has already been pointed out that the bodies observed under the light microscope are not true intranuclear inclusions but correspond to cytoplasmic elements invaginated into the nucleus. The question thus arises as to the factor responsible for the production of the bodies. The "intranuclear" inclusions occur always in strikingly enlarged hepatic cells and in nuclei considerably larger than the average nucleus. In this respect investigations into the association between the size of the cell, nucleus and nucleolus and certain cellular functions should be taken into consideration (RATHER [33], KLEINFELD [25], GRUNDMANN [19], OEHLERT and SCHULTZE [32]). Such studies have indicated that the enlargement of cells, nuclei and nucleoli is accompanied by an increased synthetic activity. TAA has been shown to increase the production of nucleic acids, especially of ribonucleic acid, in the liver of white rats (LAIRD [28], KLEINFELD and HAAM [27], STÖCKER [42], RONDEZ [35]).

In view of the close functional connection between the nucleus and the cytoplasm (CASPERSSON [8], ALTMANN [1], BRACHET [6], GRUNDMANN [19]), it may be assumed that the increased mobility of the cytoplasm occurring in hepatic cells enlarged under the effect of TAA treatment is responsible for the arisal of invaginations. The invaginated cytoplasm may improve the transport of substances between nucleus and cytoplasm. COSSEL [10] suggested on the basis of findings in human material that the intranuclear inclusions were due to the increased metabolic activity of the hepatic cells. The invaginated cytoplasm covered by the porous nuclear membrane is in close contact with the nucleoplasm.

According to our own electron microscopic investigations such cytoplasmic portions are conspicuous, first, for frequently containing elements in amounts and composition not seen in the rest of the cytoplasm; secondly for the not uncommon presence in them of high-energy substances, such as lipids and glycogen.

A further problem is the fate of the inclusions. Some micrographs indicate that the bodies may lose their double membrane and so the cytoplasmic organ-

elles may gain entrance into the nucleoplasm and become true intranuclear inclusions. At the periphery of such formations sometimes a single membrane can be observed.

It is apparent from these studies, that the "intranuclear" inclusions seen under the light microscope during TAA treatment occur always in hepatic cells characterized by considerably enlarged nuclei and correspond to cytoplasmic invaginations into the nucleus. The "inclusions" are variable in structure and their arisal seems to be associated with an increased synthetic activity of the hepatic cells and thus their presence may be regarded as an ultrastructural indicator of this function.

*

The author would like to thank Miss M. STARK for technical help and Mr. L. BENEDEK for the photography.

REFERENCES

1. ALTMANN, H.-W.: (1952) Über den Funktionsformwechsel des Kernes im exokrinen Gewebe des Pankreas. Z. Krebsforsch. **58,** 632. — 2. ALTMANN, H.-W., HAUBRICH, J.: (1964) Zur Genese hepatozellulärer Kerneinschlüße bei Colchicin-Vergiftung. Experientia (Basel) **20,** 319. — 3. ALTMANN, H.-W., OSTERLAND, U.: (1961) Über zytoplasmatische Wirbelbildungen in den Leberzellen der Ratte bei chronischer Thioacetamid Vergiftung. Beitr. path. Anat. **124,** 1. — 4. AMBROSE, A. M., DEEDS, F., RATHER, L. J.: (1950) Further Studies on Toxicity of Thioacetamide in Rats. Proc. Soc. exp. Biol. (N.Y.) **74,** 132. — 5. BALÓ, J.: (1961) A részletes kórbonctan tankönyve. Medicina, Budapest. — 6. BRACHET, J.: (1959) Ribonukleinsäure und Proteinsynthese. In Graumann—Neumann: Handbuch der Histochemie. Fischer, Stuttgart. — 7. BRODEHL, J.: (1961) Thioacetamid in der experimentellen Leberforschung. Klin. Wschr. **39,** 956. — 8. CASPERSSON, T.: (1950) Cell Growth and Cell Function. A Cytological Study. Norton et Co., New York. — 9. CHILDS, J. F. L., SIEGLER, E. A.: (1945) Compounds for Control of Orange Decay. Science **102,** 68. — 10. COSSEL, L.: (1961) Elektronenmikroskopische Befunde an den Leberepithelzellen bei Virushepatitis. Acta hepato-splenol. (Stuttg.) **8,** 333. — 11. COSSEL, L.: (1962) Elektronenmikroskopische Befunde an "Einschlußkörpern" in den Kernen menschlicher Leberepithelzellen. Z. Zellforsch. **57,** 512. — 12. DAVID, H.: (1964) Submikroskopische Ortho- und Pathomorphologie der Leber. Akademie-Verlag, Berlin. — 13. FITZHUGH, O. A., NELSON, A. A.: (1948) Liver Tumours in Rats Fed Thiourea and Thioacetamide. Science **108,** 626. — 14. GEORGII, A., MEHNERT, H.: (1961) Über Leberveränderungen durch Thioacetamid bei gleichzeitiger Belastung mit blutzuckersenkenden Substanzen der Biguanidreihe. Beitr. path. Anat. **124,** 278. — 15. GRANT, H. C., REES, K. R.: (1958) The Precancerous Liver; Correlations of Histological and Biochemical Changes in Rats during Prolonged Administration of Thioacetamide and "Butter Yellow". Proc. roy. Soc. B. **148,** 117. — 16. GRISHAM, J. W.: (1960) Electronmicroscope Study of Carcinoma of the Liver in Ethionine-fed Rats. Gastroenterology **38,** 792. — 17. GUPTA, D. N.: (1956) Acute Changes in the Liver after Administration of Thioacetamide. J. Path. Bact. **72,** 183. — 18. GUPTA, D. N.: (1956) Nodular Cirrhosis and Metastasing Tumours Produced in the Liver of Rats by Prolonged Feeding with Thioacetamide. J. Path. Bact. **72,** 415. — 19. GRUNDMANN, H.: (1964) Allgemeine Cytologie. Thieme, Stuttgart. — 20. KARNOVSKY, M. J.: (1961) Simple Method for "Staining with Lead" at High pH in Electron Microscopy. J. biophys. biochem. Cytol. **11,** 729. — 21. KENDREY, G.: (1964) Über das Verhalten der Nukleolen der Leberzellen bei Thioacetamid-Vergiftung. Acta morph. Acad. Sci. hung. **12,** 407. — 22. KENDREY, G.: (1964) Über das Verhalten und Schicksal der Nukleolen von Leberzellen bei Thioazetamid-Vergiftung. Naturwissenschaften **51,** 42. — 23. KENDREY, G.: (1964) A thioacetamidnak a nukleinsav anyagcserére kifejtett hatásáról. MTA V. Orv. Tud. Oszt. Közl. **15,** 75. — 24. KENDREY, G., NÉMETH, K.: (1965) Über die Histogenese der durch Thioacetamid induzierten Lebercarcinome. Arch. Geschwulstforsch. **26,** 1. — 25. KLEINFELD, R. G.: (1957) Early Changes in the Rat

Liver and Kidney Cells Induced by Thioacetamide. Cancer Res. **17,** 954. — 26. KLEINFELD, R. G., KOULISH, S.: (1957) Cytological Aspects of Mouse and Rat Liver Containing Nuclear Inclusions. Anat. Rec. **128,** 433. — 27. KLEINFELD, R. G., HAAM, E. v.: (1959) Effect of Thioacetamide on Rat Liver Regeneration. II. Nuclear RNA in Mitosis. J. biophys. biochem. Cytol. **6,** 393. — 28. LAIRD, A. K.: (1953) Nuclear Changes Produced in Rat Liver Cells by Thioacetamide. Arch. Biochem. **46,** 119. — 29. LEDUC, E. H., WILSON, J. W.: (1959) An Electron Microscope Study of Intranuclear Inclusions in Mouse Liver and Hepatoma. J. biophys. biochem. Cytol. **6,** 427. — 30. MILLONIG, G.: (1962) Further Observations on a Phosphate Buffer for Osmium Solutions in Fixation. Fifth International Congress for Electronmicroscopy. Philadelphia. Academic Press, New York, V. 2. p. 8. — 31. NICOLAU, S.: (1946) A járványos sárgaság. — Icterus epidemica. Sárgasággal járó, ultravírus okozta járványos májgyulladás. Orv. Lap. **2,** 577. — 32. OEHLERT, W., SCHULTZE, B.: (1960) Die Kerngröße als Ausdruck der synthetischen Aktivität des Kernes. Beitr. path. Anat. **123,** 101. — 33. RATHER, L. J.: (1951) Experimental Alteration of Nuclear and Cytoplasmic Components of the Liver Cell with Thioacetamide. Bull. John Hopkins Hosp. **88,** 38. — 34. REYNOLS, E. S.: (1963) The Use of Lead Citrate at High pH as an Electron Opaque Stain in Electron Microscopy. J. Cell Biol. **17,** 208. — 35. RONDEZ, R.: (1964) Zur Vermehrung von Kernvolumen und RNS-Gehalt bei chronischer, toxischbedingter Leberzellschädigung. Path. et Microbiol. (Basel) **27,** 429. — 36. RONDEZ, R., RÜTTNER, J. R., VOGEL, A.: (1962) Zytologische Untersuchungen an der Thioacetamid-geschädigten Rattenleber. Path. et Microbiol. (Basel) **25,** 13. — 37. RÜTTNER, J. R., RONDEZ, R.: (1960) Zur formalen Genese der TAA-Zirrhose der Ratte. Path. et Microbiol. (Basel) **23,** 113. — 38. RÜTTNER, J. R., RONDEZ, R., GASSMANN, R.: (1959) Histochemische Untersuchungen an der toxisch geschädigten Rattenleber. Schweiz. Z. allg. Path. **22,** 294. — 39. SACHS, H. W., BÄUMER, A., MENKHANS, G.: (1959) Histochemische Untersuchungen zur Giftwirkung des Thioacetamid auf die Rattenleber. Acta hepato-splenol. (Stuttg.) **6,** 286. — 40. SALOMON, J. C., SALOMON, M., BERNHARD, W.: (1962) Modifications des cellules du parenchyme hépatique du rat sous l'effet de la thioacétamide. Étude au microscope électronique des lésions précoces au cours d'une intoxication chronique. Bull. Ass. franç. Cancer **49,** 139. — 41. SCHEUER, P. J.: (1963) Histochemical Changes in Rat Liver in Senecio and Thioacetamide Poisoning. J. Path. Bact. **85,** 507. — 42. STÖCKER, E.: (1963) Die Größe des Nukleolus als Maß seiner synthetischen Aktivität. (Autoradiographische Untersuchungen mit H^3-Cytidin und H^3-Phenylalanin.) Naturwissenschaften **50,** 44. — 43. THOENES, W.: (1964) Fett im Nucleolus. J. Ultrastruct. Res. **10,** 194. — 44. THOENES, W., BANNASCH, P.: (1962) Elektronen- und lichtmikroskopische Untersuchungen am Cytoplasma der Leberzellen nach akuter und chronischer Thioacetamid-Vergiftung. Virchows Arch. path. Anat. **335,** 556. — 45. WESSEL, W.: (1958) Elektronenmikroskopische Untersuchungen von intranukleären Einschlußkörpern. Virchows Arch. path. Anat. **331,** 314.

STRUKTUR UND BEDEUTUNG INTRANUKLEÄRER INKLUSIONEN BEI THIOAZETAMID-HEPATOKARZINOGENESE

G. KENDREY

Auf einer Standarddiät gehaltene weiße männliche Ratten erhielten längere Zeit hindurch Thioazetamidgaben. Nach drei Wochen zeigten sich lichtmikroskopisch in den Kernen der Leberzellen zahlreiche pyroninophile, von einer Membran umgebene Einschlüsse. Elektronenmikroskopisch waren diese Einschlüsse von einer porösen Doppelmembran umhüllt. Außer Mitochondrien waren in den Einschlüssen alle Arten von zytoplasmatischen Organellen vertreten, in Mengen und in einer Zusammensetzung, wie sie sonst im benachbarten Zytoplasma der Leberzellen nicht vorkommen. Diese im Lichtmikroskop als intranukleäre Einschlüsse imponierenden Gebilde entsprachen in die Nuklei invaginierten Zytoplasmaelementen. Einige dieser Elemente hatten sich zu echten intranukleären Einschlußkörperchen umgewandelt, andere zeigten regressive Veränderungen. Es wird angenommen, daß die intranukleären Inklusionen, die im Laufe der Thioacetamid-Hepatokarzinogenese häufig angetroffen werden, Indikatoren einer erhöhten synthetischen Aktivität der Leberzellen sind.

СТРУКТУРА И ЗНАЧЕНИЕ ВНУТРИЯДЕРНЫХ ВКЛЮЧЕНИЙ ПРИ ГЕПАТОКАРЦИНОГЕНЕЗЕ, ВЫЗВАННОМ ДАЧЕЙ ТИОАЦЕТАМИДА

Г. КЕНДРЕИ

Автор давал белым крысам самцам, содержанным на стандартной диете, длительное время тиоацетамид. По истечении 3 недель под световым микроскопом наблюдались в ядрах печеночных клеток многочисленные пиронинофильные включения, окруженные мембраной. При электронномикроскопическом исследовании выяснилось, что эти включения покрыты пористой двойной мембраной. В этих включениях представлены, за исключением митохондриев, все виды цитоплазматических органелл, в количестве и составе, не встречаемых в цитоплазме клеток печени. Эти тельца, которые под световым микроскопом имеют вид внутриядерных включений, на самом деле являются элементами цитоплазмы, попадавшими в ядра путем инвагинации. Некоторые из них преобразовались в истинные внутриядерные включения, другие же показали признаки регрессии. Полагается, что эти «внутриядерные» включения, часто наблюдаемые при карциногенезе, вызванном тиоацетамидом, являются индикаторами повышенной синтезирующей активности печеночных клеток.

Dr. Gábor KENDREY; I. Kórbonctani Intézet, Budapest VIII. Üllői út 26. Hungary

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 65—83, 1968*

Department of Pathology (Head: Prof. K. LAPIS),
Postgraduate Medical School, Budapest

VINCALEUKOBLASTINE-INDUCED CHANGES IN THE ULTRASTRUCTURE OF ASCITES TUMOUR CELLS*

K. LAPIS

Ultrastructural changes caused in NK/Ly ascites lymphoma cells *in vivo* by vincaleukoblastine (VR^{-8}) treatment have been studied. Accumulation of membrane-bounded, electron-dense, polymorphous, lysosome-like structures was observed in a number of cells in interphase. Ultrastructural features of the nuclear changes induced by VR^{-8} (mosaic-like pattern as observed by KELLNER with light-microscope), were revealed. The ultrastructural features of the cells dividing abnormally upon the effect of the agent have been described. Oriented spindle tubules were lacking from cells arrested in mitosis. While no changes were registered in the ultrastructure of the centrioles, the treatment interfered with their orientation and migration to the poles. Centriole replication seemed to be undisturbed, nor did VR^{-8} cause ultrastructural changes in the kinetochores. Gelation in connection with the formation of the mitotic apparatus was disturbed, a phenomenon substantiated by the fact that mitochondria and other cell organelles were found in the area which under conditions of normal cell division is occupied by the mitotic apparatus and contains no other organelles. In cells arrested in mitosis conglutinated clusters of chromosomes, accumulation of layered concentric membranes were observed, whereas in a number of the multinuclear cells quadrilamellar membranes appeared. These membranes are considered to point to a disturbed reconstruction of the nuclear membrane. All these phenomena were identical with those induced *in vitro* by vicristine in HeLa cells as observed by GEORGE *et al.* The ultrastructural features of multinuclear cells arising on VR^{-8} treatment, are discussed, and it is pointed out that the organelles were well preserved in the majority of these cells.

There are numerous reports dealing with the effect of vincaleukoblastine, a dimer alkaloid isolated from *Vinca rosea* LINN. [39, 84, 85], on peripheral blood counts, bone marrow [13, 22, 91] and various animal and human tumours [1, 2, 19, 20, 21, 43, 45, 50, 51, 103, 110]; and also with comparative examination of different Vinca alkaloids [49, 86]. Their cytological effect was studied by light-microscopy [13, 20, 37, 51, 93, 101, 102, 106, 109] and it was found that vincaleukoblastine arrests the division of proliferating tumour cells at the metaphasic stage, and — depending on the dosage — gives rise to different forms of abnormal cell division. Cytological examinations showed the effect to be similar to that of colchicine and its derivatives [13, 26, 29].

Vincaleukoblastine, isolated in Hungary by JOVANOVICS *et al.* [53] under the name VR^{-8}, was biologically tested in the Oncopathological Research Institute, Budapest [33, 34, 35, 36, 57, 69, 87, 88, 89]. It was in the course of these investigations that the present author studied changes in the ultrastructure

* The author is indebted for grants from the Hungarian Scientific Health Council, the Jane Coffin Child's Memorial Fund for Medical Research, and the Anna Fuller Fund.

of ascites tumour cells elicited by the drug at issue [68]; findings made in this respect and confirmed by three series of experiments are described in the following.

Material and method

C3 fawn-coloured or Swiss white mice of both sexes and approximately equal body weight, of our laboratory stock, were inoculated intraperitoneally with 3×10^7 NK/Ly ascites lymphoma cells. The animals were divided into groups and each group received a certain dose (0.1, 1.0 and 3.0 mg/kg respectively) of VR^{-8} i.p. on the 6th or 7th day following inoculation. The dose of 1 mg/kg having been found to be the most suitable, it was administered in subsequent experiments.

Peritoneal fluid was aspirated at different intervals (1, 3, 6, 9, 12, 24, 48, 72, 96, 120 and 144 hours), and aliquots of 0.3 to 0.4 ml were fixed at $+4$ °C for one hour in a 2% solution of osmium tetroxide buffered to pH 7.4 by veronal acetate, according to PALADE [92]. After slow centrifugation, the substances were dehydrated in an alcohol series and then embedded in araldite.

For the preservation and demonstration of spindle fibers, the samples were fixed in a 2.5% solution of glutaraldehyde in Hanks' solution [38].

Sections made with the aid of Porter Blum and LKB ultramicrotomes, were studied by Hitachi HU—10, SEM—3, and JEM 6—c electron microscopes.

Since in our experience the study of chemotherapeutically induced ultrastructural alterations requires familiarity with the light-microscopic changes, the samples were submitted to parallel light- and electron-microscopic examinations.

Electron-microscopic observations

The ultrastructural features of untreated NK/Ly ascites lymphoma cells have repeatedly been described [58, 63, 65, 66, 68, 70], and only the more important ultrastructural properties of cells in metaphase will now be discussed. The electron-dense osmophilic chromosomes in the centre of the cells are situated at the equatorial plane at right angles to the mitotic spindle. Only one of the centrioles at the cell pole can be seen in the plane of section in Fig. 1. Broom-like spreading out spindle tubules are running partly to the opposite cell pole and partly to the chromosomes. Mitochondria and other cell organelles are found at the periphery of the cytoplasm but not in the area of the mitotic spindle. The spindle filaments are in no direct contact with the centriole at the cell pole; the converging spindle filaments meet at some distance from this area. On the other hand, it is by means of kinetochores or centromeres that the terminal portion of chromosomal fibres of the spindle reach and penetrate the body of the osmiophilic electron-dense chromosomes.

One to 6 hours after VR^{-8} treatment the majority of tumour cells in interphase showed neither light-microscopic nor ultramicroscopic changes. Certain proportion of the interphase-cells, however, contained spherical or oval, lysosome-like structures encircled by a single membrane; these structures were smaller than the mitochondria, and formed clusters of 8 to 10 members

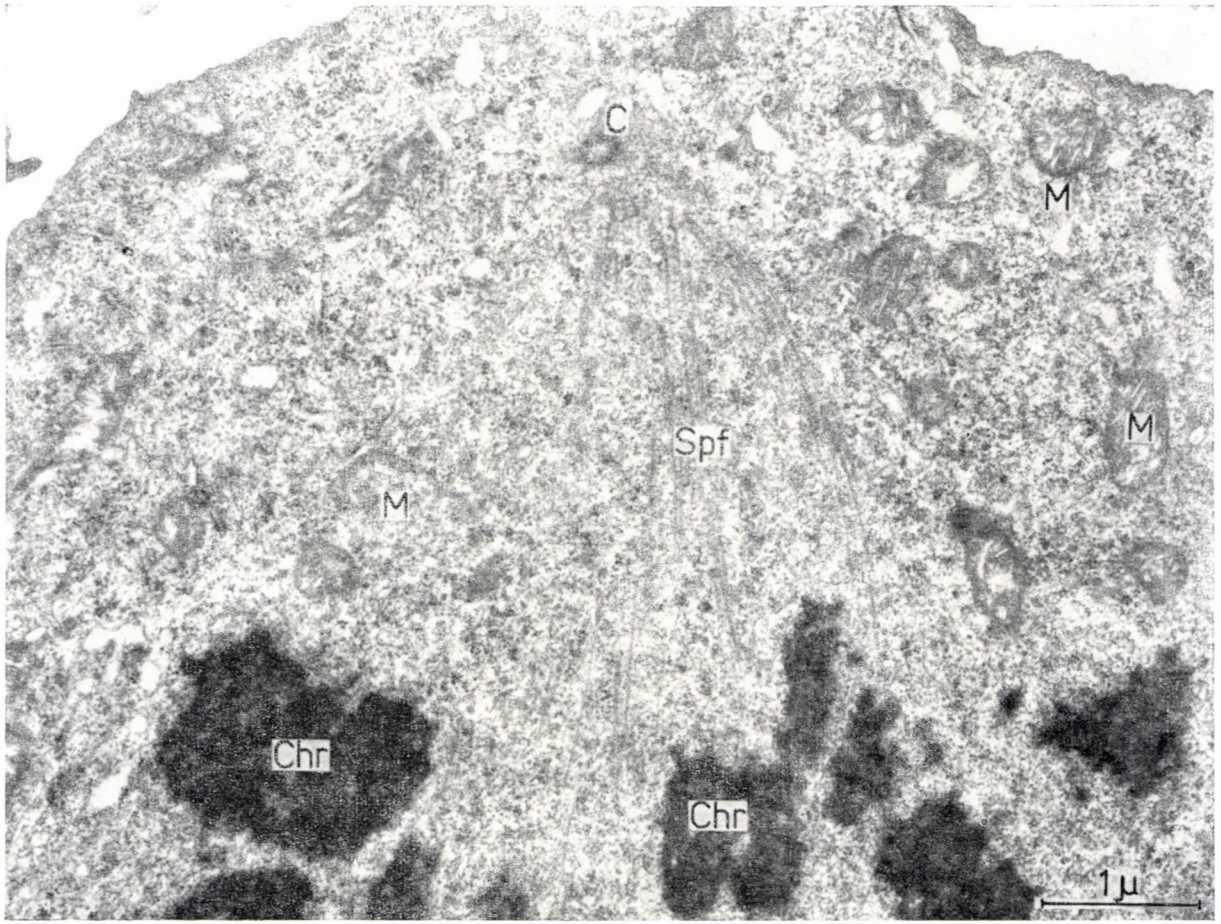
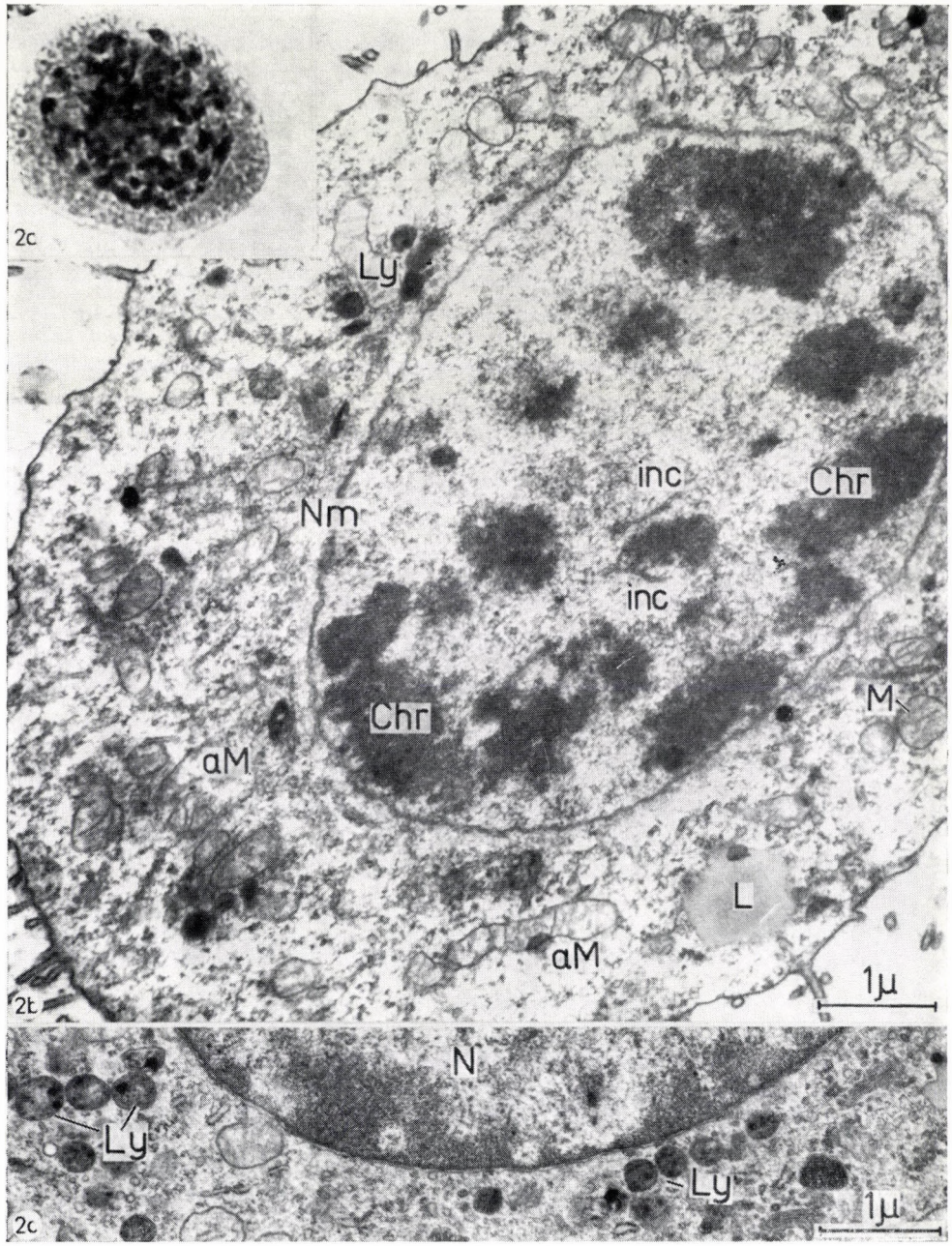


Fig. 1. Detail of dividing cell from untreated four-day old NK/Ly ascites tumour. The cell is in the metaphase; the electron-dense chromosomes are situated in the equatorial plane. Note centriole at cell pole (C). Broom-like spreading out spindle tubules (Spf) of smooth surface run from the pole partly to the chromosomes and partly to the opposite pole. Fixation in 2.5% glutaraldehyde in buffered Hanks' solution; postfixation in osmium tetroxide. Stained with uranyl acetate and lead citrate, embedded in araldite. ×29, 250


2c
Ly
Nm
inc
Chr
inc
Chr
M
aM
L
aM
1μ
2b
N
Ly
Ly
1μ
2c

(Fig. 2c). In the same cells an aggregation of free ribosomes was observed. KELLNER *et al.* [57] observed after Vinca treatment that some of the cells, while still retaining the nuclear membrane, showed a mosaic-like clumping of the chromatin (Fig. 2a). The present electron-microscopic investigations have confirmed their light-microscopic observation (Fig. 2b). While the nuclear envelope was preserved the chromatin substance of these cells took the form of electron-dense, finely granular chromosomes in the karyoplasm. The karyoplasm itself became fairly electron lucent. The chromosomes differed in size and length and displayed bulky or elongated shapes; their various aspects depended to some extent on the plane of section. The preserved nuclear envelope was undulatory and rippled. Part of the chromosomes was arranged along the nuclear membrane. Longer and shorter segments of narrow tubular structures, bounded by smooth membranes, were found in the karyoplasm near or in close contact with the chromosomes. Aggregation of ribosomes as well as accumulation and clustering of lysosome-like structures were registered also in these cells. Besides, the electron-density of the matrix of mitochondria appeared to be diminished and the cristae partly destroyed. In addition, the pores of the nuclear envelope were considerably dilated and in some cells the membrane was partially lysed (Fig. 2b).

In accordance with the light-microscopic observations the electron-microscopic preparations showed a large number of abnormal cell division 3 to 12 hours after the treatment (Figs 3a, 3c). No signs of polarization were observed in connection with cell division. No mitotic spindle or oriented spindle filaments were seen in the abnormally dividing cells, and not even structures that might have been recognized as disoriented spindle tubules. In some cells there were occasional longer or shorter tubular structures bounded by smooth membranes; these could be regarded as remnants of disoriented spindle tubules. The chromosomes of these cells never formed configurations in the equatorial

Fig. 2a. NK/Ly ascites lymphoma cell 10 hours after intraperitoneal administration of 3 mg/kg of vincaleukoblastine. Note mosaic pattern of nuclear structure. Stained with acetic orcein. × 1690

Fig. 2b. Electron-microscopic picture of an ascites cell, similar to that shown on Fig. 2a. Segregated electron-dense chromosomes are well distinguishable in the nuclear substance (Chr). Several intranuclear canaliculi of smooth-surfaced membranes (inc), can be seen in the karyoplasm of reduced electron density. The nuclear membrane (Nm) is well preserved and undulating. The cytoplasm contains unevenly distributed ribosomes, damaged mitochondria of various shapes and sizes (aM), occasional lipid droplets (L) and lysosome-like structures of high electron density (Ly). The microvilli on the cell surface are well preserved. An NK/Ly ascites lymphoma cell 3 hours after administration of 1 mg/kg of vincaleukoblastine. Fixed in osmium tetroxide, embedded in araldite. Stain: uranylacetate and lead citrate. × 21,000

Fig. 2c. Detail of cell from NK/Ly ascites tumour 6 hours after treatment with 1 mg/kg of vincaleukoblastine, fixed in osmium tetroxide, embedded in araldite. The cell is in interphase, its cytoplasm shows, next to the nucleus (N), aggregated round or oval lysosome-like structure (Ly) most of which, bounded by unit membranes, are smaller than the mitochondria. Stain: uranylacetate and lead citrate. × 23,000

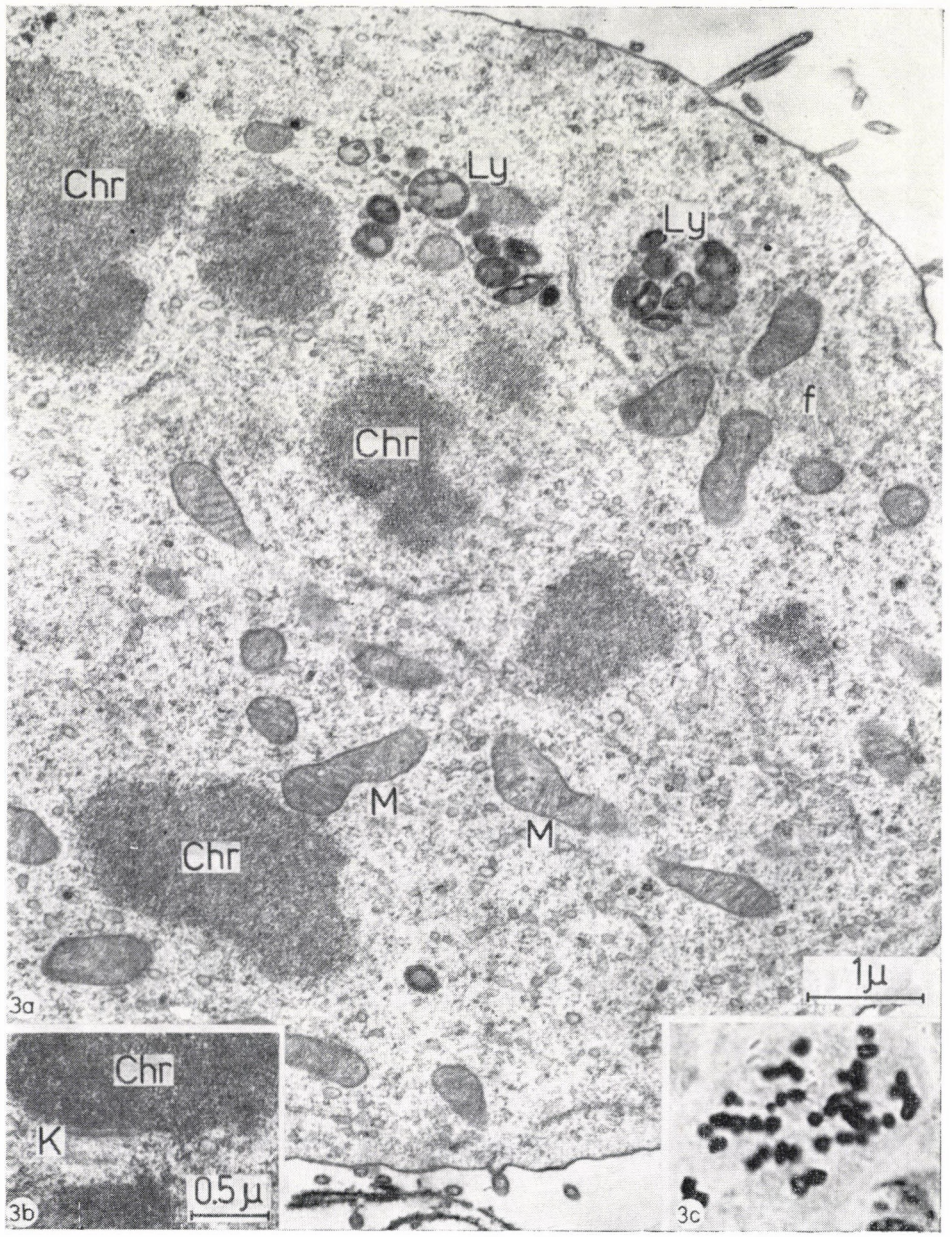
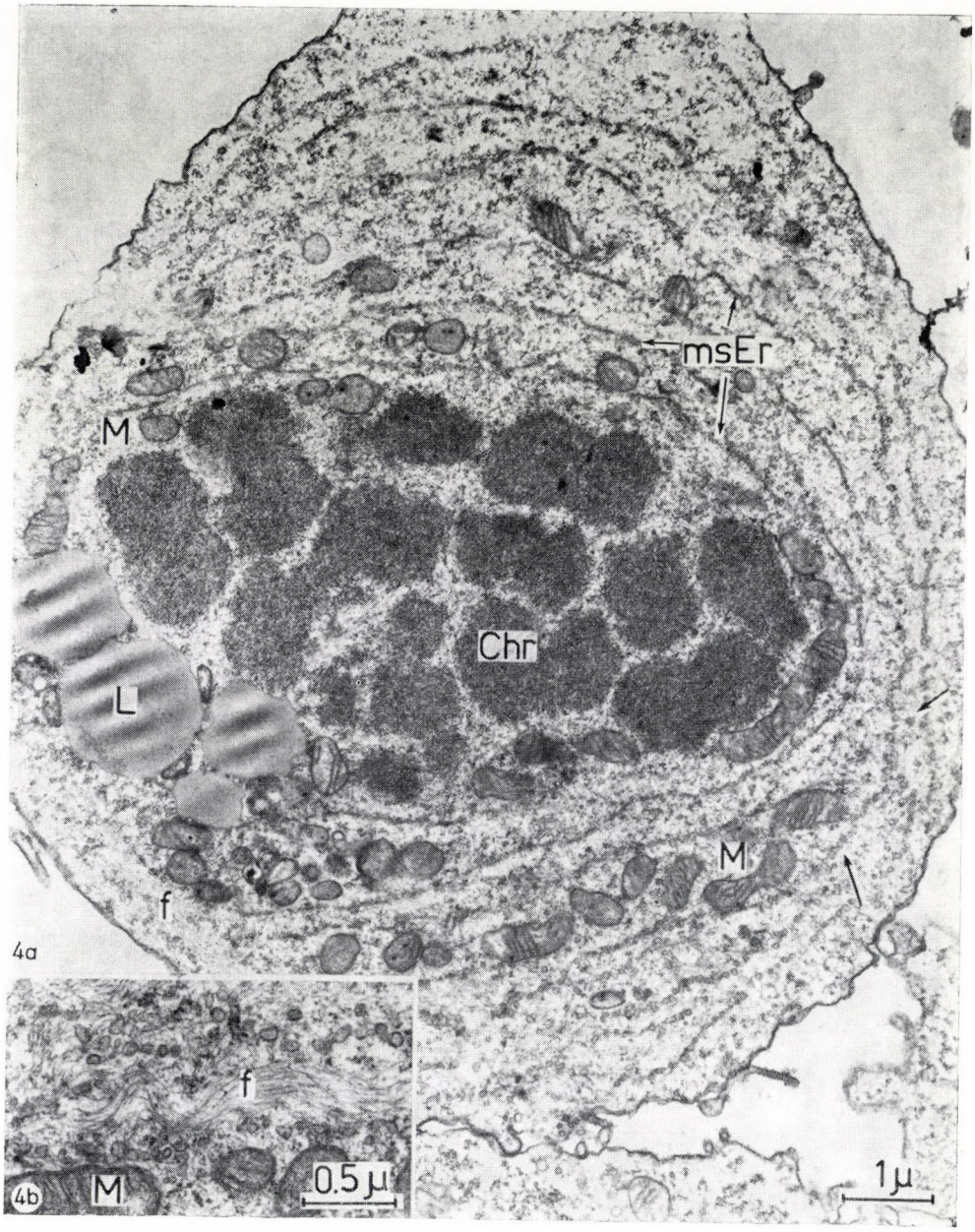

Chr
Ly
Ly
f
Chr
M
M
Chr
1μ
3a
Chr
K
0.5μ
3b
3c

plate characteristic of normal cells in metaphase (Figs 3a, 3c). They were either irregularly scattered over the cytoplasm, or formed highly electron-dense bundles with convoluted contours, while in some cells they appeared as centrally situated highly electron-dense amorphous masses. Centromeres, kinetochores were adhering to the surface of certain chromosomes (Fig. 3b). They appeared in the form of slightly curved electron-dense plates embedded in less osmiophilic granular matrices. Their shape varied according to the plane of section. No tubular structures that might have qualified as remnants of spindle fibres connected with kinetochores, were observed after vincaleukoblastine treatment. Sometimes occasional centrioles could be seen between the chromosomes in the plane of section (Fig. 3a); they were situated at the periphery of the cell or paracentrally. The centrioles revealed no structural changes, nor was there any sign to show that they served as centres of organization as they do in normal cell division. While the interchromosomal space and the space between chromosomes and centrioles contain under physiological conditions of cell division no organelles save components of the mitotic spindle, scattered mitochondria and numerous small vesicles, surrounded by smooth-surfaced membranes, were observed in the examined specimens. These serially arranged vesicles frequently formed veritable enclosures around a given chromosome (Fig. 3a). Mitochondria were in some cases near or in contact with the chromosomes. It was conspicuous that, outside the area occupied by the chromosomes, there were peripheral lysosome-like figures of various sizes and shapes, 8 to 10 of which aggregated in some instances to form inclusions (Fig. 3a). Their electron density was variable and different even within one and the same structure. The cytoplasm contained moreover peripheral bundles of delicate undulating filaments (Fig. 4a). Depending on the plane of section, they appeared either as variably shaped fields or as band-like structures.

Fig. 3a. Scattered, disoriented chromosomes (Chr) in dividing NK/Ly ascites cell 9 hours after treatment with 1 mg/kg of vincaleukoblastine and fixed in osmium tetroxide and embedded in araldite. The cytoplasm is rich in ribosomes and contains, among the chromosomes, scattered smooth surfaced vesicles which are arranged either in longitudinal rows or around a chromosome. Variably shaped and sized mitochondria (M) are also visible between the chromosomes. A centriole is likewise visible, while no coherent spindle filaments can be seen. Groups of highly electron-dense lysosome-like structures form inclusions at the periphery. Note cross sections of thin bundles of undulating filaments (f) near the inclusions. Small, peripheral vesicles form chain-like rows along the cell surface (arrow).
Stain: uranyl acetate and lead citrate. ×20,000

Fig. 3b. Detail of chromosome from a cell, the division of which has been arrested by vincaleukoblastine treatment. Note slightly curved kinetochore (K) adhering to the surface of the electron-dense chromosome. The picture shows conditions 6 hours after intraperitoneal administration of 1 mg/kg of vincaleukoblastine.
Fixed in osmium tetroxide, embedded in araldite. Stain: lead citrate. ×30,000

Fig. 3c. NK/Ly ascites lymphoma 9 hours after intraperitoneal administration of 3 mg/kg of vincaleukoblastine. Abnormal cell division, disoriented, scattered chromosomes. Stain: acetic orcein. ×1630


msEr
M
Chr
L
M
f
4a
f
4b
M
0.5 µ
1µ

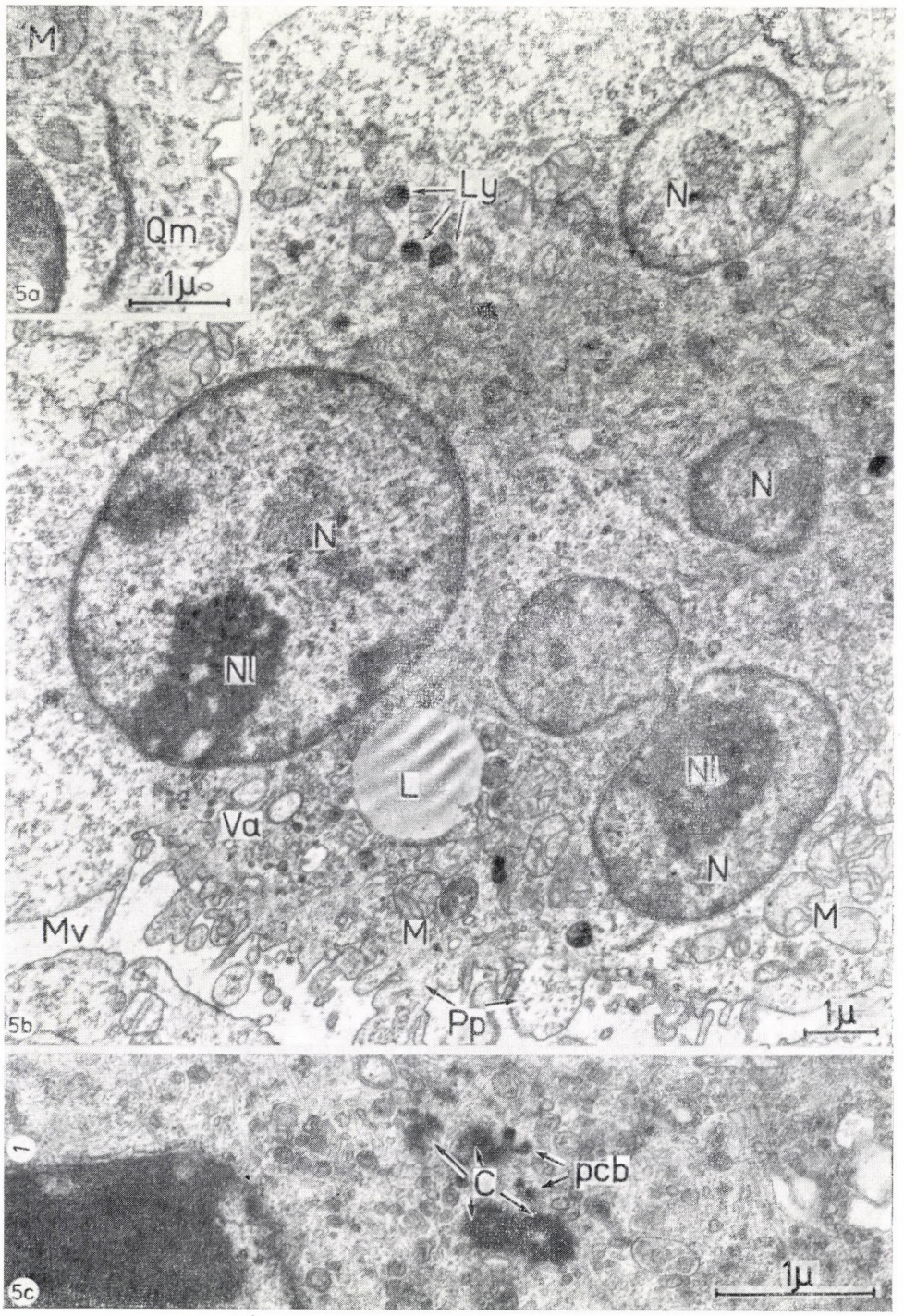
Another frequent type of abnormally dividing cell showed smooth endoplasmic reticular membranes surrounding the group of chromosomes (Fig. 4a) in the form of concentric layers. Apart from the chromosomes, the areas surrounded by the membranes contained varying amounts of mitochondria, lipid droplets and sometimes also polymorphous lysosome-like structures. Outside of, and parallel to, the concentric membranes, rows of vesicles formed chains at the periphery of the cytoplasm. Besides, bundles of filaments running along the cell membrane were observed at the cytoplasmic periphery (Figs 4a, 4b). No organized Golgi apparatus was found in any of the dividing cells.

Between 12 and 72 hours after VR^{-8} treatment no dividing cell beyond the stage of metaphase was observed.

In harmony with literary data [33, 38, 57] one of the most striking phenomenon observed by light-microscopy following VR^{-8} treatment was the appearance of increasing numbers of multinuclear giant cells (Fig. 5b). Their number was considerable at 24th and 48th hour after treatment, and reached a peak between 72 and 96 hours. Many of these giant cells were several times as large as untreated NK/Ly ascites cells. The size of the cells as well as the number and size of their nuclei showed considerable variations. It was characteristic of these cells that some of them contained nuclei of approximately equal, while others of widely different size (macro- and micronuclei). Ultrastructurally, the nuclei were similar to those of the control cells, and some of them contained well-developed nucleoli which, too, had an ultrastructure like those of untreated NK/Ly ascites cells. The internuclear cytoplasm was rich in organelles, especially in smooth-surfaced tubules and vesicles. It was usually in this area that the moderately developed and sometimes disunited Golgi complex was observed. A considerable amount of vesicular or lamellar rough-surfaced endoplasmic reticulum was also found (Fig. 5b). The centrioles were usually situated between the nuclei and displayed a practically normal structure; they were not invariably oriented at right angles. In some multinuclear giant cells four centrioles occurred close to one another in a single plane of section (Fig. 5c). In some cells around the centrioles there were small, highly

Fig. 4a. Dividing NK/Ly ascites tumour cell 6 hours after intraperitoneal administration of 1 mg/kg of vincaleukoblastine. Electron-dense chromosomes aggregated in the centre. They are surrounded by onion-leaf-like superposed layers of smooth-surfaced endoplasmic reticulum membranes (msEr). These enclose, beside the chromosomes, also mitochondria, lipid droplets (L) and lysosome-like particles. Note long row of small vesicles (arrow) running parallel with the membranes and, between them, bundles of undulating filaments.
Fixed in osmium tetroxide, embedded in araldite. Stain: uranyl acetate and lead citrate. ×17,000

Fig. 4b. Detail of abnormally dividing NK/Ly ascites tumour cell 6 hours after intraperitoneal administration of 1 mg/kg of vincaleukoblastine. Bundles of filaments (f) running near the cell surface, as described in Fig. 4a, with adjacent mitochondria.
Fixed in osmium tetroxide, embedded in araldite. Stain: uranyl acetate and lead citrate. ×33,000


M
Qm
1μ
5a
Ly
N
N
N
Nl
L
Va
Mv
M
Nl
N
M
Pp
1μ
5b
1
C
pcb
1μ
5c

electron-dense pericentriolar bodies too. Most giant cells contained also lipid droplets (Fig. 5b). The mitochondria of these cells were fairly polymorphous, of varying size, and the orientation of the crests was completely irregular. The cristae showed pronounced vesiculation in the majority of mitochondria. In addition, highly electron-dense, apparently inhomogeneous lysosome-like structures, surrounded by unit membranes were also seen in the giant cells. The lysosome-like bodies were usually smaller than the mitochondria (Fig. 5b).

Certain multinuclear cells contained longer or shorter quadrilamellar membranes that were usually situated around the nuclei. While the outer membranes were covered with ribosomes, the inner pair of membranes appeared to be smooth. An abundance of differently sized vesicles was irregularly distributed close to the cell surface. The cytoplasm of the multinuclear cells was rich in free ribosomes; the ribosome granules and their aggregates were evenly distributed over the cytoplasm. On the cell surface, a variable number of differently shaped and sized microvilli was observed. Near the inner surface of the cell membrane differently sized pinocytotic vesicles either pinched off the cell membrane or projecting into the cytoplasm were seen.

After the 12th hour following treatment, during the whole period of observation a considerable number of cells revealed signs of degeneration.

Discussion

The advance of knowledge concerning mitotic division in the last 15 years [2, 3, 7, 9, 14, 23, 25, 32, 42, 48, 75, 76, 77, 78, 80, 81, 92, 96, 98, 100] was among others, due to the results of electron-microscopic examinations. It was

Fig. 5a. Detail of multinuclear giant cell. NK/Ly ascites lymphoma 72 hours after intraperitoneal administration of 1 mg/kg of vincaleukoblastine. Note quadrilamellar membrane (Qm) with adjacent mitochondrium (M) in the cytoplasm.
Fixed in osmium tetroxide, embedded in araldite. Stain: uranyl acetate and lead citrate.
×21,000

Fig. 5b. Multinuclear giant cell from NK/Ly ascites lymphoma 72 hours after administration of 1 mg/kg of vincaleukoblastine. Cross sections of several differently sized nuclei can be seen. Ultrastructurally, the nuclei are similar to the controls. Note well developed nucleolus (Nl) in some nuclei. The cytoplasm, rich in ribosomes, contains intact and damaged mitochondria (aM), lysosome-like figures (Ly), vacuole (Va) and numerous minute vesicles. The cell surface shows microvilli (Mv) and bulky pseudopodium-like processes (Pp).
Fixed in osmium tetroxide, embedded in araldite. Stain: uranyl acetate and lead citrate.
×15,000

Fig. 5c. Detail of multinuclear giant cell, from NK/Ly ascites lymphoma 120 hours after administration of 1 mg/kg of vincaleukoblastine. Note several centrioles (C), sectioned in different planes, near one of the nuclei. Electron-dense corpuscles can be distinguished around the centrioles.
Fixed in osmium tetroxide, embedded in araldite. Stain: uranyl acetate and lead citrate.
×33,000

therefore expected that the electron-microscopic study of a vinblastine-treated cell population would shed light on the finer details of the disturbances in spindle formation and cell division. Electron-microscopic examinations were further supposed to reveal whether the treatment would provoke changes in cells at the stage of interphase.

Part of the cells in interphase contained, following treatment with VR^{-8}, accumulations and clusters of lysosome-like polymorphous structures. Their accumulation was especially conspicuous in cells with arrested mitosis. In cases of normal cell division these structures begin to form inclusions in the early prophase, an activity that becomes most pronounced in metaphase [98] so that their early accumulation must have been due to the vincaleukoblastine effect. Early accumulation of such structures following treatment with vincristine, vinblastine and colchicine has been described by other authors as well [38, 39]. The lysosomal nature of the structures in question was proved by demonstrating their acid phosphatase activity. While several workers [38, 98, 99] suggest that they derive from multivesicular bodies, others [83, 98] point to their close connection with the Golgi complex.

KELLNER [57] described a mosaic-like arrangement of the nuclear chromatin in some cells in the interphase. Electron-microscopically, these cells revealed a well preserved undulatory nuclear membrane; the karyoplasm contained segregated chromosomes. A similar behaviour of the nuclear membrane was observed [98] in cells in the normal prophase. The tubular structures observed in the nuclei of these cells were presumably analogous ones to the intranuclear canaliculi described by YASUZUMI and SUGIHARA [113, 114] in Ehrlich ascites tumour cells in the prophase.

Vinca alkaloids being spindle inhibitors [8, 27, 71, 72], we paid particular attention to the behaviour of the various components of the mitotic apparatus following treatment with VR^{-8}. Vincaleukoblastine, like vincristine [38], did not seem to affect the ultrastructure of the centrioles [7, 23, 112], only their orientation and polarization. Neither did it interfere with centriole replication [32], since we frequently observed 3 to 4 centrioles, pericentriolar bodies and satellites in the multinuclear giant cells that had formed under the effect of the drug. Like on treatment with vincristine [38] and colchicine, no organized mitotic spindles developed following the administration of VR^{-8}, and even occasional disoriented remnants of spindle filaments appeared but rarely. The action of spindle poisons may have different mechanisms. They may inhibit the synthesis of proteins required for the development and organization of spindle filaments, while it is likewise possible that they are destroying the existing filaments.

CREASEY and MARKIW [16, 17, 18] demonstrated that colchicine, vincristine and vinblastine inhibit the synthesis of soluble RNA, and suggested that, accordingly, these agents might prevent the production of specific pro-

teins required for mitosis. It has, however, been shown [75, 78, 79, 80, 81] that the mitotic apparatus is assembled from proteins synthesized prior to division and that, after treatment with the drugs at issue, even cells in the normal metaphase, i.e. cells with well developed and well functioning mitotic spindle, are unable to reach the subsequent stages of division without disturbance. It was further found [38] that cells, once division has started, are able to go through the whole process even in the presence of actinomycin-D although this substance is known to inhibit the synthesis of RNA including its soluble form. Considerations of this nature have given rise to the assumption [38] that spindle poisons of the colchicine type disturb or inhibit the organization of the filamentous structures of the mitotic spindle without significantly interfering with the synthesis of the specific components required for the assembly of the mitotic apparatus.

In contrast to that of the mitotic spindles, the ultrastructure of the centromeres or kinetochores which ensure the attachment of the spindle filaments to the chromosomes, suffered no change on treatment with vincristine or vincaleukoblastine.

The present investigations have shown that treatment with VR^{-8} (like that with colchicine and vincristine) affects the gelation process connected with mitosis.

It seems that on VR^{-8} treatment the nuclear membrane is decomposed in the course of cell division in the same manner as in the case of normal mitosis [30, 42, 82, 98, 100]. In cases of normal cell division, the nuclear membrane originates from the endoplasmic reticulum formed by a fusion of the cytoplasmic vesicles [42, 98, 111]. We regard the rows of vesicles and the concentric endoplasmic reticular membranes, observed in VR^{-8} treated cells, as the precursors or the early stage of the regeneration of the nuclear envelope. A layered accumulation of such concentric, continuous membranes has been observed in normally dividing cells only in exceptional cases, e.g. in the spermatogenic cells of Drosophila [48] and tumour cells [12]. On the other hand, structures of this nature have been observed in HeLa cell cultures treated with vincristine [38]. It would appear that Vinca alkaloids interfere in some way with the reconstruction of the nuclear envelope both *in vivo* and *in vitro*. The fact that quadrilamellar membranes running parallel to the nuclear membrane have been observed in certain multinuclear giant cells formed under the effect of VR^{-8}, points likewise to a disturbance in nuclear membrane regeneration. Similar formations have been noted in Walker-256 carcinoma cells and in daughter cells arising in the course of thymocyte division [12, 82]; we encountered such membranes [66] in cases of abnormal cell division under the effect of an alkylating agent. In view of the submicroscopic structure of these figures, we share the view of BUCK [12] who regards them as preformed nuclear membranes.

No organized Golgi apparatus was observed in cells dividing abnormally under the effect of VR^{-8}. This apparatus is known to disappear in the course of normal cell division [25, 98], and it is possible that some of the numerous vesicles observed in dividing cells originated from Golgi elements. VR^{-8} caused no significant change in the other cell organelles.

The number of cells in the ascites tumours decreased to one quarter of the original count 24 hours after treatment with VR^{-8} [33], while — according to light-microscopic observations — the remaining cells undergo rapid degeneration [35]. Electron-microscopic studies [68] have confirmed this phenomenon: we saw a considerable number of degenerating cells 3 to 12 hours after the treatment; it may have been connected with the inhibition of RNA synthesis, since vincaleukoblastine arrests RNA synthesis shortly before the beginning of cell division [16, 17, 18, 27, 96].

There are many reports to show that multinuclear giant cells arise on treatment with mustard nitrogen derivatives [5, 6, 41, 47, 54, 55, 60, 61, 62, 70] and several other chemotherapeutic agents [14, 15, 28, 40, 52, 108] as well as after X-ray irradiation [9, 61]. Production of multinuclear cells, as a result of such treatment, has been observed not only in tumours but in normal tissues [2, 4, 5, 14, 28, 59, 108] and tissue cultures as well [52, 97]. The genesis of these cells is a complex one [11, 55, 73, 74], since cell fusion, nuclear fragmentation, abortive mitosis and amitotic division may be equally involved in their formation [11, 94, 105].

The principal factors in the origin of multinuclear cells arising after VR^{-8} treatment, is presumably abortive mitosis. In the course of abnormal cell divisions caused by VR^{-8}, several nuclei may develop from the groups of chromosomes scattered throughout the cytoplasm. This is supported by the fact that nuclei and nucleoli of different size have been found in the multinuclear cells arising under the effect of VR^{-8}. Formation of micronucleoli has been reported in connection with other treatments as well [104].

Studies of the ultrastructural changes caused by various cytostatic agents [10, 64, 65, 66, 67, 68, 69, 70] revealed differences in the ultrastructure of multinuclear giant cells arising under the effect of the agents.

Opinions regarding the biological significance of multinuclear cells are divided [11, 55, 56, 95]. Morphological studies did not allow to estimate their biological value. Cellular degeneration and destruction have both light- and electron-microscopically well observable morphological criteria nevertheless. We have found that, although most of the multinuclear cells formed under VR^{-8} treatment contain a great number of lysosome-like structures, neither the cytoplasmic organelles, nor the nucleus and the nucleolus showed signs pointing to imminent degeneration. It should, of course, be borne in mind that morphological preparations reveal but the momentary situation and do not shed light on processes regarding the life cycle of cells.

REFERENCES

1. Armstrong, J. G., Dyke, R. W.: (1961) Hodgkin's Disease and other Tumours Treated with Vinblastine (vincaleukoblastine) Sulfate. Proc. Amer. Ass. Cancer Res. **3,** 206. — 2. Armstrong, J. G., Gahimer, J. E.: (1962) Hodgkin's Disease, Carcinoma of the Breast and other Tumors Treated with Vinblastine Sulfate. Cancer Chemother. Abstr. **18,** 49—71. — 3. Barer, R., Joseph, S., Meek, G. A.: (1961) Membrane Interrelationships during Meiosis. In: Electron Microscopy in Anatomy. J. D. Boyd, F. R. Johnson and J. D. Lever, eds. Williams and Wilkins, Baltimore Pp. 160. — 4. Baserga, A., Marinone, G.: (1949) Megamyeloid Cells and Aminopterin. Lancet, **2,** 1056. — 5. Baserga, A., Marinone, G.: (1952) Contributo alla conoscenza di alcune sostanze citostatiche sulle cellule mieloidi. Genesi e significato delle "cellule megamieloidi". Haematologica **36,** 717—733. — 6. Baserga, A., Marinone, G., Castellotti, P. E.: (1948) Sulle modificazioni delle cellule midollari de azotoiprite. Haematologica **33,** 229—235. — 7. Bernhard, W., De Harven, E.: (1960) L'ultrastructure du centriole et d'autres éléments de l'appareil achromatique. In: Verh. IV. Int. Kongress f. Elektronmikroskopie. Vol. 2. p. 217—227. Springer, Berlin. — 8. Biesele, J. J.: (1958) The Poisoning of Metaphase and Later Stages. In: Mitotic Poisons and the Cancer Problem. Elsevier, New York. Chapter 5. Pp. 108. — 9. Bloom, W.: (1948) Histopathology of Irradiation from External and Internal Sources. McGraw-Hill, New York. — 10. Börzsönyi, M., Lapis, K., Varga, L., Várterész, V.: (1967) Autoradiographische Untersuchung der Wirkung des Cytostaticums 1,4-Bis-(2'-mesyloxy-äthylamino)-1,4-didesoxy-mesoerythritol auf den Desoxy-ribonucleinsäure-Stoffwechsel in NK/Ly-Ascitestumorzellen. Arzneimittel-Forsch. **17,** 1596—1598. — 11. Bucher, O.: (1959) Die Amitose der tierischen und menschlichen Zelle. In: Protoplasmatologia. Handbuch der Protoplasma-Forschung. Band VI/E/1. Pp. 1—159. Springer, Wien. — 12. Buck, R. C.: (1961) Lamellae in the Spindle of Mitotic Cells of Walker 256 Carcinoma. J. biophys. biochem. Cytol. **11,** 227—236. — 13. Cardinali, G., Cardinali, G., Blair, J.: (1961) The Stathmokinetic Effect of Vincaleukoblastine of Normal Bone Marrow and Leukemic Cells. Cancer Res. **21,** 1542—1544. — 14. Cardinali, G., Piscitelli, N.: (1954) Le cellule megamieloidi nella intossicazione sperimentale de trietilenmelamina e N-ossido della metil-bis-beta-cloroetil amina. Boll. Oncol. Suppl. **1,** 635—642. — 15. Castoldi, G. L., Malacerne, P.: (1964) Aspects cytochimiques et cytogénétiques du gigantisme cellulaire provoqué par les antimitotiques. Nouv. Rev. franç. Hémat. **4/3,** 395—408. — 16. Creasey, W. A., Markiw, M. E.: (1964) Biochemical Effects of the Vinca Alkaloids. I. Effects of Vinblastine on Nucleic Acid Synthesis in Mouse Tumour Cells. Biochem. Pharmacol. **13,** 135—142. — 17. Creasey, W. A., Markiw, M. E.: (1964) Biochemical Effects of the Vinca Alkaloids. II. A Comparison of the Effects of Colchicine, Vinblastine and Vincristine on the Synthesis of Ribonucleic Acids in Ehrlich Ascites Carcinoma Cells. Biochim. biophys. Acta (Amst.) **87,** 601—609. — 18. Creasey, W. A., Markiw, M. E.: (1965) Biochemical Effects of the Vinca Alkaloids. III. The Synthesis of Ribonucleic Acid and the Incorporation of Amino Acids in Ehrlich Ascites Cells in vitro. Biochim. biophys. Acta (Amst.) **103,** 635—645. — 19. Cutts, J. H.: (1958) Biological Fffects of Extracts of Vinca rosea. Proc. Amer. Ass. Cancer Res. **2,** 289. — 20. Cutts, J. H.: (1961) The Effect of Vincaleukoblastine on Dividing Cells in vivo. Cancer Res. **21,** 168—172. — 21. Cutts, J. H., Beer, C. T., Noble, R. L.: (1960) Biological Properties of Vincaleukoblastine, an Alkaloid in Vinca rosea Linn. with Reference to its Antitumour Actions. Cancer Res. **20,** 1023—1031. — 22. Cutts, J. H., Beer, C. T., Noble, R. L.: (1957) Effects on Haematopoiesis in Rats of Extracts on Vinca rosea. Rev. canad. Biol. **16,** 487. — 23. De Harven, E., Bernhard, W.: (1956) Étude au microscope électronique de l'ultrastructure du centriole chez les Vertébrés. Z. Zellforsch. **45,** 378—398. — 24. De Harven, E., Dustin, P. Jr.: (1960) Étude au microscope électronique de la stathmocinèse chez le rat. In: Action antimitotique. et caryoclasique de substances chimiques. Coll. int. Cent. nat. Rech. Sci. Paris, **88,** 189—197. — 25. Dougherty, W. J.: (1964) Fate of Golgi Complex, Lysosomes and Microbodies during Mitosis of Rat Hepatic Cells. J. Cell. Biol. **23,** 25/A. — 26. Dustin, A. P.: (1934) Contribution à l'étude des poisons caryoclasiques sur les tumeurs animales. Bull. Acad. roy. Méd. Belg. **14,** 487—502. — 27. Dustin, P. Jr.: (1949) Mitotic Poisons in the Chemotherapy of Malignant Growth. Acta Un. int. Cancr. **6,** 466—477. — 28. Dustin, P. Jr.: (1950) The Action of Mitotic Poisons on Normal and Pathological Bloodcell Formation. Sang **21,** 297—330. — 29. Eigsti, O. J., Dustin, P. Jr.: (1955) Colchicine in Agriculture, Medicine, Biology, and Chemistry. Iowa State College Press, Ames. Iowa. — 30. Feldherr, C. M., Gall, J. G., Goldstein, L., Härding, C. V., Loewenstein, W. R., Mirsky, A. E.: (1964) The Nuclear Membrane and Nucleocytoplasmic Interchange. Protoplasmatologia. Handbuch der Protoplasmaforschung. Vol. V/2. Pp. 1—71. Springer, Wien. — 31. Fell, H. B., Allsop, C. B.: (1948) The Effect of Repeated Applications of Minute Quantities of Mustard Gas ($\beta\beta$ dichlorodiethylsulphide) on the Skin of Mice. Cancer Res. **8,** 177—181. — 32. Gall, J. G.: (1961) Centriole Replication,

a Study of Spermatogenesis in the Snail Viviparus. J. biophys. biochem. Cytol. **10,** 163—193. — 33. GÁTI, E.: (1963) Examens morphologiques et enzymochimiques avec Vincaleucoblastine sur lymphomes ascitiques. Bull. Ass. Franç. Cancer **50,** 455—462. — 34. GÁTI, E.: (1963) Morphologische und enzym-chemische Veränderungen an einem Mäuse-Ascites-Lymphom mit induzierter Resistenz. Z. Krebsforsch. **65,** 506—512. — 35. GÁTI, E.: (1965) Über die morphologische Wirkung verschiedener Chemotherapeutica auf Degranol-empfindlichen und Degranol-resistenten NK/Ly Ascites-Tumor. Z. Krebsforsch. **66,** 460—465. — 36. GÁTI, E.: (1966) A daganatellenes szerekkel szembeni resistencia kísérletes vizsgálata. Thesis, Budapest. — 37. GELFANT, S.: (1963) Inhibition of Cell Division: A Critical and Experimental Analysis. Int. Rev. Cytol. **14,** 1—39. — 38. GEORGE, PH., JOURNEY, L. J., GOLDSTEIN, M. N.: (1965) Effect of Vincristine on the Fine Structure of HeLa Cells during Mitosis. J. nat. Cancer Inst. **35,** 355—375. — 39. GORMAN, M., NEUSS, N., SVOBODA, G. H.: (1959) Vinca Alkaloids. IV. Structural Features of Leurosine and Vincaleukoblastine, Representatives of New Type of Indole-Indoline Alkaloids. J. Amer. Chem. Soc. **81,** 4745—4746. — 40. GRAMPA, G., DUSTIN, P. Jr.: (1952) Analyse par la colchicine des effets radiomimétiques de l'acide 4-amino-ptéroyl glutamique (aminoptérine). Rev. belge Path. **22,** 113—125. — 41. HADDOW, A.: (1954) Experimental and Clinical Aspects of the Action of Various Carboxylic Acid Derivatives of the Aromatic Nitrogen Mustard Series. Ciba Found. Symp. on Leukaemia Res. pp. 196—204. Churchill, London. — 42. HARRIS, P., MAZIA, D.: (1962) The Finer Structure of the Mitotic Apparatus. In: The Interpretation of Ultrastructure. Harris, R. J. C., ed. Pp. 279—305. — Academic Press, New York. — 43. HERTZ, R., LIPSETT, M. B., MOY, R. H.: (1960) Effect of Vincaleukoblastine on Metastatic Choriocarcinoma and Related Trophoblastic Tumours in Women. Cancer Res. **20,** 1050—1053. — 44. HILL, J. M., LOEB, E.: (1961) Treatment of Leukemia, Lymphoma, and Other Malignant Neoplasms with Vinblastine. Cancer Chemother. Abstr. **15,** 41—61. — 45. HODES, M. E., ROHN, R. J., BOND, W. H.: (1959) Effects of a Plant Alkaloid, Vincaleukoblastine, in Human Beings. J. Lab. clin. Med. **54,** 826—827. — 46. HODES, M. E., ROHN, R. J., BOND, W. H.: (1960) Vincaleukoblastine. I. Preliminary Clinical Studies. Cancer Res. **20,** 1041—1049. — 47. HUTH, H.: (1952) Über die Entstehung von Riesenzellen beim Mäuseascitescarcinom. Z. Krebsforsch. **58,** 219—221. — 48. ITO, S.: (1960) The Lamellar Systems of Cytoplasmic Membranes in Dividing Spermatogenic Cells of Drosophila virilis. I. biophys. biochem. Cytol. **7,** 433—442. — 49. JOHNSON, I. S., ARMSTRONG, J. G., GORMAN, Mr. BURNETT, J. P. JR.: (1963) The Vinca Alkaloids: A New Class of Oncolytic Agents. Cancer Res. **23,** 1390—1427. — 50. JOHNSON, I. S., WRIGHT, H. F., SVOBODA, G. H.: (1959) Experimental Basis for Clinical Evaluation of Antitumor Principles Derived from Vinca rosea Linn. J. Lab. clin. Med. **54,** 830—845. — 51. JOHNSON, I. S., WRIGHT, H. F., SVOBODA, G. H., VLANTIS, J.: (1960) Antitumor Principles Derived from Vinca rosea Linn. I. Vincaleukoblastine and Leurosine. Cancer Res. **20,** 1016—1022. — 52. JOURNEY, L. J., GOLDSTEIN, M. N.: (1961) Electron Microscope Studies on HeLa Cell Lines Sensitive and Resistant to Actinomycin-D. Cancer Res. **21,** 929—932. — 53. JOVANOVICS, K., MÁRK, G., HÖRÖMPŐ, L., SZÁSZ, K.: (1963) The Examination of Periwinkle Catharantus roseus seu Vinca rosea Linn. Winkler Centenary Festschrift Budapest. Akademie- Verlag. — 54. KINDRED, J. E.: (1949) The Blood Cells and Hemopoietic and Other Organs of Dogs Given Intravenous Injections of 2-chloroethyl-vesicants. Arch. Path. **47,** 378—398. — 55. KELLNER, B.: (1956) Histological Changes and Their Chronological Sequence Induced in Transplanted Tumours by Nitrogen Mustard, Mustard Gas and BCM (1,6-bis)beta-chloroaethyl-amino)-1,6-desoxy-d-mannite-dichlorhydrate. Acta morph. Acad. Sci. hung. **7,** 215—235. — 56. KELLNER, B.: (1957) The Significance of Amitosis and Multinuclear Cells in Tumour Growth. Acta biol. Acad. Sci. hung. **7,** 13—14. — 57. KELLNER, B., NÉMETH, L., TYIHÁK, E., PÁLYI, I., GRÉCZI, E.: (1962) Untersuchungen bezüglich der zytostatischen Wirkung der Vinca-rosea-Alkaloide. Ungarische Krebstagung in Budapest. 1961. Pp. 155—159. Akademie Verlag, Budapest. — 58. KLUG, H.: (1961) Licht- und elektronenmikroskopische Untersuchungen an Ascitestumorzellen. Z. Krebsforsch. **64,** 313—322. — 59. KOBLOV, G. A.: (1957) Dévelopment des structures polynucléaires dans le mésothelium du péricarde (russian) Dokl. Akad. Nauk. S.S.S.R. **115,** 1011—1014. — Ref. in: Bull. signal 19, 1598, 1958. — 60. KOLLER, P. C.: (1958) Comparative Effects of Alkylating Agents on Cellular Morphology. Ann. N. Y. Acad. Sci. **68,** 738—801. — 61. KOLLER, P. C., CASARINI, A.: (1952) Comparison of Cytological Effects Induced by X-rays and Nitrogen Mustards. Brit. J. Cancer **6,** 173—185. — 62. KÖVI, J., NÉMETH, GY.: (1961) Az amytal-ascites sarcoma cytologiája és növekedési viszonyai. Magy. Onkol. **5,** 102—112. — 63. LAPIS, K.: (1962) Cytologiai és elektronmikroszkópos vizsgálatok NK/Ly asciteslymphoma sejteken. Magy. Onkol. **6,** 57. — 64. LAPIS, K.: (1963) Kemoterápiás szerek által okozott ultrastrukturális elváltozások ascites lymphoma sejteken. In: "25 év a rákellenes küzdelem szolgálatában." Pp. 377—388. Országos Onkológiai Intézet, Budapest. — 65. LAPIS, K., BENEDECZKY, I.: (1966) Antimetabolite-induced Changes in the Fine Structure of Tumour Cells. Acta biol. Acad. Sci. hung. **17,** 199—

215. — 66. LAPIS, K., BENEDECZKY, I., BOLLA, K.: (1967) Ultrastructural Alterations Induced by Alkylating Agent (R—74) in Tumour Cells. Acta biol. Acad. Sci. hung. **18,** 303—322. — 67. LAPIS, K., BERNHARD, W.: (1965) The Effect of Mitomycin-C on the Nucleolar Fine Structure of KB Cells in Cell Culture. Cancer Res. **25,** 628—645. — 68. LAPIS, K., GUBA, F.: (1964) Electron Microscopic Studies of Ascites Lymphoma Cells Treated with Different Chemotherapeutics. Acta Un. int. Cancr. **20,** 180—184. — 69. LAPIS, K., MERCER, E. H.: (1962) Az NK/Ly egér ascites lymphoma sejtek elektronmikroszkópos morphologiája és a cytostaticus szerek okozta elváltozásai. Magy. Tud. Akad. Biol. Orv. Tud. Oszt. Közl. **13,** 345—363. — 70. LAPIS, K., MERCER, E. H.: (1963) An Electron Microscope Study of NK/Lymphoma Cells and the Changes Produced by Two Cystostatic Drugs. Cancer Res. **23,** 678—681. — 71. LETTRÉ, H.: (1948) Mitosegifte und cancerogene Faktoren als Antibiotica. Z. Krebsforsch. **1,** 5—35. — 72. LETTRÉ, H.: (1950) Über Mitosegifte. Ergebn. Physiol. **46,** 379—452. — 73. LEWIS, W. H.: (1927) Binucleate Cells and Giant Cells in Tissue Cultures and the Similarity of the Latter to the Giant Cells of Tuberculous Lesions. Tubercle (Edinb.) **8,** 317—330. — 74. LEWIS, W. H.: (1927/b) The Formation of Giant Cells in Tissue Cultures and Their Similarity to Those in Tuberculous Lesions. Amer. Rev. Tuberc. **15,** 616—628. — 75. MAZIA, D.: (1960) The Analysis of Cell Reproduction. Ann. N. Y. Acad. Sci. **90,** 455—469. — 76. MAZIA, D.: (1961) Meiosis and Mitosis: Mitosis and the Physiology of Cell Division. In: J. Brachet, A. E. Mirsky eds.: The Cell. Biochem. Physiol. Morph. Vol. **3,** 77—412. Acad. Press, New York. — 77. MAZIA, D.: (1964) Events of Mitotic Cell Division and their Control. Proc. III. Int. Congr. Chemotherapy Stuttgart, 1963. Pp. 34—42. Thieme, Stuttgart. — 78. MAZIA, D., DAN, K.: (1952) The Isolation and Biochemical Characterization of the Mitotic Apparatus of Dividing Cells. Proc. nat. Acad. Sci. (Wash.) **38,** 826—838. — 79. MAZIA, D., HARRIS, P. J., BIBRING, T.: (1960) The Multiplicity of the Mitotic Centers and the Timecourse of their Duplication and Separation. J. biophys. biochem. Cytol. **7,** 1—20. — 80. MAZIA, D., MITCHINSON, J. M., MEDINA, H., HARRIS, P.: (1961) The Direct Isolation of the Mitotic Apparatus. J. biophys. biochem. Cytol. **10,** 467—474. — 81. MAZIA, D., ROSLANSKY, J. D.: (1956) The Quantitative Relations between Total Cell Proteins and the Proteins of the Mitotic Apparatus. Protoplasma (Wien) **46,** 528—534. — 82. MURRAY, R. G., MURRAY, A. S., PIZZO, A.: (1965) The Fine Structure of Mitosis in Rat Thymic Lymphocytes. J. Cell Biol. **26,** 601—619. — 83. NALL, J., NOVIKOFF, A. B.: (1965) Effect of Arginine Deprivation, Ultraviolet Radiation and X-radiation on Cultured KB-cells. J. Cell Biol. **27,** 603—620. — 84. NEUSS, N., GORMAN, M., BOAZ, H. E., CONE, N. J.: (1962) Vinca Alkaloids. XI. Structure of Leukocristine (LCR) and Vincaleukoblastine (VLB). J. Amer. Chem. Soc. **84,** 1509—1510. — 85. NEUSS, N., GORMAN, M., SVOBODA, G. H., MACIAK, G., BEER, C. T.: (1959) Vinca Alkaloids. III. Characterization of Leurosine and Vincaleukoblastine, New Alkaloids from Vinca rosea Linn. J. Amer. Chem. Soc. **81,** 4754—4755. — 86. NEUSS, N., JOHNSON, I. S., ARMSTRONG, J. G., JANSEN, C. J.: (1964) The Vinca Alkaloids. In: Advances in Chemotherapy. Goldin, A., Hawking, F. eds. pp. 133—174. Academic Press. New York. — 87. NÉMETH, L., DÖBRÖSSY, L., GÁL, F., NÉMETH, L. JR.: (1965) Effect of Vinblastine-Sulphate (VR—8) on V—2 Rabbit Carcinoma. Neoplasma **12,** 257—263. — 88. NÉMETH, L., KELLNER, B.: (1963) On Biological Properties of Vinca rosea Alcaloids. Neoplasma **10,** 149—154. — 89. NÉMETH, L., KELLNER, B.: (1964) Growth-inhibiting Action of the Alkaloids of Vinca rosea with Special Reference to their Capacity for Preventing Intravenously Introduced Tumour Cells from Settling. Acta Un. Int. Cancr. **20,** 218—220. — 90. NOBLE, R. L., BEER, C. T., CUTTS, J. H.: (1958/a) Screening procedures for Experimental Cancer Chemotherapy: Role of Chance Observations in Chemotherapy: Vinca rosea. Ann. N. Y. Acad. Sci. **76,** 882—894. — 91. NOBLE, R. L., BEER, C. T., CUTTS, J. H.: (1958) Further Biological Activities of Vincaleukoblastine — an Alkaloid Isolated from Vinca rosea (L). Biochem. Pharmacol. **1,** 347—348. — 92. PALADE, G. E.: (1951) A Study of Fixation for Electron Microscopy. J. exp. Med. **95,** 285—298. — 93. PALMER, C. G., LIVENGOOD, D., WARREN, A. K., SIMPSON, P. J., JOHNSON, I. S.: (1960) The Action of Vincaleukoblastine on Mitosis in vitro. Exp. Cell Res. **20,** 198—265. — 94. PÁLYI, I., TÖRŐ, I.: (1959) Das Verhalten des Granulationsgewebes in Gewebekulturen. Z. mikr. anat. Forsch. **65,** 21—32. — 95. POLICARD, A.: (1950) Précis d'histologie physiologique. 5th ed. Doin, Paris. — 96. PRESCOTT, D. M., BENDER, M. A.: (1962) Synthesis of RNA and Protein during Mitosis in Mammalian Tissue Culture Cells. Exp. Cell Res. **26,** 260—268. — 97. RINALDINI, L. M.: (1952) The Antimitotic Action of an Aromatic Nitrogen Mustard on Tissue Cultures. Brit. J. Cancer, **6,** 186—196. — 98. ROBBINS, E., GONATAS, N. K.: (1964) The Ultrastructure of a Mammalian Cell during the Mitotic Cycle. J. Cell Biol. **21,** 429—463. — 99. ROBBINS, E., GONATAS, N. K.: Histochemical and Ultrastructural Studies on HeLa Cell Cultures Exposed to Spindle Inhibitors with Special Reference to the Interphase Cell. J. Histochem. Cytochem. **12,** 704—711. — 100. ROTH, L. E., OBETZ, S. W., DANIELS, E. W.: (1960) Electron Microscopic Studies of Mitosis in Amebae. I. Amoeba Proteus. J. biophys. biochem. Cytol. **8,** 207—220. — 101. RYUJIRO, S., YANAGI, K.: (1964)

Cytological Effects of Chemicals on Tumours. XXVI. The Action of Velban (Lilly) on Established Tumour Strains in vivo and in vitro. J. Fac. Sci., Hokkaido Univ. Ser. VI. **15,** 353—359. — 102. SIEBS, W.: (1963) Mitose Ablaufstörungen. Zur Wirkung von Vincaleukoblastin auf normale und maligne Zellen in vitro. Z. Zellforsch. **61,** 231—275. — 103. STECHER, G., ELLERMEIER, H., BANIHASCHEMI, A.: (1963) Zytostatische Behandlung mit Vinblastine (Velbe). Med. Welt. (Stuttg.) **10,** 519—523. — 104. TAKAHASHI, T., YOSHIDA, T. H.: (1959) Relation between Micronuclei and Chromosome Fragments after X-Irradiation. Ann. Rep. nat. Inst. Genet. **10,** 125. — 105. TÖRŐ, I., VADÁSZ, J.: (1939) Untersuchungen über die Wirkung von Colchicin und Corhormon in Gewebekulturen mit Hilfe von Filmaufnahmen. Arch. exp. Zellforsch. **23,** 277—298. — 106. VAITKEVICIUS, V. K., TALLEY, R. W., BRENNAN, M. J.: (1961) Cytological and Clinical Observations during Vinca Leukoblastine Therapy of Disseminated Cancer. Proc. Amer. Ass. Cancer Res. **3,** 275. — 107. VAITKEVICIUS, V. K., TALLEY, R. W., TUCKER, J. L., BRENNAN, M. J.: (1962) Cytological and Clinical Observations during Vinca Leukoblastine Therapy of Disseminated Cancer. Cancer (Philad.) **15,** 294—306. — 108. VERGA, L.: (1957) Anomalie citomorfologiche e meccanismo di azione della citostase. Le cellule ematopoietiche durante trattamento endovenoso con E—39. Haematologica **42,** 469—478. — 109. WALKER, D. G., WRIGHT, J. C.: (1961) The Effect of Vinca Leukoblastine on Primary Cultures of Human Neoplasms. A Preliminary Report. Cancer Chemother. Abstr. **14,** 139—144. — 110. WARWICK, O. H., DARTE, J. M. M., BROWN, T. C.: (1960) Some Biological Effects of Vinca Leukoblastine, and Alkaloid in Vinca rosea L. in Patients with Malignant Disease. Cancer Res. **20,** 1032—1040. — 111. WATSON, M. L.: (1955) The Nuclear Envelope. Its Structure and Relation to Cytoplasmic Membranes. J. biophys. biochem. Cytol. **1,** 257—270. — 112. WENT, H. A.: (1966) The Behavior of Centrioles and the Structure and Formation of the Achromatic Figure. Protoplasmatologia. Handbuch der Protoplasmaforschung. Vol. VI/G/1. Pp. 1—109. Springer, Wien. — 113. YASUZUMI, G., SUGIHARA, R.: (1965) The Fine Structure of Nuclei as Revealed by Electron Microscopy. Exp. Cell Res. **37,** 207—229. — 114. YASUZUMI, G., SUGIHARA, R.: (1965) The Fine Structure of Nuclei as Revealed by Electron Microscopy II. The Process of Nucleolus Reconstitution in Ehrlich Ascites Tumour Cell Nuclei. Exp. Cell Res. **40,** 45—55.

AUF WIRKUNG VON VINCALEUCOBLASTIN IN DER ULTRASTRUKTUR VON ASCITESTUMORZELLEN ENTSTANDENE VERÄNDERUNGEN

K. LAPIS

Verfasser beschreibt die in den NK/Ly-Asciteslymphomzellen auf Wirkung von Vincaleucoblastin (VR^{-8}) in vivo vor sich gehenden ultrastrukturellen Veränderungen. In einem Teil der in der Interphase steckengebliebenen Zellen finden sich Anhäufungen elektronendichter, polymorpher, mit einer Membrane umgebener Körperchen, lysosomartiger Gebilde. Das ultrastrukturelle Substrat der in einem Teil der in der Interphase verbliebenen Zellen auf Wirkung von Vincaleucoblastin entstehenden Kernveränderung (lichtmikroskopisch die durch KELLNER beschriebene mosaikartige Struktur) wird besprochen, ferner die ultrastrukturellen Kennzeichen der auf Wirkung des Mittels entstandenen anomalen mitotischen Zellen mitgeteilt. Der Verfasser stellt fest, daß in den mitotischen Zellen die orientierten Spindeltubuli fehlen. In der Ultrastruktur der Zentriolen finden sich keine Veränderungen, dagegen erleidet ihre Orientation und ihre Wanderung zu den Polen eine Störung. Die Reduplikation der Zentriolen scheint nicht gehemmt zu sein und auch in der Ultrastruktur der Kinetochoren kam es auf Wirkung des Mittels zu keinen Veränderungen. Auch der mit der Entstehung des mitotischen Apparates verbundene Gelbildungsprozeß erleidet eine Störung. Hierauf weise auch der Umstand, daß in den normal vom mitotischen Apparat eingenommenen und keine anderen Organellen enthaltenden Teilen der in der Mitose steckengebliebenen Zellen Mitochondrien und andere zytoplasmatische Organellen aufgezeigt werden können. In den in der Mitose verbliebenen Zellen wurden agglomerierte Chromosomenhaufen, Anhäufung der umgebenden Membranen in mehreren konzentrischen Schichten, ferner in einem Teil der mehrkernigen Zellen das Auftreten von quadrilamellaren Membranen beobachtet. Diese Membranen werden vom Verfasser als das Zeichen der auf Wirkung von Vincaleucoblastin eintretenden Störung der Kernmembranrekonstruktion aufgefaßt. Die obigen, auf Wirkung von Vincaleucoblastin auftretenden Veränderungen stimmen mit den unter Vincristin-Wirkung in HeLa-Zellen in vitro von GEORGE und Mitarbeitern beschriebenen Veränderungen überein. Die ultrastrukturellen Besonderheiten der auf Wirkung von Vincaleucoblastin entstandenen vielkernigen Zellen werden erörtert, und es wird hervorgehoben, daß die Mehrzahl dieser Zellen über Zellorganellen mit gut erhaltener Struktur verfügt.

ИЗМЕНЕНИЯ УЛЬТРАСТРУКТУРЫ АСЦИТИЧЕСКИХ ОПУХОЛЕВЫХ КЛЕТОК, ВОЗНИКАЮЩИЕ ПОД ВЛИЯНИЕМ ВИНКАЛЕЙКОБЛАСТИНА

К. ЛАПИШ

Автор сообщает об изменениях ультраструктуры, наблюдаемых в клетках асцитической лимфомы NK/Ny под влиянием винкалейкобластина (VR^{-8}) in vivo. В части клеток, находящихся в межуточной фазе, видны накопления окруженных мембраной, электроннoплотных, полиморфных телец, лизосомоподобных образований. Дается описание ультраструктурной среды изменения ядра (под световым микроскопом мозаичная ядерная структура, описанная Кельнером), возникшего под влиянием препарата, в части клеток, находящихся в межуточной фазе, а также ультраструктурные особенности ненормальных митотических клеток, образовавшихся под влиянием винкалейкобластина. Устанавливается, что в деляющихся клетках отсутствуют ориентированные веретенообразные канальцы. В ультраструктуре центриол не наблюдалось изменений, однако нарушается их ориентация и перемещение к полюсам. Редупликация центриолов, повидимому, не претерпевает торможения, и в ультраструктуре кинетохоров также не отмечаются изменения под влиянием винкалейкобластина. Нарушается и процесс образования геля, связанный с образованием митотического аппарата. На это указывает то обстоятельство, что в частях деляющихся клеток, занятых в норме митотическим аппаратом и свободных от прочих органелл, наблюдаются митохондрии и прочие цитоплазматические органеллы. В клетках, оставшихся в фазе деления, наблюдались накопления агломерированных хромосом, нескольких концентрических слоев окружающей мембраны, и в части многоядерных клеток появление четырехламеллярных мембран. Эти мембраны рассматриваются автором как знак нарушения восстановления ядерной оболочки, происшедшего под влиянием винкалейкобластина. Устанавливается, что вышеуказанные изменения, возникшие под влиянием винкалейкобластина, соответствуют изменениям, возникшим под влиянием винкристина в HeLa — клетках in vitro, описавшимся Жорж и сотрудниками. Сообщаются ультраструктурные особенности многоядерных клеток, образовавшихся под влиянием винкалейкобластина, и подчеркивается, что большинство клеток имеет клеточные органеллы с хорошо сохраненной структурой.

Dr. Károly Lapis; Orvostovábbképző Intézet, Budapest
XIII. Szabolcs u. 33. Hungary

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 85—101, 1968*

Institute of Pathological Anatomy and Histopathology (Director: J. ORMOS), University Medical School, and Institute of Hygiene and Epidemiology (Director: J. VETRÓ), Szeged

CONTRIBUTIONS TO THE DEVELOPMENT OF EXPERIMENTAL PYELONEPHRITIS

J. ORMOS, ZS. CSAPÓ and JUDIT LANTOS

Pyelonephritis, accompanied by the development of abscesses and leading to a contraction of the affected kidney, has been induced in 61% of rats by the intravenous administration of *E. coli* and ligation of one ureter for 48 hours. Leukocytic emigration started 8 hours after *E. coli* administration, but accumulation of leukocytes in certain vessels was sometimes observed after 4 hours already. The inflammatory process may start in the cortex, medulla and the wall of the renal pelvis alike; neither the cortex nor the medulla were seen to represent a striking predilectory site of initial changes. The process invariably began and spread in the interstitial spaces; parenchymal damage and passing of leukocytes into the tubules were secondary phenomena. The inflammatory process was in no case observed to have started in the glomeruli; the latter remained comparatively unimpaired despite grave interstitial involvement and extensive tubular damage. Hormonally induced renal ischaemia failed to render the kidney susceptible to intravenously introduced pathogenic organisms.

Pyelonephritis, the condition which, as regards frequency, occupies the second place after respiratory infections and the first among lethal nephropathies [12, 34], has successfully been induced experimentally by different methods [38] but views as to the development of its changes are still divided. Even textbooks are contradictory in regard to the initial manifestations of human pyelonephritis; according to some authors it originates in the medulla of the kidney [19, 20, 25, 35], while others [1, 3, 34] think that the process (at least that of haematogenic origin) starts in the cortex. The fact that the definition of the disease is not uniform, adds to the existing confusion [11]. Beside some other questions the aim of the present study was to observe the initial changes of the disease on a model similar to human pyelonephritis as far as possible.

Material and methods

Albino rats of our own breed, weighing 120 to 180 g, of both sexes and kept on a mixed diet, were used. *E. coli* was isolated from the urine of human subjects suffering from acute pyelonephritis, passed through dogs, then through rats, and 2×10^8 organisms were then injected into the caudal vein of the test animals. The strain was kept on agar slant and, in order to maintain its virulence, passed through rats every week. One day before being used, it was transferred into broth, incubated for 18 hours and centrifuged; the sediment was diluted with physiological saline to the desired concentration. We determined the number of germs in the suspension by means of a densitometer and by means of a calibration curve plotted previously in order to demonstrate the correlation between germ count and density.

The animals were anaesthetized with ether, the operative area was depilated and sterilized with an alcoholic solution of iodine. After gaining access to the abdominal cavity from the left paracostal approach and retracting the bowels to the right side, the left ureter was fixed to the psoas major muscle; by transfixing the muscle and the skin, the knot came to lie outside the skin. The suture was removed after 24 or 48 hours without opening the abdomen; in order to avoid angulation of the ureter, the kidney was palpated and pushed toward the chest. The bacterial suspension was administered between the 5th and 60th minute following ureteral ligation.

According to the method of KOVÁCS and DÁVID [26] cortical necrosis was induced by administering 1 mg of oestrone acetate (Hogival — Chinoin Budapest) subcutaneously, daily for 10 days; the last dose was combined with the administration of 10 I.U. of posterior pituitary extract (Glanduitrin — Richter Budapest).

After the spontaneous death or the sacrifice of the animals their kidneys were fixed in a 4% solution of formalin, embedded in paraffin and stained with haematoxylin-eosin and other stains if necessary. With a view to study early changes, two samples were excised from every kidney in the third experimental group, and serial sections were made.

When determining the bacterial count, the kidney removed under sterile conditions was cut in half. Both parts were weighed separately; one was fixed in formalin, while the other half was homogenized in a mortar with quartz sand and 3 ml of physiological saline, diluted 10 and 100 fold, and then plated on agar broth. The colonies were counted after 18 hours incubation at 37 °C; the bacterial count was referred to 1 g of renal tissue.

The animals were divided in 4 groups. The *first* contained 15 rats whose left ureter was ligated for 24 hours. They survived 2 to 10 days (spontaneous death or killing). This group was disregarded in the following because a ligature of 48 hours, as applied in the second group, proved to be more suitable for our purposes.

The *second group* comprised 191 rats. These were subjected to *E. coli* infection combined with ligation of the left ureter for 48 hours. Animals that died spontaneously within 48 hours were disregarded (Table I).

Table I

Distribution of pyelonephritic (+) and intact (—) cases in group 2

Time of death		2	3—4	5—8	9—16	20—22	40—60	More than 80	Total
		days							
Killed	+	24	6	13	4	2	3	13	65
	—	1	4	6	5	4	2	4	26
Died spontaneously	+	5	39	8	2	2	0	1	57
	—	2	32	5	1	1	2	0	43

Early alterations were studied on the 48 animals constituting the *third group*. Save for the 12 animals that had died spontaneously, 2—4 members of each of this group were killed 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48 hours after ureteral ligation and *E. coli* administration.

Renal necrosis was induced by hormonal treatment in the 86 rats that constituted the *fourth group*. *E. coli* was administered in a quantity of 2×10^8 to 27 animals immediately before, and to 29 animals one hour, to 15 animals two hours and to 15 rats 48 hours after the injection of Glanduitrin.

Results

One hundred of 191 animals, forming the second group, died spontaneously later than 48 hours, while 91 were killed. Pyelonephritis has been induced on the side of the temporary ligature in 57 animals among the spontaneously died ones and in 65 among the killed ones. The time of the deaths

and the frequency of the pyelonephritis is shown in Table I. The kidney of 40 rats of the third group were sectioned serially. Among the latter in 19 cases suppurative inflammation was observed, in particular in 7 cases only in the cortex, in 4 cases only in the medulla, and in both cortex and medulla in 8 cases.

Renal lesions in positive cases of groups 2 and 3

Gross appearance. The contents of the dilated renal pelvis and ureter became purulent sometimes in 28 to 32, and invariably in 48 hours; this suppuration disappeared gradually after removal of the ligature. The

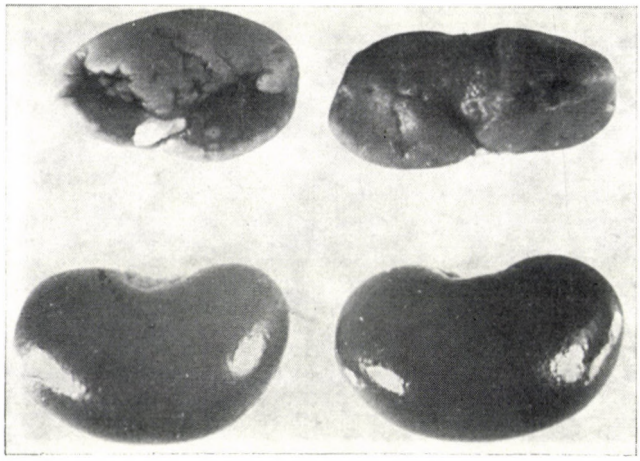


Fig. 1. 99 and 112-day old contracted kidneys

kidneys grew somewhat larger and patchy at 12 to 16 hours. Minute abscesses appeared in the cortex and in smaller number in the medulla after 28 to 32 hours; they grew to pinhead size at 48 hours, emerged from the surface, and coalesced at some points. They became more compact after 7 days but were still projecting from the surface. At 20 days, the surface showed depressions at the site of the abscesses. Different retractions of the surface were accompanied by shrinkage at 40 days, and the latter became subsequently more marked (Fig. 1). No acute inflammation was registered in the contralateral kidney. After 2 days the weight of the temporarily ligated kidneys exceeded by 29% that of the contralateral organ; the difference decreased after 3 to 4 days and disappeared by the 5th to 8th day. With advancing shrinkage, the weight ratio turned into the opposite so that in animals having lived longer than 80 days the temporarily ligated kidneys weighed 0.13 to 0.75 % (mean, 0.48) of that of the control organs.

Microscopic appearance. Some tubular dilatation and hyperaemia appeared in two hours. Tubular dilatation then became more pronounced to

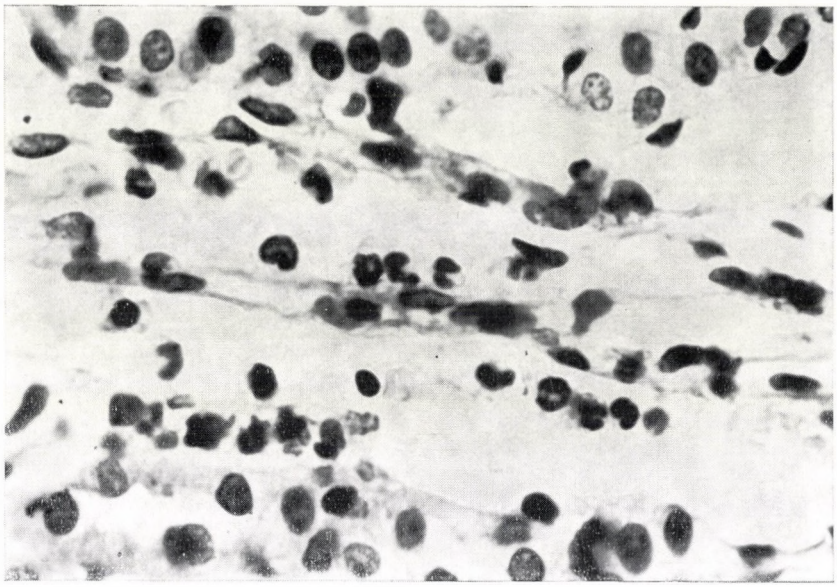


Fig. 2. Accumulation of leukocytes in the medullary vessels after 4 hours. Haematoxylin-eosin, ×900

diminish subsequently, but was still conspicuous after a week. At some sites numerous polymorphonuclear leukocytes were observed after 4 hours in the cortical capillaries and the medullary veins, especially at the base of

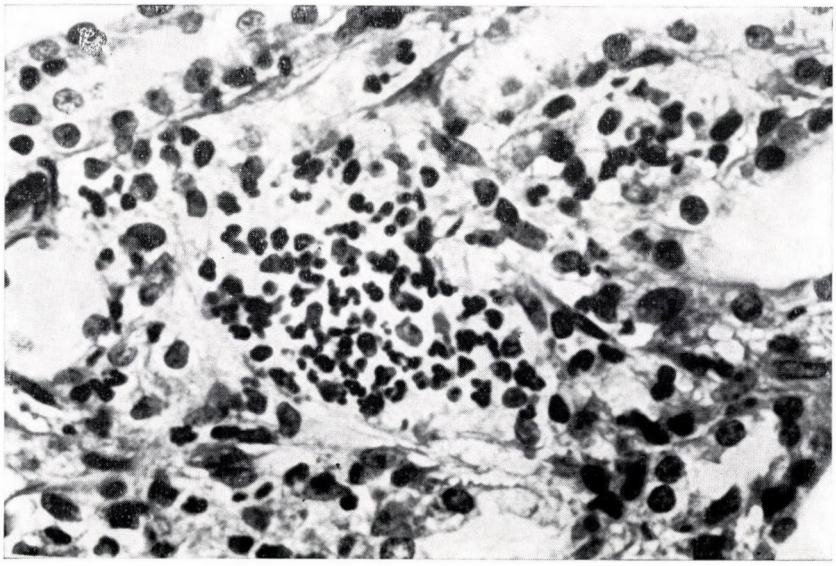


Fig. 3. Fresh suppurative infiltration in the outer zone of the medulla after 8 hours. Haematoxylin-eosin, ×560

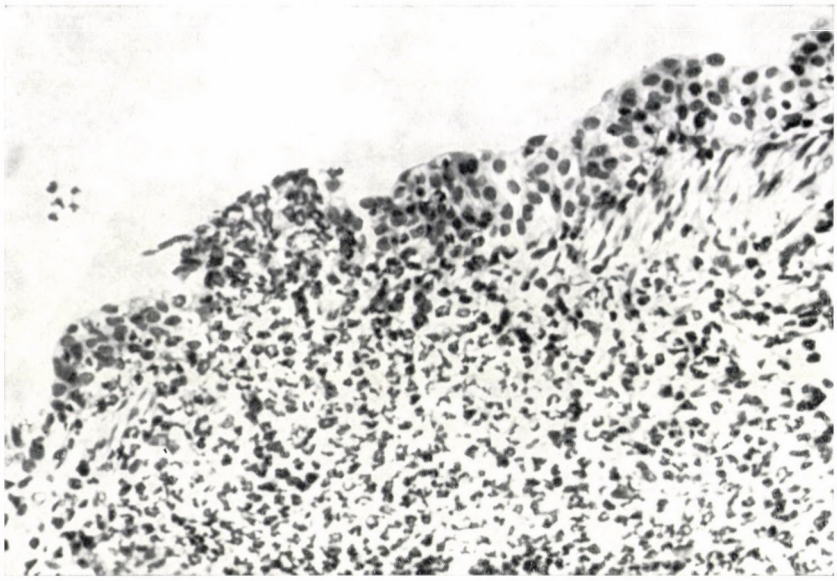


Fig. 4. Abscess of pelvic wall penetrating into the lumen after 8 hours. Haematoxylin-eosin, ×360

the pyramids (Fig. 2). After 8 to 12 hours beside intravascular leukocyte accumulations occasional leukocyte aggregations and minute abscesses were encountered sometimes interstitially in the cortex and medulla, and in the

Fig. 5. Band-like suppurative infiltrations in the cortex after 32 hours. Haematoxylin-eosin, ×90

wall of the renal pelvis (Figs 3, 4). By 16—20 hours the abscesses became larger, more numerous and coalescent. Although their number was somewhat higher in the cortex, initial infiltrations and abscesses could be seen all over the kidney and even in the wall of the renal pelvis. After 24 to 48 hours larger, bandlike infiltrations and suppuration developed (Fig. 5). At 32 to 36 hours, triangular or rather conical infiltrations with their apex projecting into the papillae, and at 40 hours even papillary necroses were observed. After the first week, the site of the leukocytes became gradually occupied by round cells and fibroblasts; all polymorphonuclears had disappeared from the interstitium after the third week.

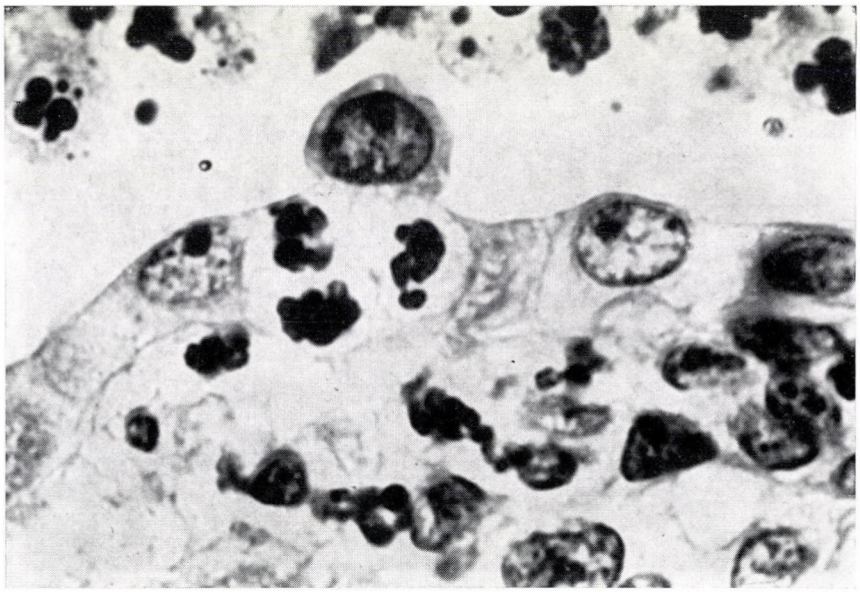


Fig. 6. Migration of leukocytes across the preserved tubular epithelium after 6 days. Note leukocytic cast in lumen. Haematoxylin-eosin, ×1800

Through the preserved epithelium lying in infiltrates emigration of polymorphonuclears into the lumen could occasionally be observed (Fig. 6). More frequent were the necrosis and desquamation of the epithelium, especially in the heavily infiltrated areas (Figs 7, 9, 10). Probably in this way had a large number of leukocytes gained access into the tubular lumina with the result that leukocyte casts developed already in 20 hours and their number was increasing thereafter (Fig. 8). Leukocyte casts were often observed after several weeks and even months, although at that time the infiltrations contained no or hardly any leukocytes. Most of the tubules in the larger infiltrations were destroyed in 3 to 4 days (Figs 9, 10), while there remained

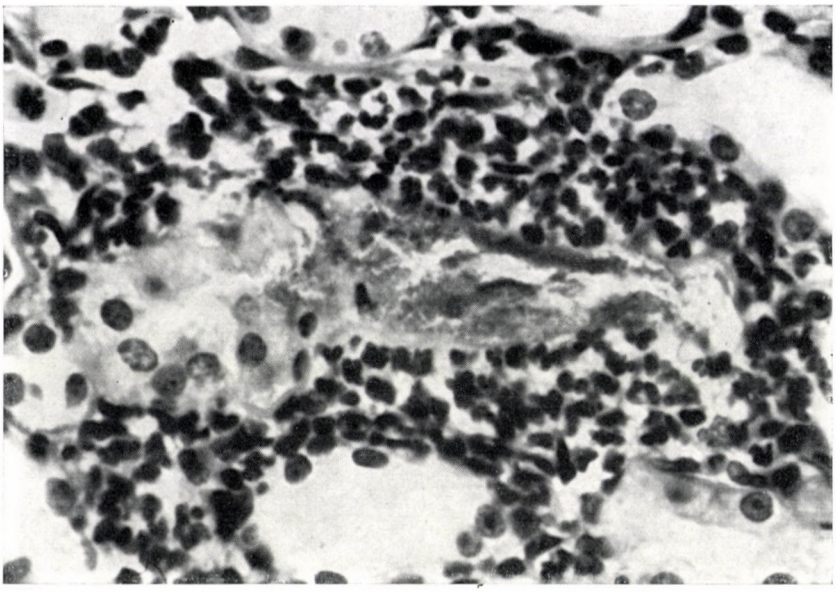


Fig. 7. Necrotized tubule in suppurative infiltration, at 32 hours. Haematoxylin-eosin, ×900

great masses of epithelium without lumen, they even showed signs of regeneration. After several weeks and months, it was hardly or not at all possible to distinguish the remaining epithelial cells from the connective tissue cells

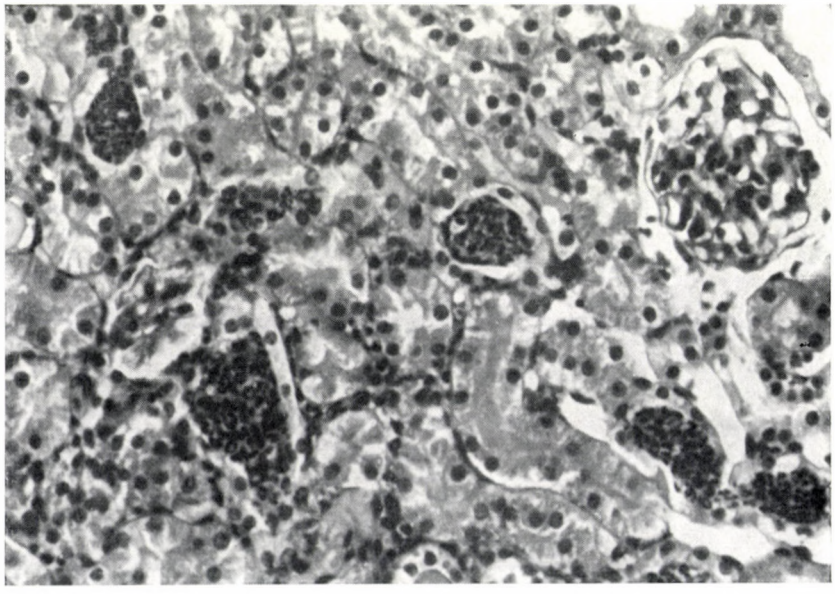


Fig. 8. Early leukocyte casts, at 20 hours. Haematoxylin-eosin, ×300

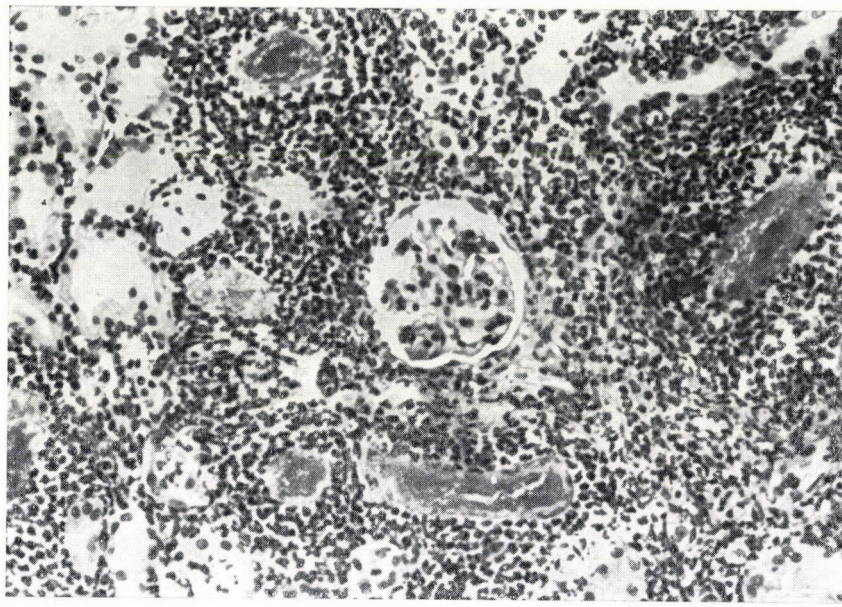


Fig. 9. Note intact glomerulus beside necrotized tubules in infiltrated area, at 32 hours. Haematoxylin-eosin, ×220

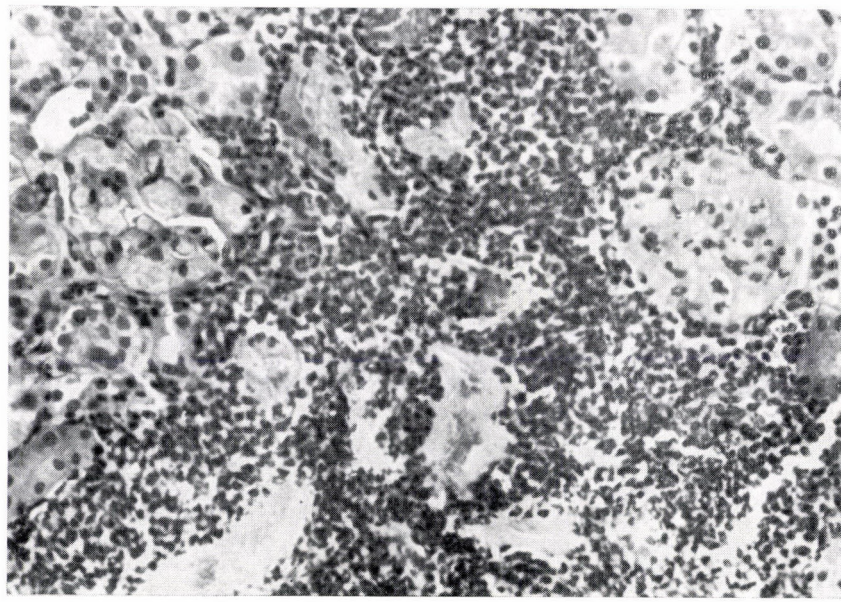


Fig. 10. Conditions at 32 hours. A necrotized glomerulus beside the tubules in the leukocytic infiltration; the glomerulus at the periphery of the infiltrated area shows changes suggestive of haemorrhagic infarction. Haematoxylin-eosin, ×220

entangled with them. In such areas the PAS reaction revealed remnants of basement membranes (Fig. 11).

Compared to the tubules, the glomeruli in the infiltrations appeared to be considerably less damaged (Fig. 9). In no case had the inflammatory process been seen to originate in the glomeruli of an otherwise uninjured part of the kidney; only exceptionally was an occasional collection of leukocytes seen on the surface of a glomerular loop even in involved areas. Some glomeruli within infiltrated areas were necrotic, others showed signs of haemorrhagic infarction (Fig. 10). Owing to the extensive tubular destruction, the surviving glomeruli came to lie closely to one another, and the wall of the loops became more or less thicker ("Schlingen-Kollaps") (Figs 11, 12).

Pus in the renal pelvis presumably originated from the leukocyte casts of the tubules and partly (mainly?) from the passage of leukocytes across the pelvic epithelium, the break-through of abscesses from the wall to the pelvic lumen; the latter phenomenon could be observed 8 hours after the infection already (Fig. 4).

After an initial decrease, the bacterial count in the kidneys increased rapidly until the 48th hour. It decreased thereafter, but was still high after a week. The sudden decrease after the 48th hour may have been caused by the release of the ligature which made possible the removal of a great number of bacteria with the urine (Table II and Fig. 13).

Group 4. Administration of *E. coli* elicited no pyelonephritis if cortical necrosis had been induced by hormonal treatment. The mild inflammatory changes were not more pronounced than those developing at such cortical necrosis or induced by *E. coli* without any other intervention. Irrespective of whether the animals received the bacterial suspension immediately before, or 1, 2 or 48 hours after the administration of Glanduitrin, bacterial count in the kidneys decreased rapidly; the rate of decrease was equal in the test animals and in the controls which received only *E. coli* (Table II and Fig. 13).

Discussion

Although pyelonephritis could be induced by the intravenous introduction of bacteria without any other intervention in different frequencies [4, 8, 9, 14, 17, 23, 28, 40], it seemed more similar to the human pathomechanism to enhance the kidney's susceptibility to the bacteria circulating in the blood. This was usually achieved by artificial urinary congestion. In principle, constriction of the ureter [16, 31] was the solution at hand, but practice has proved the permanent or provisional ligation of the ureter or its obstruction in some other — anatomical or functional — way an easier method. This

Table II

Bacterial count in the kidney,

	Time between infection and sacrifice	10′	15′	20—30′	60′	2 hours	6 hours
	Control animals (received only bacterial suspension)	$2.0 \cdot 10^5$ $4.4 \cdot 10^4$ $4.2 \cdot 10^4$ $3.8 \cdot 10^4$ $1.8 \cdot 10^4$		$2.9 \cdot 10^4$ $2.9 \cdot 10^4$ $2.7 \cdot 10^4$ $2.4 \cdot 10^4$ $2.4 \cdot 10^4$ $2.3 \cdot 10^4$ $2.1 \cdot 10^4$ $1.5 \cdot 10^4$ $1.4 \cdot 10^4$ $1.3 \cdot 10^4$ $1.2 \cdot 10^4$ $9.7 \cdot 10^3$ $4.5 \cdot 10^3$ $3.9 \cdot 10^3$ $2.0 \cdot 10^3$	$5.0 \cdot 10^5$ $1.0 \cdot 10^4$ $3.6 \cdot 10^3$ $3.5 \cdot 10^3$ $2.0 \cdot 10^3$ $8.0 \cdot 10^2$	$8.8 \cdot 10^3$ $8.4 \cdot 10^3$ $7.6 \cdot 10^3$ $6.1 \cdot 10^3$ $6.0 \cdot 10^3$ $3.8 \cdot 10^3$ $3.2 \cdot 10^3$ $2.1 \cdot 10^3$ $1.7 \cdot 10^3$	$4.1 \cdot 10^3$ $3.9 \cdot 10^3$ $3.3 \cdot 10^3$ $2.5 \cdot 10^3$ $2.4 \cdot 10^3$ $1.9 \cdot 10^3$ $1.1 \cdot 10^3$ $9.5 \cdot 10^2$
Hormonally-treated animals	Infection immediately before the administration of pituitary extract			$3.4 \cdot 10^4$ $3.2 \cdot 10^4$ $1.8 \cdot 10^4$ $1.5 \cdot 10^4$ $3.6 \cdot 10^3$ $1.3 \cdot 10^3$ $5.0 \cdot 10^2$ $1.9 \cdot 10^2$		$3.9 \cdot 10^4$ $3.8 \cdot 10^4$ $8.5 \cdot 10^3$ $8.4 \cdot 10^3$ $6.2 \cdot 10^3$	
	Infection 1 hour after the administration of pituitary extract	$1.6 \cdot 10^5$ $1.0 \cdot 10^5$ $5.4 \cdot 10^4$		$2.8 \cdot 10^4$ $1.5 \cdot 10^4$ $6.6 \cdot 10^3$ $1.4 \cdot 10^3$	$4.5 \cdot 10^3$ $1.0 \cdot 10^3$		$6.9 \cdot 10^3$ $6.0 \cdot 10^1$ $4.5 \cdot 10^1$
	Infection 2 hours after the administration of pituitary extract			$1.4 \cdot 10^4$ $1.3 \cdot 10^4$ $6.7 \cdot 10^3$ $5.1 \cdot 10^3$			$1.4 \cdot 10^3$ $1.1 \cdot 10^3$ $1.0 \cdot 10^3$ $8.6 \cdot 10^2$
	Infection 48 hours after the administration of pituitary extract						
	Infection combined with 48 hours ligation of left ureter		$3.5 \cdot 10^4$ $1.3 \cdot 10^4$ $9.4 \cdot 10^3$ $9.2 \cdot 10^3$			$6.0 \cdot 10^3$ $6.0 \cdot 10^3$ $5.0 \cdot 10^3$ $4.9 \cdot 10^3$	

referred to 1 g of renal tissue

24 hours	2 days	3 days	4 days	5 days	6 days	7 days	8 days
$7.0 \cdot 10^4$ $2.2 \cdot 10^4$ $4.1 \cdot 10^3$ $4.0 \cdot 10^3$ $3.4 \cdot 10^3$ $8.2 \cdot 10^2$ $7.8 \cdot 10^2$ $6.0 \cdot 10^2$ $3.2 \cdot 10^2$ $2.4 \cdot 10^2$ $2.0 \cdot 10^2$ $1.5 \cdot 10^2$ $1.2 \cdot 10^2$ $1.1 \cdot 10^2$ $6.4 \cdot 10^1$ $5.0 \cdot 10^1$ $2.0 \cdot 10^1$	$6.0 \cdot 10^2$ $4.6 \cdot 10^2$ $4.4 \cdot 10^2$ $1.0 \cdot 10^2$ $7.0 \cdot 10^1$		$1.5 \cdot 10^2$ $2.4 \cdot 10^1$ 0 0	$1.0 \cdot 10^3$ $6.8 \cdot 10^2$ $6.1 \cdot 10^2$ $5.7 \cdot 10^2$ $5.4 \cdot 10^2$ $2.6 \cdot 10^2$ $1.1 \cdot 10^2$ $3.8 \cdot 10^1$			$7.8 \cdot 10^1$ $3.8 \cdot 10^1$ 0 0
$1.9 \cdot 10^4$ $6.8 \cdot 10^2$ $6.0 \cdot 10^2$ $2.0 \cdot 10^2$							$9.8 \cdot 10^1$ 0 0 0 0
$2.4 \cdot 10^5$ $3.3 \cdot 10^4$ $8.8 \cdot 10^2$ $8.4 \cdot 10^2$ $7.2 \cdot 10^2$ $8.7 \cdot 10^1$	$4.6 \cdot 10^2$ 0			$8.4 \cdot 10^1$ $7.4 \cdot 10^1$ $5.0 \cdot 10^1$ 0 0			0 0 0 0 0
$6.7 \cdot 10^3$ $1.4 \cdot 10^3$ $4.0 \cdot 10^2$ $7.8 \cdot 10^1$			$9.7 \cdot 10^1$ 0 0				
		$2.0 \cdot 10^2$ 0 0 0 0			0 0 0 0 0 0		
$1.9 \cdot 10^8$ $1.3 \cdot 10^7$ $6.4 \cdot 10^6$	$3.0 \cdot 10^8$ $1.8 \cdot 10^8$ $1.2 \cdot 10^8$ $2.4 \cdot 10^7$		$2.1 \cdot 10^8$ $1.8 \cdot 10^6$ $5.4 \cdot 10^5$ $6.0 \cdot 10^3$			$6.0 \cdot 10^5$ $5.2 \cdot 10^5$ $1.0 \cdot 10^5$ $5.0 \cdot 10^3$ $1.4 \cdot 10^3$	

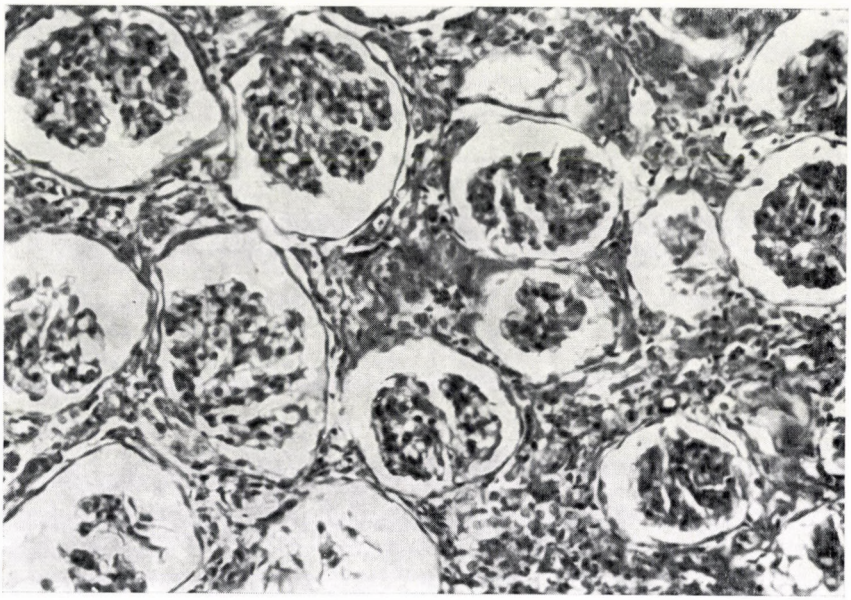


Fig. 11. Conditions at 112 days. Note closely packed glomeruli in the shrunken cortex. The connective tissue contains remnants of tubular epithelium and basement membranes. PAS stain, ×220

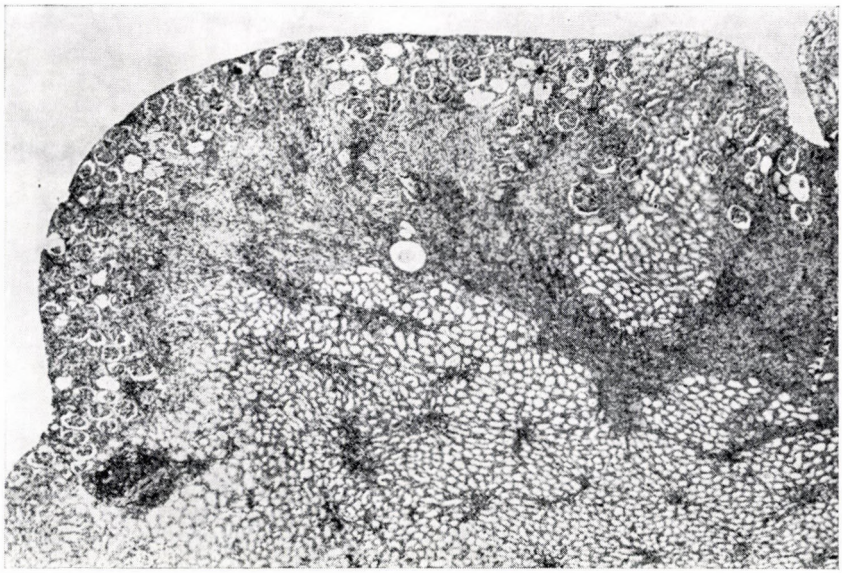


Fig. 12. Detail of shrunken kidney after 99 days. Haematoxylin-eosin, ×35

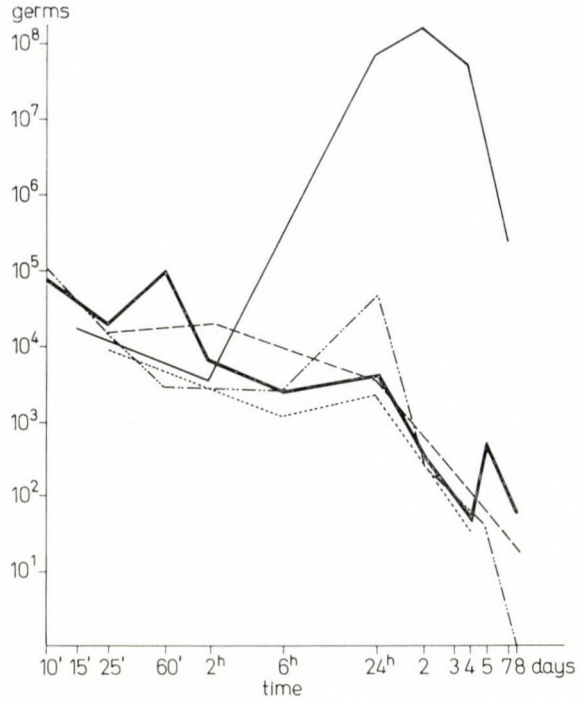


Fig. 13. Mean values of bacterial counts shown in Table II. Data regarding the 48-hour group of hormonally treated animals are not indicated

▬▬ = controls; — — — — = hormonal treatment and *E. coli* infection immediately before administration of pituitary extract; — · · — · · — · · = hormonal treatment and infection 1 hour after administration of pituitary extract; · · · · · · · · · · = hormonal treatment and infection 2 hours after the administration of pituitary extract; ▬▬ = ureteric ligation for 48 hours

procedure was usually combined with the introduction of the bacteria into the blood [6, 7, 13, 15, 18, 23, 27, 29, 30, 36, 39] or the urinary tract [2, 21, 37].

It was intended to employ in this study a method that seemed to imitate human pathomechanism as closely as possible. Pyelonephritis is mostly of haematogenic origin [41]; the experiments of RÉNYI-VÁMOS and HORVÁTH [33] have shown that even urogenital infections induce pyelonephritis by the way of blood-vessels, although in this case their spread by the urinary channels or lymphatics would seem to be more obvious. Led by such considerations, we decided to apply haematogenous infection using *E. coli*, the most frequent bacterium in human pyelonephritis [20, 34], and rendered the kidney more susceptible by a temporary ligation of the ureter because, wherever the infection originates, a disturbance of urinary passage is the most important predisposing factor [34], and temporary impediment of urine flow is probably the factor concerned most frequently [5]. We regard the changes provoked

in the present experiments as pyelonephritis since they consisted in a lesion which had started with an acute inflammatory process of the kidney and frequently also the renal pelvis, involving gradually both the cortex and medulla, producing a chronic lesion of interstitial location but causing tubular destruction and finally renal shrinkage. This process did not alter considerably the renal function for a long time, for animals usually did not die of renal failure even if the contralateral kidney had been removed.

We believe that the present experiments admit of certain inferences to the pathomechanism of human pyelonephritis. We found, in harmony with the rabbit experiments of MALLORY *et al.* [27], that the development of the disease depended to a great extent on the virulence of the bacterial strain. Like BRUMFITT and HEPTINSTALL [6] we, too, observed a correlation between the duration of ureteral ligation and the frequency of pyelonephritis; the time of ligation can, however, not be extended beyond some limit lest irreversible pyonephrosis develops (48 hours seemed to be the most appropriate length of time).

It is not known why the kidney becomes susceptible in cases of bacteraemia [5, 9]; the present study did not, moreover, allow inferences to why disturbed urine flow was an important factor in the pathogenesis of pyelonephritis. The theory has been advanced that renal ischaemia may, as regards infection, create a site of lessened resistance [5, 38]. In this respect we wished to obtain information by inducing ischaemic necrosis combined with hematogenous infection. The result was negative, as we did not succeed in producing pyelonephritis, although the two procedures had been combined in several time-relations. That the number of bacteria in the kidneys of the test animals was practically equal to that of the controls points to the fact that the failure of causing pyelonephritis was not due to a vasospasm-conditioned inability of the bacteria to gain access to the kidney in sufficient numbers; it showed furthermore that the hormonally induced renal injury offers no such favourable conditions for bacterial proliferation as did arterial ligation in the studies of HEPTINSTALL and STRYKER [24].

We paid particular attention to initial changes. In contradiction to certain earlier [27] and more recent [41] observations the inflammatory process did not seem to originate from the glomeruli or the tubules; it always started around the interstitial vessels. As noted also by HEPTINSTALL [22], leukocytes usually invaded the tubules across the damaged epithelium, although emigration was observed also between apparently unimpaired epithelial cells.

While the hyperaemia two hours after the intervention may have been due solely to ureteral ligation, the accumulation of leukocytes, observed in the vessels sometimes already after four hours, and especially the interstitial leukocytic infiltrates seen at certain points after eight hours, had to be considered the initial stage of the inflammatory process. Our observations did not

support HEPTINSTALL's [22] suggestion that subepithelial leukocytic infiltration in the renal pelvis developed only in case of ascending infection. We found that haematogenous infection might commence in cortex and medulla alike, and even in the renal pelvis, giving rise to the starting point of the inflammatory process in any of these areas. Spreading, these foci manifest themselves as pyelonephritis. Our observations do not, therefore, support the theory that initial changes are mostly or exclusively restricted to a certain part of the kidney [9]. Intrarenal spread of the process occurs in the interstitial spaces and the experiments of RÉNYI-VÁMOS [32] seem to show that the lymph vessels are not involved in this respect. It is, of course, indeniable that the site of the changes may differ according to the animal's species, the type of the microorganism and to the experimental conditions. Owing to its richer blood supply, a presumably larger number of microorganisms gain access to the cortex; the rabbit experiments of FREEDMAN and BEESON [10] have, however, shown that the cortex requires more bacteria than the medulla for the development of changes, so that these two factors may balance each other to some extent. Human observations as well as animal experiments support the assumption that pyelonephritis is usually a bacterial inflammatory renal disease of haematogenic origin which may start at any point of the kidney or the pelvis. As it has recently been emphasized [11, 41], the renal pelvis is not necessarily involved in the process.

REFERENCES

1. ANDERSON, W. A. D.: (1961) Pyelonephritis. In: Pathology, Mosby, St. Louis. 4th ed., Pp. 601—602. — 2. BABICS, A., RÉNYI-VÁMOS, F.: (1952) Die aszendierende Pyelonephritis. Acta med. Acad. Sci. hung. **3,** 15—33. — 3. BALÓ, J.: (1961) Pyelonephritis. A vese gennyes gyulladása. In: Részletes kórbonctan. Medicina, Budapest. Pp. 352—354. — 4. BONSDORFF, A. VON: (1899) Experimentelle Untersuchungen über die Ausscheidung der Streptokokken durch die Nieren. Beitr. path. Anat. **25,** 181—205. — 5. BROD, J.: (1964) Pyelitis und Pyelonephritis. In: Die Nieren. Volk und Gesundheit, Berlin, Pp. 660—708. — 6. BRUMFITT, W., HEPTINSTALL, R. H.: (1958) Experimental Pyelonephritis: The Influence of Temporary and Permanent Ureteric Obstruction on the Localization of Bacteria. Brit. J. exp. Path. **39,** 610—617. — 7. DATTA, S. K., CHAKRAVARTI, R. N.: (1966) Experimental Pyelonephritis in the Rhesus Monkey. Nature, **209,** 90—91. — 8. ERLANDSON, A. L. JR., GAGLIARDI, L. A.: (1961) The Pathogenesis of Experimental Enterococcal Pyelonephritis in Mice. J. infect. Dis. **108,** 181—188. — 9. FOSTER, W. D.: (1965) The Pathology of Early Stages of Experimental Staphylococcal Pyelonephritis in the Guineapig and Rat. J. Path. Bact. **89,** 657—663. — 10. FREEDMAN, L. R., BEESON, P. B.: (1958) Experimental Pyelonephritis. IV. Observations on Infections Resulting from Direct Inoculation of Bacteria in Different Zones of the Kidney. Yale J. Biol. Med. **30,** 406—414. — 11. GLOOR, F.: (1966) Pathologische Anatomie der Pyelonephritis. In: Die Pyelonephritis. Eds. Losse-Kienitz. Thieme, Stuttgart. Pp. 56—74. — 12. GÖMÖRI, P., SZENDEI, A.: (1959) A chronikus pyelonephritisről. Orv. Hetil. **100,** 965—970. — 13. GORRILL, R. H.: (1956) The Effect of Obstruction of the Ureter on the Renal Localization of Bacteria. J. Path. Bact. **72,** 59—71. — 14. GORRILL, R. H.: (1965) The Fate of Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli in the Mouse Kidney. J. Path. Bact. **89,** 81—88. — 15. GUZE, L. B., BEESON, P. B.: (1956) Experimental Pyelonephritis. I. Effect of Ureteral Ligation on the Course of Bacterial Infection in the Kidney of the Rat. J. Exp. Med. **104,** 803—815. — 16. GUZE, L. B., BEESON, P. B.: (1958) Experimental Pyelonephritis. II. Effect of Partial Ureteric Obstruction on the Course of Bacterial Infection in the Kidney of the Rat and the Rabbit. Yale J. Biol. Med. **30,** 315—319. — 17. GUZE, L. B., GOLDNER, B. H.,

KALMANSON, G. M.: (1961) Pyelonephritis. I. Observations on the Course of Chronic Non-obstructed Enterococcal Infection in the Kidney of the Rat. Yale J. Biol. Med. **33,** 372—385. — 18. HAGEMANN, I., DUTZ, H., BIENENGRÄBER, A., STEFFEN, W.: (1963) Über die Beeinflussung der experimentellen Rattenpyelonephritis durch Oxytetracyclin. Z. ges. exp. Med. **137,** 561—573. — 19. HAMPERL, H.: (1957) Vom Nierenbecken ausgehende (aszendierende) eitrige Nephritis (Pyelonephritis). In: Lehrbuch der allgemeinen Pathologie und der pathologischen Anatomie. Springer, Berlin—Göttingen—Heidelberg, 22—23rd ed. Pp. 622—625. — 20. HARANGHY, L.: (1966) A heveny gennyes pyelonephritis. Az idült pyelonephritis. In: A kórbonctan elemei. Medicina, Budapest, Vol. 2, Pp. 592—594. — 21. HEPTINSTALL, R. H.: (1964) Experimental Pyelonephritis. Ascending infection of the Rat Kidney by Organisms Residing in the Urethra. Brit. J. exp. Path. **45,** 436—441. — 22. HEPTINSTALL, R. H.: (1965) Experimental Pyelonephritis: a Comparison of Blood-borne and Ascending Patterns of Infection. J. Path. Bact. **89,** 71—80. — 23. HEPTINSTALL, R. H., GORRILL, R. H.: (1955) Experimental Pyelonephritis and its Effect on Blood Pressure. J. Path. Bact. **69,** 191—198. — 24. HEPTINSTALL, R. H., STRYKER, M.: (1962) Experimental Pyelonephritis. A Study of the Susceptibility of the Hypertensive Kidney to Infection in the Rat. Bull. Johns Hopkins Hosp. **111,** 292—306. — 25. KETTLER, L. H.: (1965) Pyelonephritis. In: Lehrbuch der speziellen Pathologie. VEB. Gustav Fischer, Jena. Pp. 436—441. — 26. KOVÁCS, K., DÁVID, M.: (1963) Hormonokkal előidézett vesekéregnekrozis. Orv. Hetil. **104,** 1405—1407. — 27. MALLORY, G. K., CRANE, A. R., EDWARDS, J. E.: (1940) Pathology of Acute and of Healed Experimental Pyelonephritis. Arch. Path. **20,** 330—347. — 28. NAVASQUEZ, S. DE: (1950) Experimental Pyelonephritis in the Rabbit Produced by Staphylococcal Infection. J. Path. Bact. **62,** 429—436. — 29. PRÁT, V., BENESOVÁ, D., CERVINKA, F.: (1959) Experimentálni pyelonefritida. II. Vznik svrastelé pyelonefritické ledviny u králika. Čas. Lék. čes. **98,** 461—468. — 30. PRÁT, V., BENESOVÁ, D., PÁVKOVÁ, L., CERVINKA, F.: (1959) The Relationship of Urinary Obstruction to Experimental Chronic Pyelonephritis in the Rabbit. Acta med. scand. **165,** 305—311. — 31. PRÁT, V., PÁVKOVÁ, L., BENESOVÁ, D., CERVINKA, F.: (1958) Experimentálni pyelonephritis. I. Vyznam zuzeni mocovodu pri vylucováni E. coli moci u králiku. Čas. Lék. čes. **97,** 1465—1472. — 32. RÉNYI-VÁMOS, F.: (1952) Über die „lymphogene" Aszension der Pyelonephritis. Acta med. Acad. Sci. hung. **3,** 7—13. — 33. RÉNYI-VÁMOS, F., HORVÁTH, L.: (1962) Neuere experimentelle Angaben zur Entstehung der hämatogenen Pyelonephritis. Acta chir. Acad. Sci. hung. **3,** 261—267. — 34. ROBBINS, S. L.: (1957) Pyelonephritis. In: Textbook of Pathology, ed.: W. B. Saunders, Philadelphia—London, Pp. 915—920. — 35. SAPHIR, O.: (1958) Hydronephrosis, Pyelitis and Destructive Pyelonephritis. In: A Text on Systemic Pathology, ed.: Grune and Stratton, New York — London, Vol. **1,** Pp. 460—465. — 36. SENECA, H., ZINSSER, H. H., LATTIMER, J. K.: (1962) Experimental Enterococcal Pyelonephritis: I. Effect of Tetracycline, Prednisone and Tetracycline plus Prednisone. J. Urol. (Baltimore) **87,** 213—219. — 37. THELEN, A., ROTHER, K., SARRE, H.: (1956) Experimentelle Untersuchungen zur Pathogenese der pyelonephritischen Schrumpfniere. Urol. int. **3,** 359—393. — 38. UEBEL, H.: (1965) Methodik der experimentellen Pyelitis und Pyelonephritis. In: Eichler—Farah—Herken—Welch eds. Handbuch der experimentellen Pharmakologie, Springer, Berlin—Heidelberg—New York Vol. **16,** Part 4. Pp. 338—357. — 39. VIVALDI, E., COTRAN, R., ZANGWILL, D. P., KASS, E. H.: (1959) Ascending Infection as a Mechanism in Pathogenesis of Experimental Non-obstructive Pyelonephritis. Proc. Soc. exp. Biol. Med. (N. Y.) **102,** 242—244. — 40. WOODS, J. W.: (1960) Non-obstructive Escherichia coli Pyelonephritis in the Rat. Proc. Soc. exp. Biol. Med. (N. Y.) **104,** 116—119. — 41. ZOLLINGER, H. U.: (1966) Die Pyelonephritis. In: Doerr—Uehlinger eds. Spezielle pathologische Anatomie. Springer, Berlin — Heidelberg — New York, Vol. **3,** Pp. 433—487.

BEITRÄGE ZUR ENTSTEHUNG DER EXPERIMENTELLEN PYELONEPHRITIS

J. ORMOS, ZS. CSAPÓ und J. LANTOS

An Ratten konnte mittels intravenöser Injektion einer Escherichia-coli-Suspension und 48stündiger Konstriktion des einen Ureters an der abgebundenen Seite bei 61% der Tiere eine zunächst mit Abszeßbildung einhergehende, dann sich in eine Schrumpfniere umwandelnde Pyelonephritis herbeigeführt werden. Die früheste Leukozytenemigration wurde 8 Stunden nach der Einspritzung der Bakteriumsuspension beobachtet, doch war in einzelnen Gefäßen bereits 4 Stunden nach der Injektion bisweilen eine Leukozytenanhäufung zu sehen. Als Ausgangspunkte des pathologischen Prozesses konnten die in der Rinden-, in der Marksubstanz bzw. in der Nierenbeckenwand auftretenden entzündlichen Veränderungen gleicherweise

dienen. Weder die Rinden-, noch die Marksubstanz zeigte eine bedeutende Prädisposition zu anfänglichen Veränderungen. Der Prozeß setzte jeweils im Interstitium ein und pflanzte sich darin fort; die Schädigung des Nierenparenchyms sowie das Eindringen von Leukozyten in das Lumen der Kanälchen waren sekundäre Erscheinungen. In den Glomerula einsetzende Entzündungsprozesse wurden nicht beobachtet, und die Glomerula blieben selbst bei schwerer interstitieller Entzündung und ausgedehntem Kanälchenuntergang gegenüber der Schädigung relativ resistent. Die auf hormonalem Wege durch Nierenischämie herbeigeführte Nierenrindennekrose bewirkte keine erhöhte Empfänglichkeit der Nieren gegenüber den intravenös eingespritzten Krankheitserregern.

ДАННЫЕ К РАЗВИТИЮ ЭКСПЕРИМЕНТАЛЬНОГО ПИЕЛОНЕФРИТА

Й. ОРМОШ, Ж. ЧАПО и Й. ЛАНТОШ

В экспериментах на крысах путем внутривенного введения взвеси кишечной палочки (Escherichia coli) и 48-часового сжатия одного мочеточника, на стороне сжатия в 61% животных удалось вызвать пиелонефрит с образованием абсцессов, преобразовывающийся впоследствии в сморщенную почку. Самая ранняя эмиграция лейкоцитов наблюдалась через 8 часов после введения взвеси микробов. Подчас уже через 8 часов после введения микробов в отдельных сосудах было отмечено накопление лейкоцитов. Исходной точкой патологического процесса равным образом могли послужить воспалительные изменения коркового или мозгового вещества почек, как и стенки почечной лоханки. Значительной предрасположенности к начальным изменениям не наблюдалось ни в корковом слое, ни в мозговом веществе почки. Патологический процесс всегда начинался и распространялся в интерстициальной щели. Поражение паренхимы и попадание лейкоцитов в просвет канальцев были вторичными явлениями. Воспалительного процесса, начавшегося в клубочках, не наблюдалось. Даже при наличии тяжелых интерстициальных воспалительных изменений и распространенного некроза канальцев, клубочки оказались относительно резистентными в отношении поражения. Некроз коркового слоя почек, вызванный гормональным путем посредством почечной ишемии, не повысил восприимчивость почек в отношении патогенных микробов.

Dr. Jenő Ormos
Dr. Zsolt Csapó
Kórbonctani Intézet, Szeged, Hungary
Dr. Judit Lantos; Közegészségügyi és Járványügyi Állomás, Szeged, Hungary

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 103—114, 1968*

Laboratories of the Lakeshore General Hospital and
McGill University, Montreal, Que., Canada

ENDEMIC CARDIOMYOPATHY OF BEER CONSUMERS

G. Rona

Quebec and Omaha investigators have described in heavy beer drinkers an endemic primary myocardiopathy with a characteristic symptomatology. Some cases terminated fatally within 24 hours following hospitalization. The study describes the histopathology of 25 cases. The histopathological changes of the heart are characterized by a combination of acute hyaline myofibre necrosis, chronic vacuolar dystrophic myocardiopathy and the absence of any significant reactive change.

Over a period extending from August, 1965, to May, 1966, fortyseven male patients were studied in Quebec City presenting the following combination of clinical findings: heavy beer drinkers, shortness of breath, epigastric pain, greatly reduced cardiac output, congestive heart failure and electrocardiographic abnormalities [1]. Twenty patients died soon after admission whereas most of the others, who survived the first 24 hours in the hospital, recovered. The patients almost exclusively were residents of the same section of Quebec City, called Lower Town situated between the St. Lawrence river and the high bluff, known as Cape Diamond [2].

Coinciding with the Quebec outbreak a similar syndrome was reported by Omaha investigators [3]. All twenty-six patients studied were young or middle aged males, who had a history of drinking. The ingestion of the alcoholic beverage, primarily beer, ranged from 27 to 144 ounces daily for 10 to 15 years. The syndrome was characterized by acute dyspnoea and weakness followed by predominantly right sided congestive failure. Other findings were oedema, hepatic tenderness, tachycardia, hypotension, electrocardiographic abnormalities, marked cardiomegaly and pericardial effusion. Laboratory findings in some patients indicated thiamine deficiency and lactic acid acidosis. Ten patients died within 72 hours of admission. At autopsy the cardiac change was considered to be nonspecific diffuse myocardiopathy or myocarditis. A pertinent pathological finding was severe centrilobular hepatic necrosis and congestion. In 8 survivors biopsy revealed a varying degree of cirrhosis. These hepatic changes suggested a nutritional deficiency secondary to excessive alcohol ingestion.

Material and method

Early in the outbreak of Quebec cases, the author through the courtesy of Dr. Jean L. BONENFANT, Associate Professor in Pathology, Laval University, had the opportunity to study the histopathological changes in 17 autopsied cases. When the Omaha cases had become known, he also investigated the autopsy protocols and slides available to May 1966 at the Omaha Veterans Administration Hospital through the courtesy of Dr. James F. SULLIVAN, Professor of Medicine, Creighton University. These two groups are comparable and for this reason they are discussed together in the present paper.

I. Autopsy findings

The heart was generally enlarged. The heart weight in Quebec ranged from 350 to 690 g, and in the Omaha series 425 to 600 g. The average weight was 500 and 538 g respectively with a combined average of 512 g (Table 1).

Table 1

No. of cases	Quebec	Omaha	Combined
	17	8	25
Heart			
Infarct	1	—	1
Hypertrophy	13	8	21
Average weight, g	500	538	512
Coronary sclerosis	6	2	8
Mural thrombi	4	2	6
Hydrothorax	15	5	20
Hydropericardium	13	4	17
Ascites	11	2	13
Thrombo-embolic phenomena	6	2	8
Lung			
Congestion	8	7	15
Haemorrhage	5	—	5
Infarct	2	1	3
G.I. tract			
Haemorrhagic oesophagitis	5	4	9
Gastric or duodenal ulceration	2	3	5
Gastric haemorrhage	9	3	12
Liver			
Congestion	11	3	14
Steatosis	4	—	4
Cirrhosis	1	1	2
Splenomegaly	3	1	4
Lipoid nephrosis	2	—	2

The cardiac chambers were dilated. The myocardium was flabby and pale. In 8 cases there was mild to moderate degree of coronary atherosclerosis. The lumen of the coronary arteries, with one exception, did not show any significant reduction. In one case a macroscopic infarct was discernible, which apparently was unrelated to occlusive coronary artery disease. Mural endocardial thrombi were present in 6 cases.

The most frequent extracardiac findings were fluid accumulation in serous cavities in the form of hydrothorax, hydropericardium and ascites, and thrombo-embolism involving the renal, iliac, femoral arteries and abdominal aorta; the branches of the pulmonary artery were the site of thrombosis or embolism leading in 6 cases to haemorrhagic consolidation and infarction of the lungs. Gastrointestinal lesions were frequent in the form of diffuse haemorrhagic necrosis of the oesophagus, acute peptic gastric and duodenal ulcers and erosive gastritis with haemorrhage. Hepatic lesions encountered were congestion (14 cases), steatosis (4 cases) and nutritional cirrhosis (2 cases) associated in 1 case with haemosiderosis. Splenomegaly (4 cases) and lipoid nephrosis (2 cases) were other findings.

II. Histopathological studies

1. Cardiac changes

Based on the study of 25 cases the components of the cardiac lesion can be classified into three categories: acute and chronic retrogressive myocardial alteration and reactive changes of the stromal elements (Table 2).

A) Acute myocardial alteration in the form of hyaline necrosis was observed in 14 cases. The lesion involved all chambers equally; no specific subendocardial predilection characteristic of anoxaemic myocardial necrosis was noted. In 3 cases the necrosis was infarct-like and in 11 cases focal (Fig. 1). Although cellular reaction was insignificant, the site of the lesion was distinctly demarcated from the adjacent intact areas by cytoplasmic eosinophilia (Fig. 2). In PAS sections there was disappearance of intracytoplasmic glycogen. PTAH stain revealed a loss of cross striation. The alteration of the contractile elements was more advanced than were the nuclear changes. The enlarged, irregular nuclei presented a condensation of chromatin; with progression of the necrobiotic process together with the contractile elements the nuclei also disappeared.

B) Vacuolar degeneration was the most conspicuous chronic myofibre change (Fig. 3). The cytoplasmic vacuoles were generally multiple and particularly prominent on cross section and less apparent in longitudinal sections. The vacuoles were different in shape and size and produced a fusiform swelling

Table 2

Components of histological changes

	Quebec	Omaha	Combined
No. of hearts examined	17	8	25
A) *Acute myocardial changes*			
Necrosis	12	2	14
focal	9	2	11
infarct-like	3	—	3
B) *Chronic myocardial changes*			
Vacuolar change	17	8	25
Lipochrome	5	3	8
Hypertrophy	6	4	10
Atrophy and shrinkage	12	6	18
Dystrophy	12	6	18
Myocytolysis and fading	13	5	18
Empty sarcolemmal sheath	12	7	19
C) *Reactive changes*			
Oedema	6	4	10
Focal inflammation	5	4	9
Fibrosis	4	2	6
Capillary proliferation	7	3	10
Arteriolar swelling	8	5	13

of the muscle fibres (Fig. 4). A segment of certain muscle bundles rather than certain specific layers of the myocardium was effected. Swelling, uneven staining of the cytoplasm and disorganization of the contractile elements can be interpreted as evidence of myofibre dystrophy. Groups of enlarged fibres showed coarse irregular myofibrils. The latter presented a characteristic peripheral arrangement leaving at the central portion of the distended muscle fibres a pale structureless basophilic area. Less frequently the contractile elements exhibited a haphazard distribution. Some other fibres were shrunken and exhibited a dense basophilic cytoplasm (Fig. 5). There was an increase of lipochrome content of the fibres. The nuclei were of irregular shape and size and rich in chromatin. Dystrophic and vacuolar fibres alternated (Fig. 6). The fading fibres and empty sarcolemmal sheaths gave to the myocardium a moth-eaten appearance (Fig. 7). In other foci, together with contractile elements and other cytoplasmic components the sarcolemmal sheaths also disappeared leading to myocytolysis. Fat tissue replacement (lipomatosis) of the vanished myofibres completed the process. Frozen sections stained with

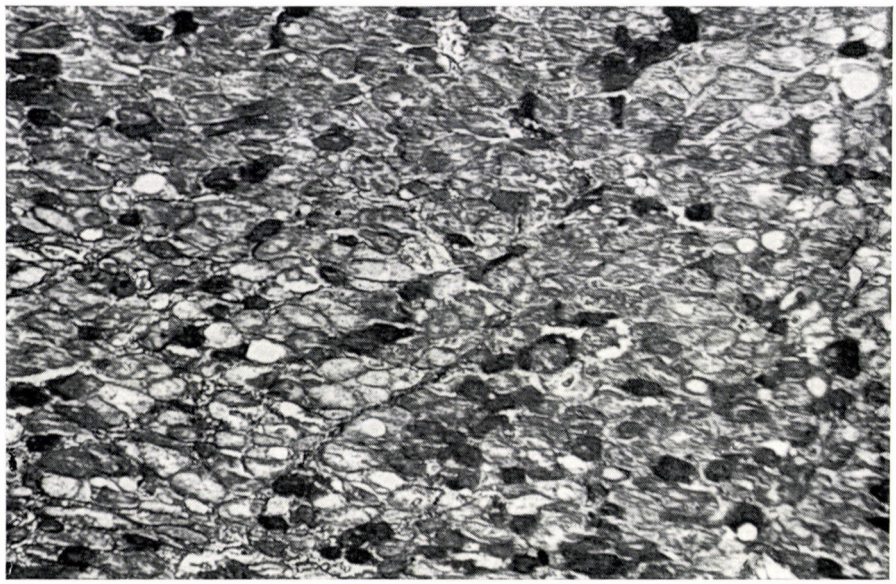


Fig. 1. The darkly staining myocardial fibres are devoid of cross striation and represent focal hyaline myofibre necrosis. PTAH. ×125

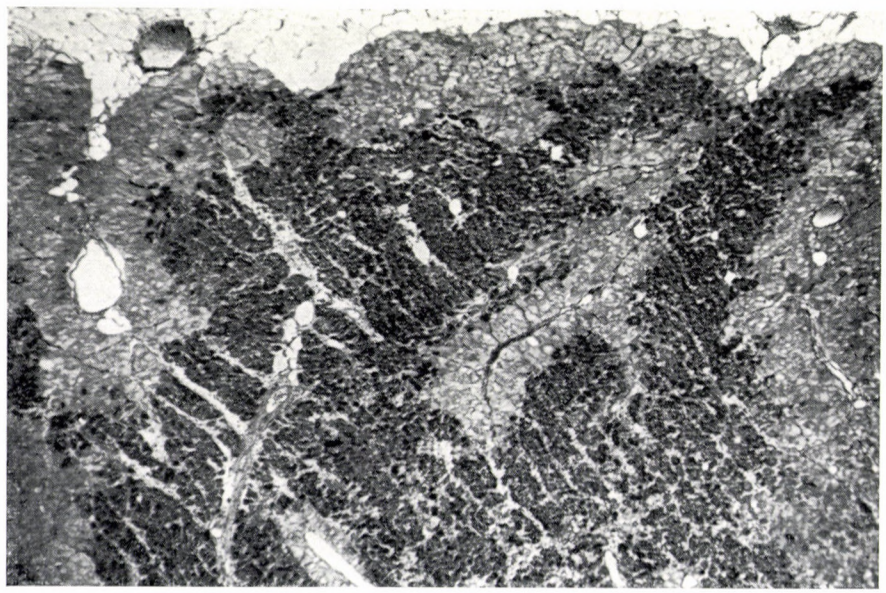


Fig. 2. Massive infarct-like well demarcated myocardial necrosis. PTAH. ×40

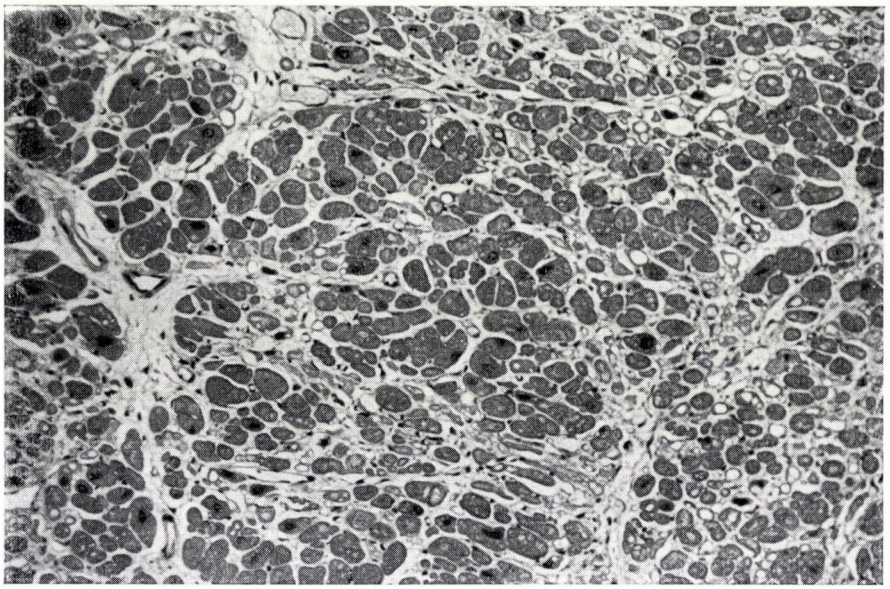


Fig. 3. Vacuolar degeneration of the myocardial fibres. H. et E. ×250

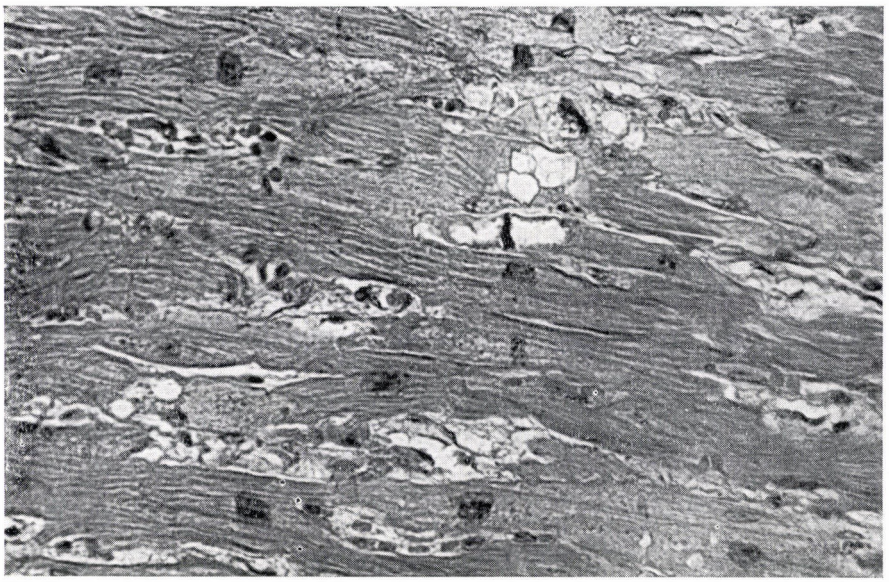


Fig. 4. Intracytoplasmic vacuoles situated along the longitudinal axis and leading to fusiform swelling of the muscle fibres. H. et E. ×125

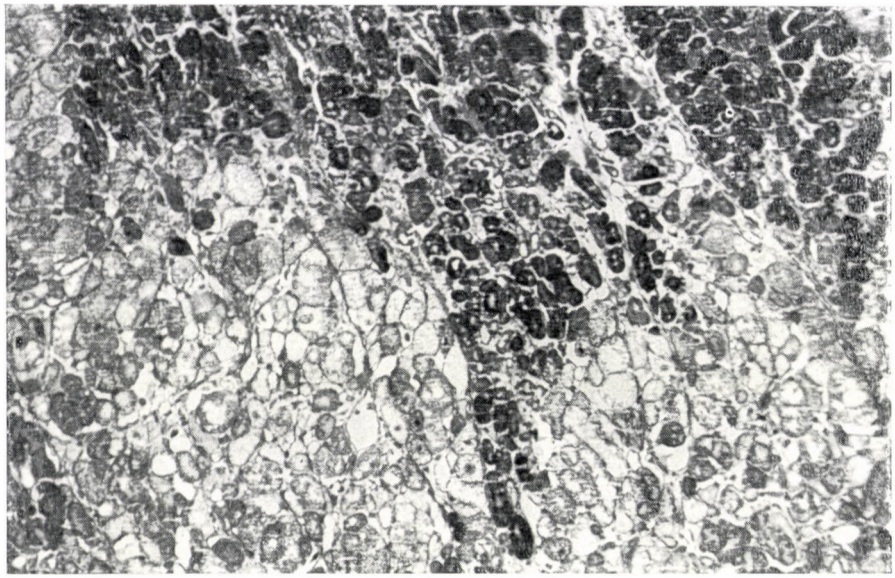


Fig. 5. The combination of acute and chronic myocardial changes with muscle fibres presenting dense basophilic cytoplasm. PTAH. ×400

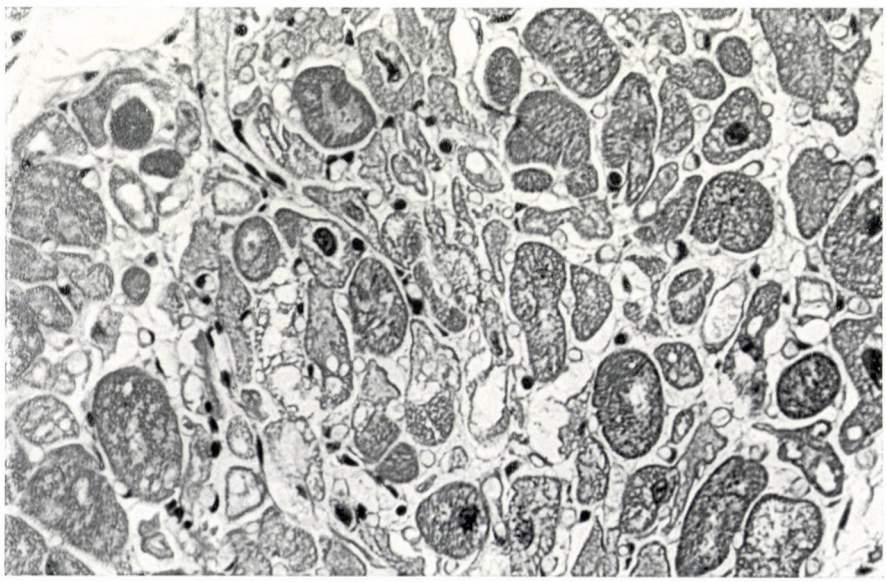


Fig. 6. Dystrophic myocardial fibres presenting peripheral condensation of myofibres. PTAH. ×125

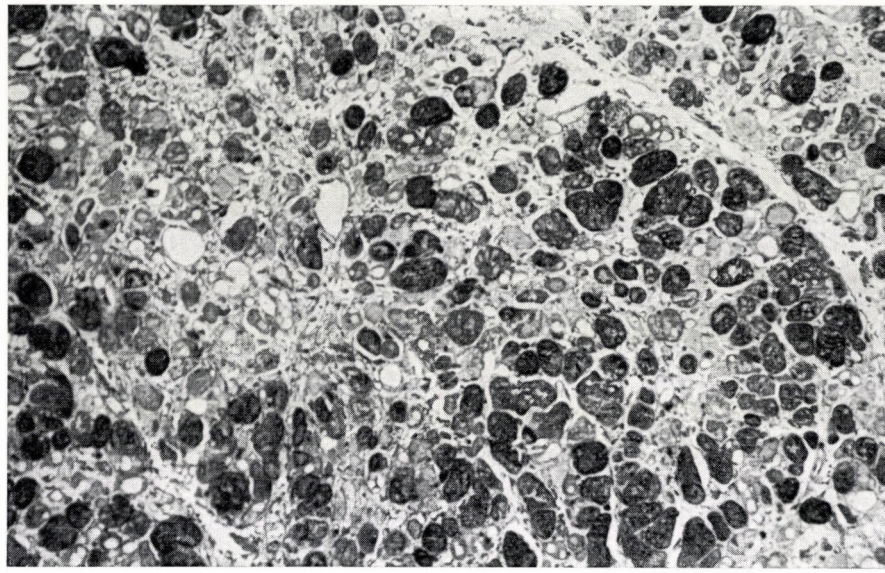


Fig. 7. Empty sarcolemmal sheaths and moth-eaten appearance of the myocardium. PTAH. ×125

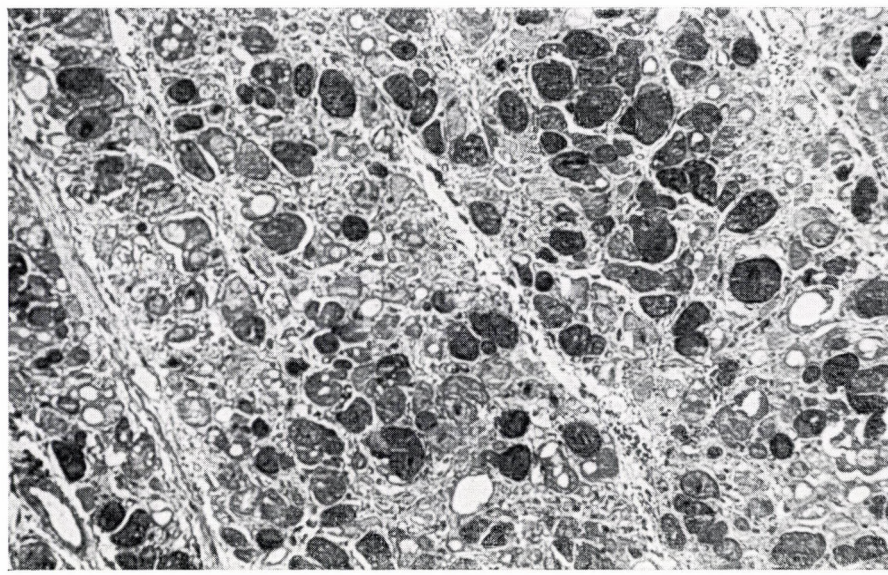


Fig. 8. Disappearance of myocardial fibres leading to collapse fibrosis. PTAH. ×125

Sudan IV were available in 7 cases. In all of these fatty metamorphosis of varying intensity was noted in the form of fine intracytoplasmic droplets.

C) The pertinent feature of the myocardial lesions was the remarkable lack of reactive changes. Associated with hyaline necrosis there was edema and minimal cellular reaction, consisting mainly of mononuclear cells. Pronounced cellular reaction was recognized only in one case, which showed eosinophils, histiocytes and mast cells; this finding was regarded as an exception. The apparent fibrosis in chronic cases resulted from the myofibre collapse rather than from fibrous connective tissue proliferation (Fig. 8). The scars contained engorged capillaries.

A peculiar pathognomonic change was noted in the small myocardial arteries, arterioles and capillaries. The wall of the involved vessels was swollen. The central portion was occupied by a watery substance which separated the intimal and adventitial layers apart and gave rise to the formation of a double contour image (Fig. 9).

2. Extracardiac lesions

The most frequent extracardiac lesion was widespread central or confluent hepatic necrosis associated with fatty metamorphosis and minimal cellular reaction (Fig. 10). In two cases the hepatic changes were consistent with nutritional cirrhosis. Sections from the lung showed congestion, oedema and haemosiderosis. There was no significant thickening of the alveolar septa. Other histopathological findings were consistent with, and supported, the gross observations.

Discussion

Even though the association of chronic alcoholism and heart disease has been known for a long time, the clinical findings and the sudden unexpected death of beer drinkers in Quebec and Omaha were regarded as a medical mystery [2, 4]. The role of beer in producing cardiac damage is reflected by the term "Münchener Bierherz" described in 1884 [5]. The real nature of this entity has never been clarified. An undue number of cardiomyopathy cases in alcoholics were reported by BRIGDEN and ROBINSON [6] in 1964; one third of their patients were exclusively beer drinkers with an average daily consumption of 8.5 litres. Alcoholic cardiomyopathy, however, usually accompanies ingestion of concentrated alcoholic beverages rather than beer [7] and the ensuing myocardial hypertrophy, fibrosis and cellular reaction [8] as well as interstitial and intracytoplasmic oedema [9] are quite different from the extensive necrobiotic myocardial alteration herein described and designated by BONENFANT [10] as myocardosis.

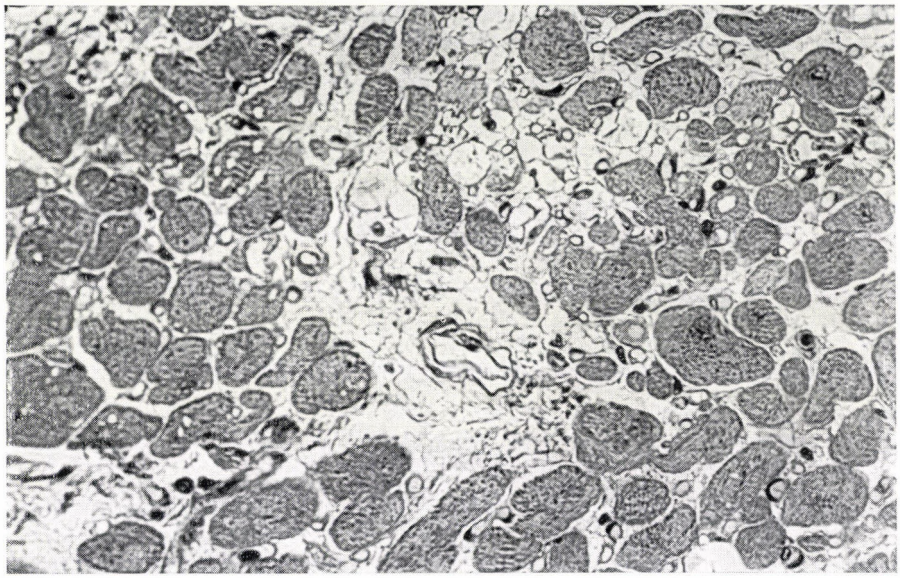


Fig. 9. The characteristic double contour image of a small artery formed by the compressed intimal and adventitial coats. PTAH. ×400

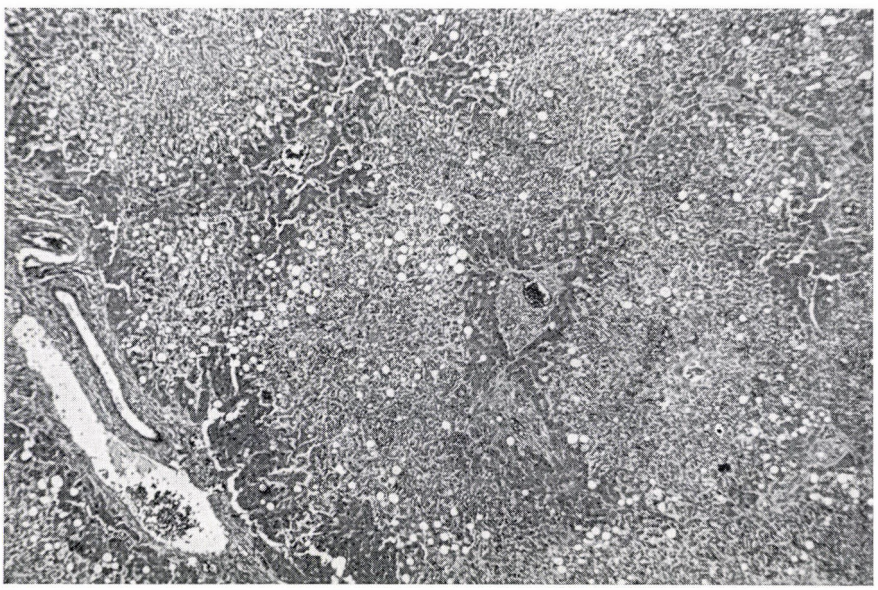


Fig. 10. Widespread hepatic necrosis associated with fatty change. H. et E. ×40

Chronic alcoholism results in thiamine deficiency. In this hemisphere the alcohol conditioned beri-beri heart disease differs considerably from the classical syndrome described in the Orient [11]. Evidences for differentiating the clinical symptomatology [12, 13] and pathological changes [14, 15] of alcoholic cardiomyopathy and beri-beri heart are inconclusive [16]. HUDSON [17] who regards alcoholic cardiomyopathy as a clinical entity doubts its specific pathological nature. Alcoholic heart disease prevails in spite of the fact that brewers are well aware of the slur cast on their products for deficient content in thiamine and want to improve this condition.

The endemic cardiomyopathy of beer consumers resembled clinically acute fulminating (Shoshin) cardiac beri-beri [18]. In follow-up studies, however, thiamine had not therapeutic effect, the recovery of patients was prolonged and some cardiac changes were irreversible [2, 19]. The histopathology was vaguely comparable with that of other rare chronic smouldering myocardial damage such as observed in cardiomyopathy of pregnancy [20], muscular dystrophy [21, 22], and myocardial disease of undetermined aetiology occurring in Cali, Columbia [23]; nevertheless the combination of hyaline myofibre necrosis, chronic dystrophic and vacuolar myocardial changes and the absence of any significant cellular reaction and fibroblastic response together with the peculiar double contour appearance of the myocardial blood vessels could be regarded as pathognomonic for the present cases. The characteristic myocardial pathology allowed the recognition of new cases and helped in the identification of the Omaha series [19].

BRIGDEN and ROBINSON [6] suggested that in addition to the direct toxic effect of alcohol other factors play a role in conditioning and perpetuating the cardiac process. The morphological changes observed in the present series similarly point to a complex pathomechanism. For this reason in experimental studies several conditions which may be implicated in the evolution of the human syndrome are being investigated. These are the direct toxic effect of alcohol and beer or beer components, associated infection or altered immune response, interference with the nutrition and intracellular metabolism, thiamine deficiency and blocking of an enzyme or enzyme systems by the beer constituents. It is hoped that a proper experimental model could be evolved that may help to elucidate the nature of the mysterious cardiomyopathy and death cases of beer consumers.

Acknowledgement

The author wants to express appreciation to Dr. J. L. BONENFANT, Associate Professor in Pathology, Laval University, Quebec; Dr. James F. SULLIVAN, Professor of Medicine, Creighton University, Omaha; and Dr. C. I. CHAPPEL, Director, Bio-Research Laboratories, Montreal; for allowing a study of their clinical data and pathological material. Acknowledgement is made to Mrs. Gisela HENDERSON for her help in preparing this manuscript.

REFERENCES

1. MORIN, Y.: (1966) A New Syndrome. Letter to the Editor, Canad. med. Ass. J., **94,** 1062. — 2. Editorial: (1966) Quebec's Medical Mystery. J. Amer. med. Ass. **196,** 25—26. — 3. MCDERMOTT, P. H., DELANEY, R. L., EGAN, J. D., SULLIVAN, J. F.: (1966) Nutritional Deficiency in Myocardiopathy, American Society for Clinical Nutrition, 6th Annual Meeting, April 30th. — 4. Editorial: (1966) Quebec's Medical Mystery Spreads. J. Amer. med. Ass. **197,** 27—28. — 5. BOLLINGER, O.: (1884) Ueber die Häufigkeit und Ursachen der idiopathischen Herzhypertrophie in München, Dtsch. med. Wschr. **10,** 180. — 6. BRIGDEN, W., ROBINSON, J.: (1964) Alcoholic Heart Disease, Brit. med. J., **2,** 1283—171. — 7. EVANS, W.: (1964) Alcoholic Myocardiopathy. Prog. Cardiovasc. Dis. **7,** 151—171. — 8. ELIASER, M., GIANSIRACUSA, F. J.: (1956) The Heart and Alcohol. Calif. Med., **84,** 234. — 9. PINTAR, K., WOLANSKYJ, B. M., GUBBAY, E. R.: (1965) Alcoholic Cardiomyopathy, Canad. med. Ass. J., **93,** 103—107. — 10. BONENFANT, J. L.: (1966) Personal Communication. See also No. 2. — 11. BENCHIMOL, A. B., SCHLESINGER, P.: (1953) Beriberi Heart Disease. Amer. Heart J., **46,** 245—262. — 12. GUBBAY, E. R.: (1966) Beriberi Heart Disease. Canad. med. Ass. J., **95,** 21—27. — 13. ALEXANDER, C. S.: (1966) Idiopathic Heart Disease. I. Analysis of 100 Cases, with Special Reference to Chronic Alcoholism. Amer. J. Med., **41,** 213—228. — 14. FERRANS, V. J., HIBBS, R. G., WEILBAECHER, D. G., BLACK, W. C., WALSH, J. J., BURCH, G. E.: (1965) Alcoholic Cardiomyopathy, a Histochemical Study. Amer. Heart J. **69,** 748—765. — 15. ALEXANDER, C. S.: (1966) Idiopathic Heart Disease, II. Electron Microscopic Examination of Myocardial Biopsy Specimens in Alcoholic Heart Disease. Amer. J. Med. **41,** 229—234. — 16. FERRANS, V. J.: (1966) Alcoholic Cardiomyopathy. Amer. J. med. Sci. **252,** 123—138. — 17. HUDSON, R. E. B.: (1965) Cardiovascular Pathology. Arnold, London. Pp. 899—907. — 18. WOLF, P. L., LEVIN, M. B.: (1960) Shöshin Beriberi. New Engl. J. Med., **262,** 1302—1306. — 19. MCDERMOTT, P. H., DELANEY, R. L., EGAN, J. D., SULLIVAN, J. F.: Myocardosis in Beer-Drinkers. To be published. — 20. CONNOR, R. C. R., HUME ADAMS, J.: (1966) Importance of Cardiomyopathy and Cerebral Ischaemia in the Diagnosis of Fatal Coma in Pregnancy. J. Clin. Path., **19,** 244—249. — 21. BAJUSZ, E., HOMBURGER, F.: (1966) Myopathies. Science **152,** 1112—1113. — 22. PERLOFF, J. K., DELEON, JR. A. C., O'DOHERTY, D.: (1966) The Cardiomyopathy of Progressive Muscular Dystrophy. Circulation. **33,** 625—648. — 23. CORREA, P., RESTREPO, C., GARCIA, C., QUIROZ, A. C.: (1963) Pathology of Heart Diseases of Undetermined Etiology which Occur in Cali, Columbia. Amer. Heart J. **66,** 584—596.

ENDÄMISCHE KARDIOMYOPATHIE DER BIERTRINKER

G. RONA

Forscher in Quebec und Omaha beobachteten bei starken Biertrinkern eine endemische primäre Kardiomyopathie mit charakteristischer Symptomatologie. Einige Fälle endeten tödlich innerhalb von 24 Stunden nach der Hospitalisation. Die Histopathologie von 25 Fällen wird beschrieben. Die histopathologischen Veränderungen des Herzens waren durch akute hyaline Muskelfasernekrose, chronische vakuoläre dystrophische Myokardiopathie und das Fehlen signifikanter reaktiver Veränderungen gekennzeichnet.

ЭНДЕМИЧЕСКАЯ КАРДИОМИОПАТИЯ У ЛИЦ, ПОТРЕБЛЯЮЩИХ БОЛЬШОЕ КОЛИЧЕСТВО ПИВА

Г. РОНА

Квебекские и Омахайские исследователи описали случаи эндемической первичной кардиопатии у больных с «пивным сердцем» с характерной симптоматологией. Некоторые случаи в пределах 24 часов после госпитализации кончились смертью. Дается описание гистопатологии 25 случаев. Гистопатологические изменения сердца характеризуются сочетанием острого гиалинового некроза мышечных волокон, хронической вакуольной дистрофической миокардиопатии и отсутствия достоверных реактивных изменений.

Dr. G. RONA; Laboratories of the Lakeshore General Hospital and McGill Univ., Montreal, Que, Canada

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 115—119, 1968*

First Department of Pathological Anatomy and Experimental Cancer Research (Director: Prof. J. Baló), University Medical School, Budapest

AUTORADIOGRAPHIC INVESTIGATION OF DNA SYNTHESIS IN THE CHROMOSOMES OF THE JBK ASCITES SARCOMA

M. Sellyei

DNA synthesis of hyperdiploid cells of JBK ascites sarcoma was investigated by autoradiography after the administration of ^{3}H-thymidine. In telocentric chromosomes the parts distant from the centromere were found to replicate later. The early replication of the parts around the centromere speaks for the formation of the mediocentric marker chromosome by means of translocation.

Ascites sarcoma JBK was developed by Juhász, Baló and Kendrey, [6] by administering to mice a 1% solution of amobarbital intraperitoneally. In seven months, one of the animals developed retroperitoneal sarcoma which was successfully transplanted subcutaneously, then transferred into ascitic form and maintained through more than 500 passages. The tumour proved suitable in experimental work. Kövi and Németh reported on its cytology and growth conditions [7], further on the chromosomes of the tumour cells [8]. The stem-line chromosome count turned out to be 43. The tumour cells contained two long marker chromosomes, different from each other and similar to the chromosomes type A and B, respectively, observed in Ehrlich's hyperdiploid ascites tumour [1]. There occurred 2—3 minute chromosomes as well. The rest of chromosomes were telocentric with no difference either in size or form from the normal chromosomes of the mouse [9]. In about 15% of mitotic cells in JBK ascites sarcoma, the number of chromosomes ranged near the tetraploid value of 80.

The DNA which is of the greatest importance in determining hereditary qualities of the cells is contained in the nuclei — i.e. during mitosis, in the chromosomes. DNA contains thymine and RNA contains uracyl, whilst three other nucleotides (adenine, guanine and cytosine) are ingredients of both DNA and RNA. For the exact investigation of chromosome replication Taylor *et al.* [17] recommended to use tritium-labelled thymidine which becomes built into DNA during its synthesis in an elective way. Tritium is emitting beta-rays, the spreading distance of electrones in photosensitive emulsion being about 3 μ [15]. Thus, the site of incorporated ^{3}H-thymidine can be detected by autoradiography [13].

Lima-de-Faria [10] was the first to show that in the chromosomes of the spermatocytes of the grasshopper Melanoplus differentialis, DNA synthesis

takes place in an asynchronous way. A delayed synthesis of DNA by genetically inactive chromosomes could be observed in mammalian tissue cultures too, since there was an increased uptake of ^{3}H-thymidine at the end of the phase of synthesis [4, 11, 12, 16].

The aim of the present investigation was to characterize the chromosomes of JBK ascites sarcoma from the point of view of DNA replication.

Material and methods

White mice weighing 22—24 g and from our own breed were inoculated intraperitoneally using a suspension containing $2 \cdot 10^6$ cells of JBK ascites sarcoma. 10—12 days after the implantation, 70 μC of ^{3}H-thymidine (Cea-Cen-Sorin, Mol, Belgium; Activity: 12 000 mC/mM) were administered intraperitoneally, when then 6, 10 and 14 hours later the animals were sacrificed. 4—5 hours after a solution containing 25 μg demecolcine was injected intraperitoneally. The ascites was centrifuged and the cells were incubated at 37 °C for 20 minutes in hypotonic saline. After renewed centrifuging the cells were fixed in absolute ethanol and glacial acetic acid 3 : 1, resuspended in 60% acetic acid, and spread on slides covered with chromegelatin. The preparations were stained with acetic orcein, dried and covered with Kodak AR.10 stripping film. After 14—30 days exposition, the slides were developed, and 100 mitotic cells of each animal were examined for ^{3}H-labeling. Well labeled mitoses were photographed, then the silver grains and the gelatin removed according to BIANCHI *et al.* [2], the preparations restained and the mitoses photographed again.

Results

In comparison with the previous observation [7], an increased rate of tetraploid cells was found. Varying from animal to animal, 20—40% of the mitotic cells contained 72—84 chromosomes while 20—24% of the cells, 43 chromosomes. This can be considered the JBK ascites sarcoma's modality count. Chromosome counts of the rest of the mitoses were also within the diploid range.

We found only one labeled mitosis out of 200 mitotic cells (0.5%) when examined 6 hours after the administration of ^{3}H-thymidine. After 10 hours the rate was 18%, and after 14 hours, 38%. The mitoses took up the labeled substance with different intensity. In telocentric chromosomes, uptake was more expressed in the parts far from the centromere than in those around it (Fig. 1). The chromatides of the marker chromosome type A showed a secondary constriction (the primary one indicating the centromere). Above it, in spite of a lower DNA content, the intensity of labeling was similar to that of the neighbouring parts. The mediocentric marker chromosome type B took up the ^{3}H-thymidine poorly in the region of the centromere, and a difference was obvious between the two arms (Figs 1, 2). Most minute chromosomes were labeled.

In the marked mitotic cells we failed to find those typical, "hot" chromosomes, characterized by late replication and by massive uptake of ^{3}H-thymidine, and observed e.g. in tumours of the golden hamster [5].

Discussion

According to POTTER, ^{3}H-thymidine is rapidly built into the cellular DNA; otherwise it becomes disintegrated and eliminated within one hour. Consequently, the labeled mitoses in our material received ^{3}H-thymidine only

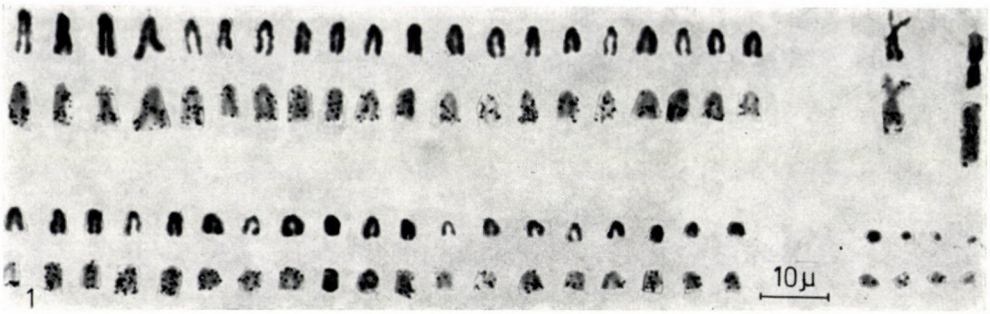


Fig. 1. Karyotype of JBK ascites sarcoma cell. Right: group of marker chromosomes. Bottom: same chromosomes after ^{3}H-thymidine autoradiography. Distal parts of telocentric chromosomes show slightly increased ^{3}H-thymidine uptake

for a short period of DNA synthesis — namely in a later one, since even 14 hours after the administration of labeled thymidine only 38% of the mitotic cells showed uptake. It follows that the unlabeled parts of the chromosomes had become replicated before administration of ^{3}H-thymidine.

In telocentric chromosomes, the parts around the centromere took up less of the labeled substance than the distal parts of chromatides. The environs of the centromere in the type B marker chromosome remained unlabeled (Fig. 2). This phenomenon suggests the formation of this mediocentric chromosome by the fusion of two telocentric forms (translocation).

The constriction of the type A marker chromosome showed no decrease of ^{3}H-thymidine uptake in contrast to expectations. This might have been due to the lateral radiation of tritium in the neighbouring parts of the chromosome resulted in the appearance of silver grains above the secondary constriction.

EVANS *et al.* [3] found that in the chromosomes of the mouse's somatic cells (bone marrow, spleen, thymus) in the later phase of DNA synthesis the ^{3}H-thymidine was built in especially into the proximal parts of the chromosome, near the centromere. The case was just the opposite in the telocentric chromosomes of JBK ascites sarcoma. The aim of our further investigations will be to clarify whether the late replication of the distal parts of chromosomes represents a phenomenon characteristic of the JBK tumour.

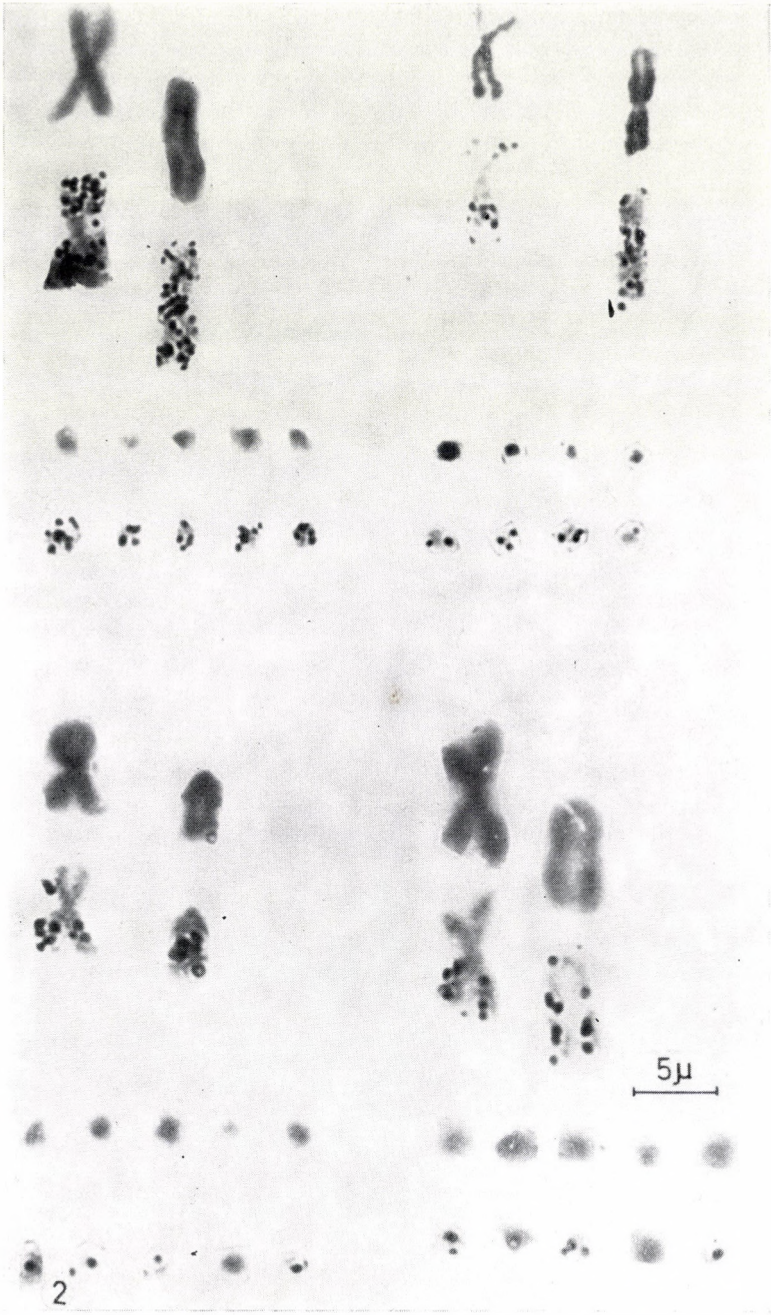


Fig. 2. Marker chromosomes of four tumour cells presented separately. The upper arm of the long metacentric chromosome incorporated relatively little amount, while the region around the centromere, no ^{3}H-thymidine

REFERENCES

1. Bayreuther, K.: (1952) Der Chromosomenbestand des Ehrlich-Ascites-Tumors der Maus. Z. Naturforsch. **7/B,** 554. — 2. Bianchi, N., Lima-de-Faria, A., Jaworska, H.: (1964) A Technique for Removing Silver Grains and Gelatin from Tritium Autoradiographs of Human Chromosomes. Hereditas (Lund) **51,** 207. — 3. Evans, H. J., Ford, C. E., Lyon, M. F., Gray, J.: (1965) DNA Replication and Genetic Expression in Female Mice with Morphologically Distinguishable X Chromosomes. Nature (Lond.) **206,** 900. — 4. Gilbert, C. W., Muldal, S., Lajtha, L. G., Rowley, J.: (1962) Time-sequence of Human Chromosome Duplication. Nature (Lond.) **195,** 869. — 5. Haemmerli, G., Zweidler, A., Stäuli, P.: (1966) Transplantation Behaviour and Cytogenetic Characteristics of a Spontaneous Reticulum Cell Sarcoma in the Golden Hamster. Int. J. Cancer **1,** 599. — 6. Juhász, J., Baló, J., Kendrey, G.: (1955) Ein neuer experimenteller Geschwulststamm: das Amytal-Ascitessarkom. Acta morph. Acad. Sci. hung. **5,** 243. — 7. Kövi, J., Németh, Gy.: (1961) Az amytal-ascites-sarkoma cytológiája és növekedési viszonyai. Magy. Onkol. **5,** 102. — 8. Kövi, J., Németh, Gy.: (1961) Über vergleichende Chromosomenuntersuchungen an den Zellen des Amytal-Aszitessarkoms und des tetraploiden Ehrlich-Aszitestumors. V. Ungarische Krebstagung, Akademie Verlag, Budapest. Pp. 112—115. — 9. Levan, A., Hsu, T. C., Stich, H. F.: (1962) The Idiogram of the Mouse. Hereditas (Lund) **48,** 677. — 10. Lima-de-Faria, A.: (1959) Differential Uptake of Tritiated Thymidine into Hetero- and Euchromatin in Melanoplus and Secale. J. biophys. biochem. Cytol. **6,** 457. — 11. Moorhead, P. S., Defendi, V.: (1963) Asynchrony of DNA Synthesis of Human Diploid Cells. J. Cell Biol. **16,** 202. — 12. Morishima, A., Grumbach, M. M., Taylor, J. H.: (1962) Asynchronous Duplication of Human Chromosomes and the Origin of Sex Chromatin. Proc. nat. Acad. Sci. (Wash.) **48,** 756. — 13. Perry, R. P.: (1964) Quantitative Autoradiography. In Prescott, D. M. (ed.): Methods in Cell Physiology. Vol. I. Academic Press, New York. Pp. 305—326. — 14. Potter, V. R.: (1959) Metabolic Products Formed from Thymidine. In Stohlman, F. (ed.): The Kinetics of Cellular Proliferation. Grune and Stratton, New York. Pp. 104—113. — 15. Schmid, W.: (1965) Autoradiography of Human Chromosomes. In Yunis, J. J. (ed.): Human Chromosome Methodology. Academic Press, New York. Pp. 91—110. — 16. Taylor, J. H.: (1960) Asynchronous Duplication of Chromosomes in Cultured Cells of Chinese Hamster. J. biophys. biochem. Cytol. **7,** 455. — 17. Taylor, J. H., Woods, P. S., Hughes, W. L.: (1957) The Organization and Duplication of Chromosomes as Revealed by Autoradiographic Studies Using Tritium-labeled Thymidine. Proc. nat. Acad. Sci. (Wash.) **43,** 122.

AUTORADIOGRAPHISCHE UNTERSUCHUNG DER DNS-SYNTHESE IN DEN CHROMOSOMEN DES JBK-ASCITES-SARKOMS

M. SELLYEI

Die DNS-Synthese in den hyperdiploiden Zellen des JBK-Ascites-Sarkoms wurde nach Einführung von ^{3}H-Thymidin mit der autoradiographischen Methode untersucht. Die Replikation der vom Zentromer entfernter liegenden telozentrischen Chromosomen erfolgt relativ später. Durch die frühe Replikation der um das Zentromer liegenden Teile wird das im Wege der Translokation vor sich gehende Entstehen der mediozentrischen Marker-Chromosomen bestätigt.

АВТОРАДИОГРАФИЧЕСКОЕ ИССЛЕДОВАНИЕ СИНТЕЗА ДЕЗОКСИРИБОНУКЛЕИНОВОЙ КИСЛОТЫ В ХРОМОСОМАХ АСЦИТНОЙ САРКОМЫ ЙБК

М. ШЕЙЕИ

Синтез дезоксирибонуклеиновой кислоты гипердиплоидными клетками асцитной саркомы ЙБК исследовался методом авторадиографии после введения H^3-тимидина. Репликация находящихся более отдаленно от центромеры участков телоцентрических хромосом происходит сравнительно позже. Ранняя репликация участков, находящихся около центромеры, подкрепляет предположение о возникновении медиоцентрической хромосомы-маркера путем транслокации.

Dr. Mihály Sellyei; Budapest, VIII. Üllői út 26. Hungary

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 121—128, 1968*

Department of Morphology (Head: Prof. I. Törő), Institute of Experimental Medicine (Director: Prof. I. Rusznyák), Hungarian Academy of Sciences, Budapest

ULTRASTRUCTURAL LOCALIZATION OF A CRYSTALLINE SUBSTANCE IN THE ADRENAL ZONA FASCICULATA OF THE RAT

D. Szabó

Electron microscopy showed the intracellular occurrence of a crystalline substance assuming the shape of a longish rectangular body in adult rats with a normally, less than normally, and excessively functioning zona fasciculata, as well as in that of 21-day-old rat embryos. The rectangular bodies are electron-optically empty and closely surrounded by an electron-dense substance of acid phosphatase activity. The result of the digitonin reaction adapted for electron microscopy makes it seem probable that the crystalline substance is cholesterol or a cholesterol derivative.

It has been known for more than half a century that in addition to the sudanophil substance seen under the light microscope the adrenal cortex contains birefringent crystals detectable by polarization microscopy (Albrecht and Weltmann [1], Landau and McNee [10], Hoerr [8], Bennett [4], Yoffey and Baxter [20]).

In recent years the sudanophil lipid droplets and the osmiophil lipid droplets, observed with the electron microscope, were found to be identical. Whereas several authors (Belt [2, 3], Zelander [21], Carr [5], Yamori, Matsuura and Sakamoto [18], Nishikawa, Murone and Sato [13], Holzmann and Lange [9]) have studied the lipids and the change in their amount in various functional states of the adrenal cortex, no attempts have, to our knowledge, been made to identify by means of electron microscopy the crystal substance demonstrable in the cortex with the polarization microscope.

In earlier electron microscopic studies of the distribution of acid phosphatase activity in the zona fasciculata of groups of ACTH-treated and of hypophysectomized rats (Szabó, Stark, Pósalaky and Varga [16], Szabó, Stark and Varga [17]), the appearance in both groups of peculiar rectangular bodies attracted our attention. This incidental observation made us raise the question whether the substance of these bodies might not be the same as that of the crystals seen in the polarization microscope. The present work was undertaken with the purpose of answering this question.

Materials and methods

Ninety Wistar rats weighing 140 to 180 g, maintained on a standard diet and kept in a constant temperature room (23 °C ±0.5, 65% rel. humidity), were divided into three experimental and one control group.

In group 1, each animal was injected intramuscularly with 2 U of depot ACTH (Cortrophin Z) in the morning and evening hours for three consecutive days, and in the morning hours of the fourth day with a single dose of crystalline ACTH (Cortrophin) administered subcutaneously. One hour later all animals were killed by decapitation.

In group 2, the rats were hypophysectomized by the parapharyngeal approach, and decapitated 11 days later.

In group 3, the animals were hypophysectomized as in group 2, and six weeks later treated with ACTH in the same manner as the rats in group 1. One hour after the last injection the animals were killed.

The rats in the control group has been individually housed for between 24 and 48 hours. Those serving as controls of animals treated with ACTH (groups 1 and 3) were injected with physiological salt solution in volumes equal to the ACTH injections.

In all four groups, the adrenals of each animal were removed immediately after decapitation and cut into small blocks.

In a separate series, the adrenals of seven 21-day-old embryos were extirpated instantly after the mothers decapitation, and fixed within one minute.

For electron microscopy, the blocks were fixed with buffered (MILLONIG [12]) 1% osmium tetroxide for 1 1/2 hours at 4 °C, or they were subjected to double fixation. In the latter case, the blocks were fixed first for 2 hours at 4 °C with 0.1 M cacodylate buffered to pH 7.2 and containing 4.5% glutaraldehyde. This was followed by washing for 24 hours at 4 °C in 0.13 M cacodylate buffered to pH 7.4 and containing 0.22 M sucrose, thereafter by a second fixation for 1 1/2 hours at 4 °C with 1% osmium tetroxide buffered according to MILLONIG [12] and finally by rinsing for a short period of time.

For the purpose of studying the distribution of acid phosphatase activity, blocks were fixed in glutaraldehyde in the manner described above, then washed, and sectioned in a cryostat at 40 μ. The sections were allowed to float in 0.05 M acetate buffer at pH 5 for 15 minutes. Incubation for 30 minutes in a medium prepared according to GOMORI [7] was followed by washing with the same acetate buffer for another 30 minutes.

Using a number of adrenal blocks fixed in glutaraldehyde and washed, the digitonin reaction as adapted by ÖKRÖS [14] to electron microscopy was induced with 0.5% digitonin dissolved in 35% ethanol.

The material for electron microscopy was dehydrated in a graded series of ethanol and embedded in Durcupan ACM via propylene oxide. During fixation, pieces of 1 cu.mm of material were cut out under a stereoscopic microscope and so oriented at embedding as to include the zona fasciculata. The material was sectioned with glass knives on a Porter-Blum I microtome. Before ultrathin sections, 1 μ (half-thick) sections were cut. These were stained with toluidine blue and examined in a light microscope to make sure that the zona fasciculata was actually included. The ultrathin sections were mounted on Formvar-coated and carbon-stabilized copper grids. A number of them were stained with 20% uranyl acetate in methanol, and the rest with lead citrate as described by REYNOLS [15], excepting a few to which both stains were applied. The stained preparations were examined with a JEM—6AS electron microscope operated at 80 KV accelerated voltage and 30 μ objective aperture, and photographed on Gevaert Scientia 23 D 50 plates.

Observations

In many of the cells of the zona fasciculata long rectangular bodies, with the four corners slightly rounded off, were observed in all experimental groups as well as in the control group. They appeared electron-optically empty and varied in length between 0.7 and 1.4 μ, and in width between 0.08 and 0.15 μ. They occurred in single, in irregular groups (Figs 1 to 4), or in parallel arrangement (Fig. 5). A fine granular and intensely electron-dense substance could be seen either to form a thin wrapping around these rectangular bodies (Figs 1 and 3) or to enclose them completely as a more or less large mass (Figs 5 and 7). In some cells this substance could be observed as microbodies dispersed near the rectangular bodies (Fig. 6).

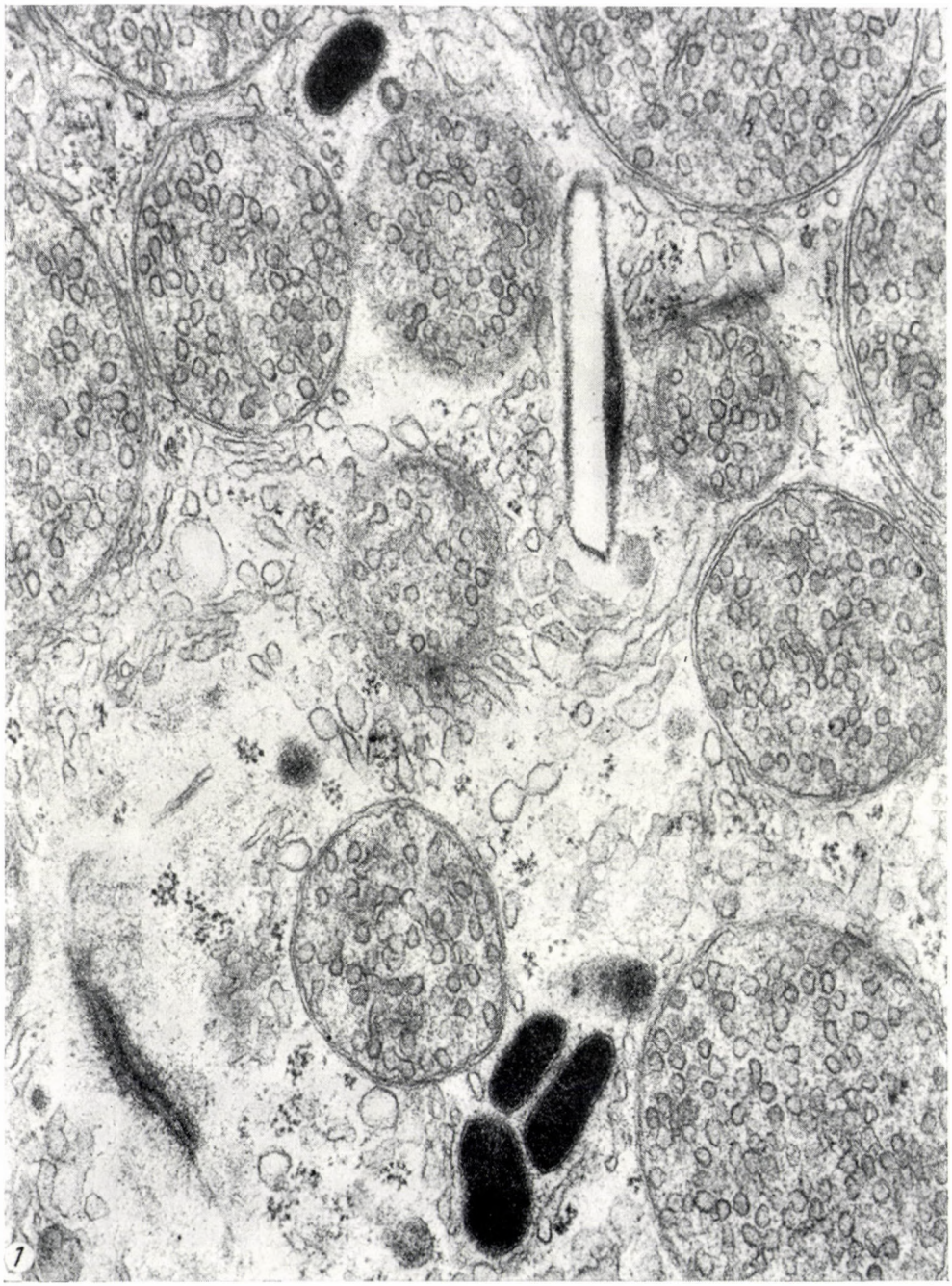
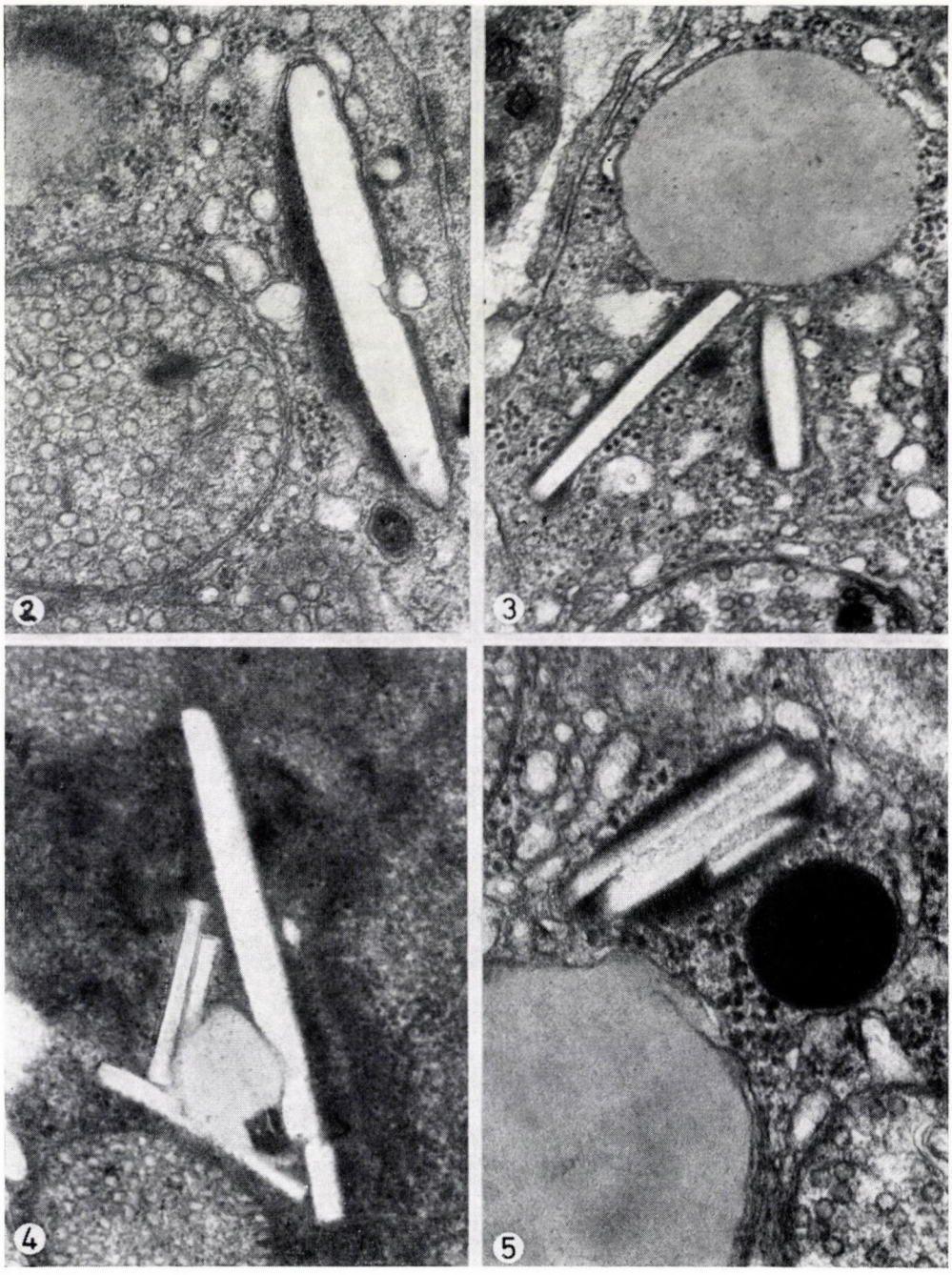


Fig. 1. Zona fasciculata of untreated rat. Among vesicular mitochondria an electron-optically empty rectangular body with a thin wrapping of a granular substance which is of the same density as that of the microbodies. (Arch. No 347/65) ×45,000


2
3
4
5

The acid phosphatase reaction resulted in the accumulation of a lead-containing product in the electron-dense substance (Fig. 7).

Both the rectangular bodies and the electron-dense mass enclosing them showed elective affinity for digitonin (Fig. 9).

Rectangular bodies and lipid droplets appeared near each other (Figs 3 and 5), adhering to each other (Fig. 7), or as a conglomerate (Fig. 8). Their number was the largest in rat embryos immediately before birth.

Discussion

With the electron microscope, YATES [19] observed crystalline bodies of a textil-like pattern in the adrenocortical cells of Syrian hamsters treated with triparanol. Crystalline patterns were noted by FAWCETT and BURGOS [6] in human testicular interstitial cells. LENNEP and MADDEN [11] saw rectangular vacuoles in the human corpus luteum. These authors state that "it is possible that such angular 'vacuoles' represent crystals of comparatively pure cholesterol".

The authors of the first half of the century, mentioned in the introductory part of this paper, believed the birefringent crystals consisted of cholesterol and cholesterol esters.

In our opinion, the structural elements visible with the electron microscope as rectangular bodies are actually those which under the polarization microscope show as doubly refractive crystals. Underlying this opinion is our finding that each of the two types of structure forms a bond with digitonin. This linkage manifests itself in intensified double refraction and in elective affinity for digitonin of the rectangular bodies and the electron-dense substance enclosing them.

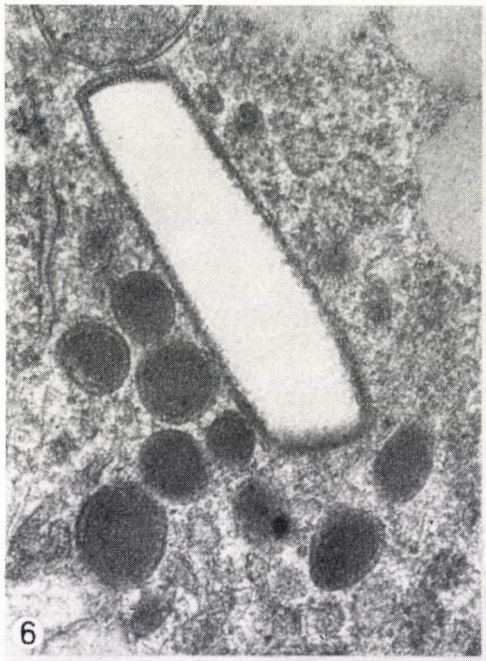
Digitonin is known to form a complex with cholesterol as well as with the 3β-hydroxysteroids. In the zona fasciculata, cholesterol, the important precursor of corticosterone, is present in large amounts, while 3β-hydroxysteroids are there in traces only. Light microscopy reveals that in the digitoninophil areas the cholesterol reaction of SCHULTZ yields positive results, which is additional evidence that the substance detected by digitonin is cholesterol.

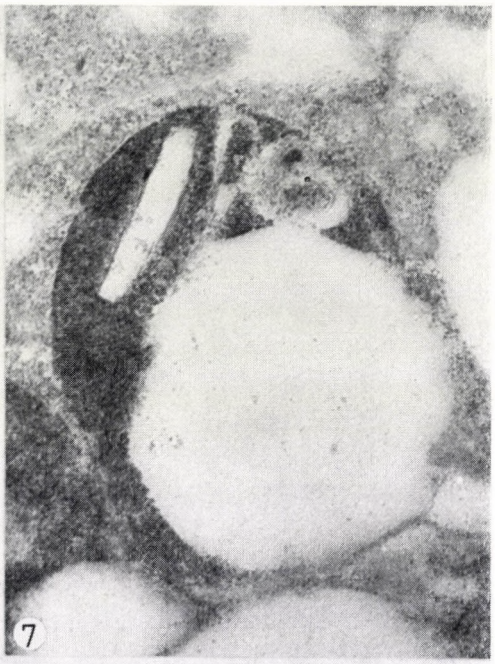
Fig. 2. Zona fasciculata of rat treated with ACTH. Next to an enlarged mitochondrium a rectangular body surrounded by a granular electron-dense substance. (Arch. No 1022/66) ×48,500

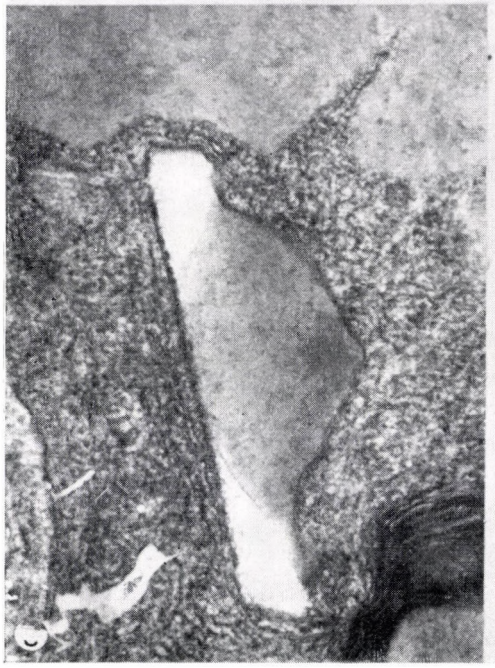
Fig. 3. 21-day-old rat embryo. Near a lipid droplet a rectangular body with electron-dense wrapping. (Arch. No 1332/66) ×40,000

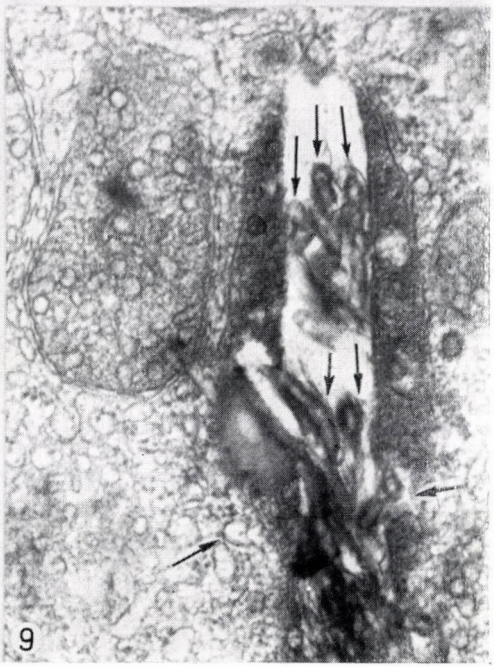
Fig. 4. 21-day-old rat embryo. Groups of rectangular bodies and a lipid droplet among mitochondria in an electron-dense field. (Arch. No 1188/66) ×44,000

Fig. 5. 21-day-old rat embryo. Near a lipid droplet one round and one oval microbody, the latter containing three parallel rectangular bodies. (Arch. No 990/65) ×63,000


6


7




9

It may be argued that the rectangular bodies merely represent vacuoles at sites where cholesterol has been dissolved during dehydration. Nevertheless, the cholesterol—digitonin complex will always form when the digitonin reaction has been performed before dehydration.

The contention that the rectangular bodies might be artifacts can be safely refuted on the strength of the finding that acid phosphatase activity was never absent from the electron-dense substance enclosing them.

I acknowledge with gratitude the helpful advice, comments and assistance of Dr. E. STARK, Dr. B. VARGA, Dr. I. ÖKRÖS and Dr. B. BUKULYA, all of this Institute.

REFERENCES

1. ALBRECHT, A., WELTMANN, O.: (1911) Über das Lipoid der Nebennierenrinde. Wien. klin. Wschr. **24,** 483—484. — 2. BELT, W. D.: (1958) The Origin of Adrenal Cortical Mitochondria and Liposomes: A Preliminary Report. J. biophys. biochem. Cytol. **4,** 337—340. — 3. BELT, W. D.: (1960) Fine Structural Changes in the Adrenal Cortex of the Hamster during Starvation. Anat. Rec. **136,** 162—163. — 4. BENNETT, H. S.: (1940) The Life History and Secretion of the Cells of the Adrenal Cortex of the Cat. Amer. J. Anat. **67,** 151—227. — 5. CARR, I.: (1961) The Ultrastructure of the Human Adrenal Cortex before and after Stimulation with ACTH. J. Path. Bact. **81,** 101—106. — 6. FAWCETT, D. W., BURGOS, M. H.: (1960) Studies on the Fine Structure of the Mammalian Testis. II. The Human Interstitial Tissue. Amer. J. Anat. **107,** 245—270. — 7. GOMORI, G. W.: (1952) Microscopic Histochemistry. Principles and Practice. University of Chicago Press, Chicago. — 8. HOERR, N. L.: (1936) Histological Studies on Lipids. II. A Cytological Analysis of the Liposomes in the Adrenal Cortex of the Guinea-pig. Anat. Rec. **66,** 317—342. — 9. HOLZMANN, K., LANGE, R.: (1966) Zytologische Beobachtungen an der hyperplastischen Nebennierenrinde des Menschen. Z. Zellforsch. **69,** 80—92. — 10. LANDAU, M., MCNEE, J. W.: (1914) Zur Physiologie des Cholesterinstoffwechsels. Beitr. path. Anat. **58,** 667—699. — 11. LENNEP, E. W., MADDEN, L. M.: (1965) Electron-microscopic Observations on the Involution of the Human Corpus Luteum of Menstruation. Z. Zellforsch. **66,** 365—380. — 12. MILLONIG, G.: (1962) Further Observations on a Phosphate Buffer for Osmium Solutions in Fixation. In: 5th Int. Congr. Electron Microscopy, ed.: Breese, S. S. Jr., Vol. 2, p. 8, Academic Press, New York. — 13. NISHIKAWA, M., MURONE, I., SATO, T.: (1963) Electron Microscopic Investigations of the Adrenal Cortex. Endocrinology **72,** 197—209. — 14. ÖKRÖS, I.: (1966) Digitonin Reaction Adapted to Electron Microscopy. Acta morph. Acad. Sci. hung. **14,** 346. — 15. REYNOLS, E. S.: (1963) The Use of Lead Citrate at High pH as an Electron Opaque Stain in Electron Microscopy. J. Cell Biol. **17,** 208—212. — 16. SZABÓ, D., STARK, E., PÓSALAKY, Z., VARGA, B.: (1966) Electron-microscopic Studies of the Adrenal Cortex of the Rat in Various Functional Conditions. Acta physiol. Acad. Sci. hung. **29,** 380—381. — 17. SZABÓ, D., STARK, E., VARGA, B.: (1966) Electron-microscopic Study of the Functional Changes and the Acid Phosphatase Reaction on the Fasciculate Zone of the Rat Adrenal. Acta morph. Acad. Sci. hung. **14,** 342. — 18. YAMORI, T., MATSUURA, S., SAKAMOTO, S.: (1961) An Electron-microscopic Study of the Normal and Stimulated Adrenal Cortex in the Rat. Z. Zellforsch. **55,** 179—199. — 19. YATES, R. D.: (1966) The Effects of Triparanol on Adreno-

Fig. 6. Zona fasciculata of rat treated with ACTH 6 weeks after hypophysectomy. A group of microbodies around a rectangular body. (Arch. No 330/66) ×57,000

Fig. 7. Zona fasciculata of rat 11 days after hypophysectomy. Acid phosphatase reaction: a lead-containing reaction product accumulated in the substance embracing a lipid and electron-dense granules. (Arch. No 112/66) ×70,500

Fig. 8. 21-day-old rat embryo. Conglomerate of rectangular body and lipid droplet surrounded by electron-dense substance. Lipid droplets seen near it. (Arch. No 1325/66) ×51,000

Fig. 9. Untreated rat. Digitonin reaction: Strongly osmiophil reaction product (arrows) within a rectangular body and in the electron-dense granular substance surrounding it. Endoplasmic reticulum and mitochondria free from reaction product. (Arch. No 1269/66) ×41,500

cortical Cells of the Zona Fasciculata of Syrian Hamsters. Z. Zellforsch. **71,** 41—52. — 20. YOFFEY, J. M., BAXTER, J. S.: (1947) The Formation of Birefringent Crystals in the Suprarenal Cortex. J. Anat. (Lond.) **81,** 335—342. — 21. ZELANDER, T.: (1959) Ultrastructure of the Mouse Adrenal Cortex. An Electron Microscopical Study in Intact and Hydrocortisone-treated Male Adults. J. Ultrastruct. Res., Suppl. **2,** 1—111.

DIE ULTRASTRUKTURELLE LOKALISATION EINER KRISTALLINEN SUBSTANZ IN DER ZONA FASCICULATA DER RATTENNEBENNIERE

D. SZABÓ

Elektronenmikroskopisch wurde in der normal, schwach und extrem stark funktionierenden Zona fasciculata von erwachsenen Ratten, sowie von 21tägigen Rattenembryonen das intrazelluläre Vorhandensein einer kristallinen Substanz von länglicher Rechteck-Struktur aufgezeigt. Elektronenoptisch sind diese Strukturen leer und eng von einer elektronendichten Substanz mit saurer Phosphatasenaktivität umgeben. Die für Elektronenmikroskopie adaptierte Digitoninreaktion ließ vermuten, daß diese kristalline Substanz Cholesterol oder ein Cholesterolderivat darstellt.

УЛЬТРАСТРУКТУРНАЯ ЛОКАЛИЗАЦИЯ КРИСТАЛЛИЧЕСКОГО ВЕЩЕСТВА В ZONA FASCICULATA НАДПОЧЕЧНИКОВ У КРЫС

Д. САБО

В электронном микроскопе было выявлено внутриклеточное наличие кристаллического вещества продолговатой прямоугольной структуры у взрослых крыс с нормальной, пониженной и с крайне повышенной функцией zona fasciculata, так же как и у 21-дневных зародышей крыс. Электроннооптически эти структуры пустые, и они тесно окружены электронноплотным веществом с активностью кислой фосфатазы. На основе пробы с дигитонином, адаптированной к электронной микроскопии, предполагается, что кристаллическое вещество представляет собой холестерин или производное холестерина.

Dr. Dezső SZABÓ; MTA Kísérleti Orvostudományi Kutató Intézet, Budapest VIII. Szigony u. 12. Hungary

Acta morphologica Acad. Sci. hung. **16** *(1), pp. 129—143, 1968*

Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana

REGULATION OF ACTIVITY AND BIOSYNTHESIS OF ENZYMES CONTROLLING GLUCONEOGENESIS AND GLYCOLYSIS

G. WEBER

1. Recent advances in the study of regulation of enzyme activity and biosynthesis have been discussed.

2. Nutritional and hormonal factors frequently result in chronic adaptive changes involving an altered rate in the biosynthesis of enzymes. The biosynthetic responses of groups of enzymes in carbohydrate metabolism in gluconeogenic conditions such as starvation, diabetes, and after steroid injection, were reported. The synchronous behavioural patterns described are in accord with the predictions of the functional genic unit concept.

3. As a contrast to the responsiveness of the enzyme forming systems to nutritional and hormonal stimulation, a lack of responsiveness in liver cancer cells was observed. The correlation of the activities of key enzymes of carbohydrate metabolism with the growth rate of hepatomas provides an understanding for alterations in the behaviour of overall metabolic pathways at the molecular level.

4. Acute adaptation involves the action of regulatory signal molecules on the enzyme itself. The selective action of free fatty acids in the control of carbohydrate metabolism was pinpointed at the enzyme activity level. Free fatty acids act as feedback inhibitors of the key enzymes of glycolysis, direct oxidative pathway, and the Krebs cycle. In further studies the reciprocal control of liver pyruvate kinase activity by fructose 1,6-diphosphate and ATP has been described.

5. The chief mechanisms operating in acute and chronic adaptation in various nutritional and hormonal conditions have been discussed.

Professor Joseph BALÓ is greatly admired by his students, colleagues, and friends. It is a pleasure and a privilege to join the ranks of those who pay homage to him by a contribution to this volume. I am especially happy that I was asked to do so because of the stimulation and encouragement I received from Professor BALÓ when, as a student working on serum lipase in the Pathophysiological Institute, on recommendation of Professor Joseph Sós, I approached him for advice. Professor BALÓ had worked on lipase years before; his practical advice, his enthusiasm, and his steady interest in the progress of this problem played an important role in my continued investigations on this subject at that time. Because of Professor BALÓ's interest in enzymes, I would like to describe some of our recent research and concepts on regulation of certain key enzymes in liver.

Materials and methods

Male Wistar rats of 100 to 200 g weight were maintained in separate cages with Purina laboratory chow and water available *ad libitum*, unless otherwise specified.

Experimental methods for preparation of tissue extracts, assay of individual enzymes, the basis for expression of enzyme activity, and the methods of interpretation were outlined elsewhere [4, 8—13, 16].

Results and discussion

Role of key enzymes in regulation of gluconeogenesis and glycolysis

Gluconeogenesis is a vital process which provides an adaptive mechanism to produce glucose from non-carbohydrate precursors. The maintenance of glucose homeostasis is necessary for the central nervous system and consequently gluconeogenesis comes into operation in starvation or when the diet contains no carbohydrate. It was demonstrated that the operation of this pathway is subject to nutritional and hormonal regulation. The rate and direction of the opposing pathways in the liver, gluconeogenesis and glycolysis, may be determined at three strategic branching points of metabolism where enzymes mediating one-way reactions oppose each other [1]. The ratios of the key enzymes of glycolysis (glucokinase, hexokinase, phosphofructokinase, pyruvate kinase) to the key enzymes of gluconeogenesis (glucose 6-phosphatase, fructose 1,6-diphosphatase, phosphoenolpyruvate carboxylase, pyruvate carboxylase) are shown in Table 1 [2]. Pathological alterations in the direction

Table 1

Ratios of activities of key enzymes of gluconeogenesis and glycolysis

Activities are expressed in micromoles of substrate metabolized/g wet weight per hr at 37 °C. The assays were carried out under optimal substrate and cofactor conditions at pH 7.4 as cited in the Methods section

Enzymes	Activity	Ratio
Glucose 6-phosphatase / Glucokinase	1,000 / 150	6.7
Fructose 1,6-diphosphatase / Phosphofructokinase	600 / 150	4.0
Phosphoenolpyruvate carboxylase* / Pyruvate kinase	50 / 5,000	0.01
Glucose 6-phosphatase / Glucokinase + hexokinase	1,000 / 200	5.0
Fructose 1,6-diphosphatase / Phosphofructokinase	600 / 150	4.0
Phosphoenolpyruvate carboxylase + pyruvate carboxylase* / Pyruvate kinase	200 / 5,000	0.04

* Data calculated from results of KREBS [18].

and operation of gluconeogenesis and glycolysis may occur as a result of changes in these ratios, and the clinical symptoms and signs are the consequences of such alterations at the molecular level.

The role of enzymes in homeostasis: chronic and acute adaptation. In the normal control of physiological homeostasis, the organism is able to provide long-term adaptation to nutritional or hormonal challenges. This takes place through enzyme adaptation involving a change in *enzyme amount* through biosynthesis. On the other hand, a rapid rise or increase in the overall pathway activity may be achieved through the action of signal molecules affecting only the *activity of the enzyme.* Investigations in our laboratories led us to the formulation of the functional genic unit (FGU) [3] concept. This concept postulated that the key gluconeogenic enzymes are produced on the same unit in the genome and the key glycolytic enzymes on another FGU. It was further assumed that the enzymes which function in the direction of both gluconeogenesis and glycolysis, the "bifunctional enzymes", phosphohexose isomerase, aldolase, lactate dehydrogenase, etc., are produced on a third FGU. The evidence for these assumptions was published in detail [3—5]. The operational advantage of the FGU concept is that it provides a framework of predictions which may be verified experimentally. The concept also simplified investigation of mechanisms of carbohydrate metabolic controls, by focussing experimentation on the key enzymes.

Regulation of hepatic carbohydrate metabolism through enzyme biosynthesis

Studies of the key enzymes revealed that the biosynthetic responses of these enzymes represented the chronic adaptation pattern under various physiological and pathological conditions. The three groups of enzymes follow a characteristic biosynthetic pattern under the conditions examined. Fig. 1 shows that in *starvation* the gluconeogenic enzymes were preferentially maintained, and the bifunctional enzymes decreased parallel with the nitrogen level, whereas the glycolytic enzymes were preferentially decreased [5]. In starvation there is a rapid drop in circulating insulin [6, 7], and in consequence there is a relative preponderance of the gluconeogenic and lipolytic hormones such as the glucocorticoids, growth hormone, glucagon, and epinephrine. The key gluconeogenic enzymes which are under the suppressive action of insulin [8] tend to rise as the insulin level decreases. With the limitation of the inflow of biosynthetic precursors imposed by the fasting state they do not increase and are maintained in normal range. On the other hand, the key glycolytic enzymes which are insulin-dependent for their biosynthesis rapidly decreased to low activities when the plasma insulin dropped [9—11].

In *alloxan-diabetes* the insulin level decreased; however, the animals were hyperphagic, providing an increased nutritional supply of biosynthetic

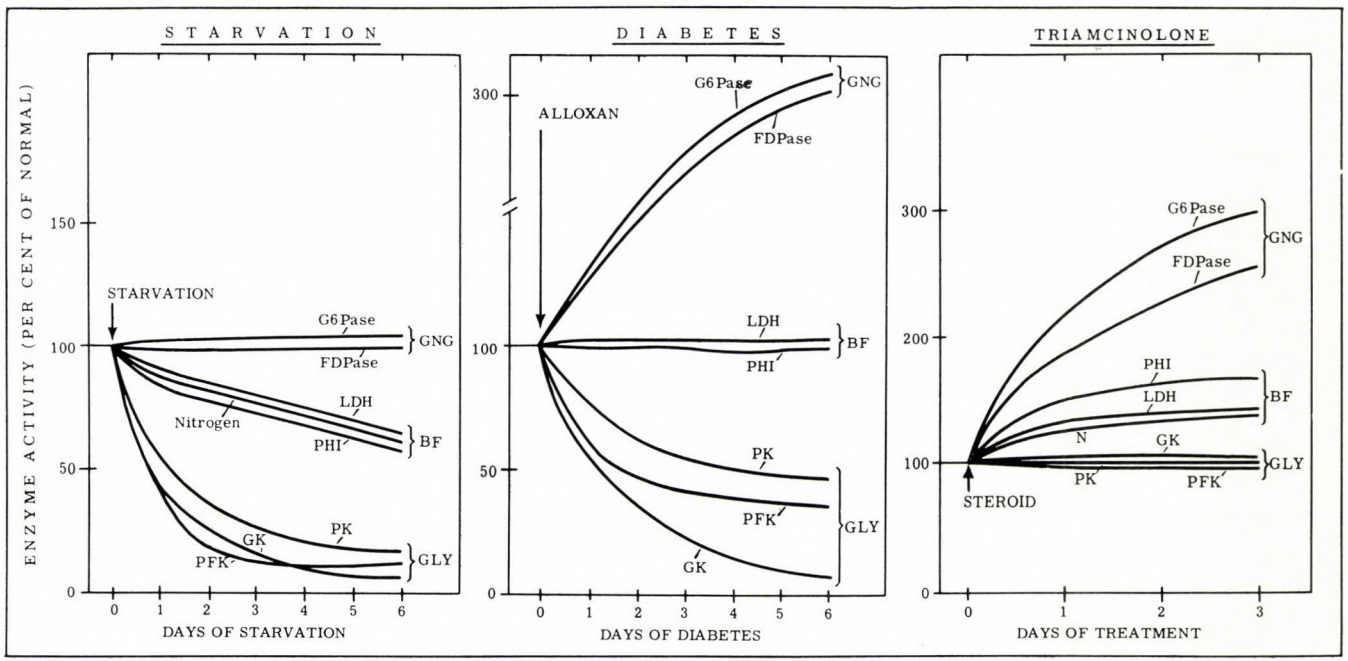


Fig. 1. The regulatory pattern of liver enzymes involved in carbohydrate metabolism is shown in the coordinated responses of gluconeogenic (GNG), bifunctional (BF), and glycolytic (GLY) enzymes during starvation, diabetes, and following glucocorticoid (triamcinolone) stimulation. Animals were starved, with water available *ad libitum*. Diabetes was induced in rats starved for 30 hr by injection of alloxan (12 mg/100 g rat, i.p.). Triamcinolone was injected daily (1 mg/100 g rat). WEBER *et al.* [5]

precursors. As a result, with the release from insulin suppression and in presence of the relative preponderance of gluconeogenic hormones the biosynthesis of gluconeogenic enzymes increased. In contrast, the insulin-dependent key glycolytic enzymes decreased to low activities. The bifunctional enzymes which are not affected by insulin remained in normal range [3, 5, 8, 9].

The action of the *glucocorticoid hormone*, triamcinolone, also showed a typical behavioural pattern for the three groups of enzymes. The hepatic nitrogen level and the activities of the bifunctional enzymes increased together. However, the key gluconeogenic enzymes rose to higher levels as a result of steroid-induced preferential biosynthesis. In contrast, the key glycolytic enzymes were not affected. Further detailed evidence for a synchronous biosynthetic pattern, characteristic for each of the three groups of the enzymes of carbohydrate metabolism, was published [3, 5, 8, 9].

Such nutritional and hormonal alterations of the enzyme biosynthetic pattern are usually reversible and respond to regulatory influences upon refeeding or restoration of hormone balance. Thus, the enzyme forming systems which represent the manifestations of the genetic potential of the cell are responsive to biosynthetic regulatory influences. However, when genetic alterations occur, such as in cancerous cells, the disturbances of the code may not permit normal responses to nutritional or hormonal regulation. Detailed studies in our laboratories described the lack of response to starvation and refeeding steroid stimulation of the various enzymes of carbohydrate metabolism [12]. The markedly altered enzyme biosynthetic pattern appears to be fixed in the cancerous cells of the liver and different strains of liver cancer show a step-wise progression of the pathological pattern of certain key enzymes [13—15].

Behaviour of enzymes in liver tumours of different growth rate. In recent reviews I and my associates have surveyed our "Molecular Correlation Concept of Neoplasia" which deals with the correlation of growth rate with the enzymatic and metabolic alterations in a spectrum of liver tumours [13—15]. I will take this example to demonstrate the following point. In a spectrum of liver tumours of increasing growth rate we observed that the key gluconeogenic enzyme activities were the lower the more rapidly the tumor examined was growing. On the other hand, lactate production [16, 17] and the activities of the key glycolytic enzymes increased parallel with the rise in growth rate (Fig. 2). The activities of the bifunctional enzymes showed no correlation [13—15]. The enzymatic indications suggesting marked disturbances in gluconeogenesis and glycolysis were confirmed by isotope studies of the production of glucose from pyruvate or by following the generation of lactate from glucose [13—17].

Acute adaptation: Effects of signal molecules on activities of key enzymes. The alterations of the key enzymes are indicators of the behaviour of the

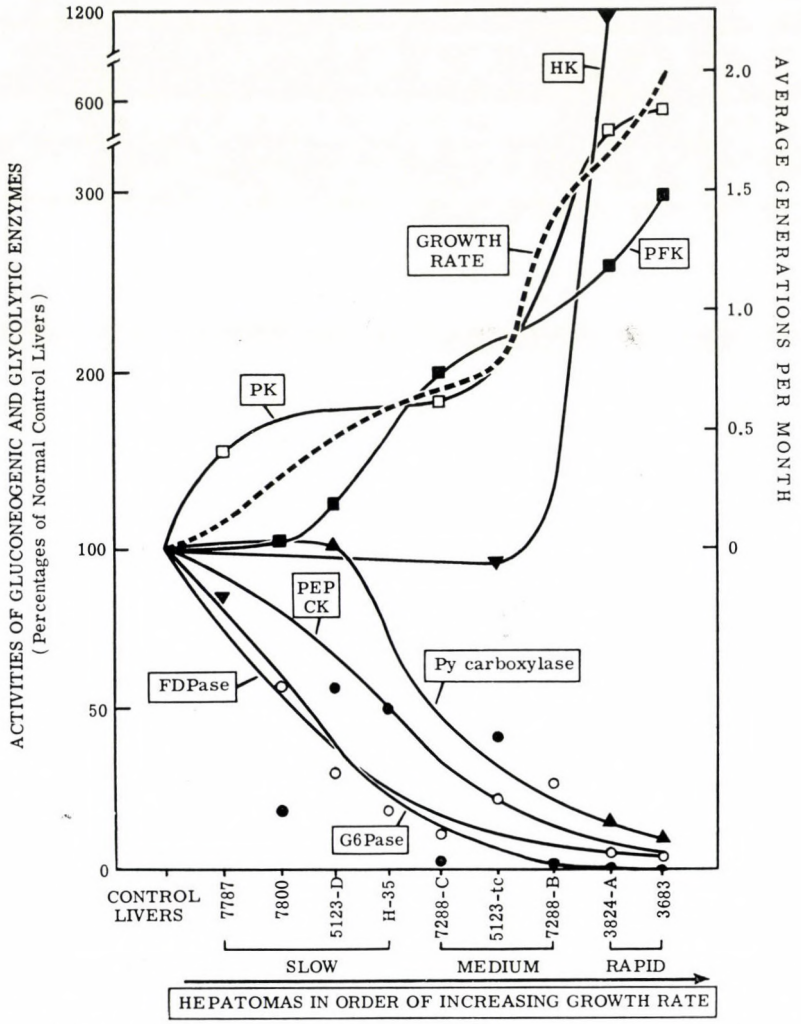


Fig. 2. Correlation of the activities of key gluconeogenic and glycolytic enzymes with the growth rate of experimental hepatomas in rat. WEBER and LEA [15]

overall metabolic pathway. These enzymatic alterations represent chronic adaptation and the described rise or decay in enzyme activities in starvation, diabetes, or after steroid injection, required a number of hours or days.

Such alterations in the biosynthetic rate of these enzymes do not provide a satisfactory explanation for the rapid, acute changes which have been observed in the direction of gluconeogenesis and glycolysis in liver. Consequently, as one facet of our investigations, we studied a number of factors which cause rapid changes in the activities of key enzymes.

Pyruvate kinase: a strategic enzyme in gluconeogenesis and glycolysis. Table 1 shows the ratios of activities of key enzymes which antagonize one

another in hepatic gluconeogenesis and glycolysis. These ratios indicate that in the later and final steps of gluconeogenesis the activities of the gluconeogenic enzymes (glucose 6-phosphatase, fructose 1,6-diphosphatase) dominate those of the glycolytic enzymes (glucokinase, phosphofructokinase). In contrast, at the crucial early step of gluconeogenesis the glycolytic enzyme (pyruvate kinase) is dominant over the activities of the two opposing gluconeogenic enzymes (phosphoenolpyruvate and pyruvate carboxylases). The significance of pyruvate kinase activity in opposing gluconeogenesis is impressive when the activity of this enzyme (5,000 μmoles/g wet weight per hr at 37 °C) is contrasted with the activity of the opposing rate-limiting enzyme of gluconeogenesis, phosphoenolpyruvate carboxylase (50 μmoles/g per hr at 37 °C). As a result of this dominance of pyruvate kinase activity, phosphoenolpyruvate, as soon as it could be formed from oxaloacetate, would immediately be recycled to pyruvate. The unfavourable ratio at this early strategic step of gluconeogenesis suggested to us that there should be mechanisms operating which are capable of overcoming the pyruvate kinase activity barrier. Since the literature provided examples showing that gluconeogenesis may be increased suddenly, we searched for a mechanism by which hepatic pyruvate kinase may be inhibited rapidly. The inhibition of this enzyme would then prevent recycling and permit the operation of the gluconeogenic processes.

Effect of free fatty acids on key enzymes of glycolysis, direct oxidative pathway and the Krebs cycle. Recent studies on the regulation of gluconeogenesis and glycolysis directed our attention to the effect of free fatty acids (FFA). Studies in *slices* showed that the addition of FFA caused an increase in gluconeogenesis from lactate as shown first by KREBS *et al.* [29]. HAYNES reported that octanoate (3 mM) enhanced glucose production from alanine in liver slices [20]. These studies in slices were corroborated by HERRERA *et al.* using *liver perfusion* technique. They found an increased conversion of alanine to glucose when linoleate was infused to rat liver at a rate of 300 μmoles/hr [21]. It is important that addition of FFA also resulted in a decreased production of CO_2 from alanine [21]. In similar liver perfusion experiments STRUCK *et al.* described that infusion of oleic acid markedly stepped up the glucose production from lactate [22]. WILLIAMSON *et al.* demonstrated that addition of oleate in the course of liver perfusion resulted in a prompt decrease in glucose production from alanine and this was concomitant with a decrease in lactate production [23]. FRIEDMANN *et al.* demonstrated that injection of 1 mmole of octanoate in rat caused in 1 hour a 5-fold increase in gluconeogenesis as measured by incorporation of label into blood glucose 1 hour after an intraperitoneal injection of 1 mmole pyruvate $3\text{-}^{14}C$ [24]. It was also established in these *in vitro* and *in vivo* experiments that the labelled FFA did not provide radioactivity to the glucose. Since FFA did not act as a precursor to glucose, its action required explanation at the molecular level.

Our attention was attracted since the gluconeogenic conditions which are associated with an increase in plasma FFA include those where biosynthetic enzyme adaptations have been described. An increase in plasma FFA occurs in such biological conditions as starvation, carbohydrate deprivation, diabetes, excess of growth hormone and glucocorticoids in acromegaly or in Cushing's syndrome or following the administration of these hormones. Since under *acute* gluconeogenic conditions such as diabetes produced by injection of anti-insulin-serum, starvation, or steroid injection there was a rapid rise in plasma FFA, we postulated that such an acute gluconeogenic adaptation should involve the inhibition of pyruvate kinase activity by FFA. For the FFA to operate as an effective *metabolic directional switch* it seemed that liver pyruvate kinase must be strongly inhibited by physiological levels of FFA because without inhibition of this enzyme gluconeogenesis cannot proceed [25].

Detailed investigations showed that in fact pyruvate kinase was progressively inhibited by increasing concentrations of FFA [25]. Using sodium octanoate as a model, inhibitory effects, dependent on dose and preincubation time, were found for the other glycolytic enzymes, phosphofructokinase, glucokinase and hexokinase. The enzymes of direct oxidative pathway, glucose 6-phosphate and 6-phosphogluconate dehydrogenases, were also inhibited. The selectivity of the action of the FFA was shown by the fact that the key gluconeogenic enzymes (glucose 6-phosphatase, fructose 1,6-diphosphatase) and the bifunctional enzymes examined (phosphohexose isomerase, aldolase, lactate dehydrogenase) were not inhibited by FFA. Further work also demonstrated that in the Krebs cycle isocitrate dehydrogenase and fumarase were inhibited but malic dehydrogenase and the malic enzyme were not [25].

These observations throw light on the action of FFA in promoting gluconeogenesis and inhibiting their own biosynthesis (Fig. 3). Fatty acids may be considered as an end product of glucose metabolism. With respect to the metabolic flow of carbon to FFA these results suggest that FFA may function as a feedback inhibitor acting (a) on key enzymes of glycolysis (glucokinase, hexokinase, phosphofructokinase, pyruvate kinase), and (b) on the key enzymes of direct oxidative pathway which are involved in the generation of NADPH involved in the reductive biosynthesis of FFA. In consequence, by such a two-pronged feedback action on both glycolysis and the pentose phosphate pathway, free fatty acids can synchronously block their own biosynthesis [25]. The FFA may be either produced in the liver or transported from the periphery in gluconeogenic conditions, when the insulin level drops, or when there is a preponderance of gluconeogenic or lipolytic hormones such as glucocorticoids, growth hormone, glucagon, or epinephrine.

These interpretations are also in accord with reports that long-chain acyl-coenzyme A esters, which increase in liver during gluconeogenic conditions, inhibited enzymes that operate at later stages of lipogenesis, such as

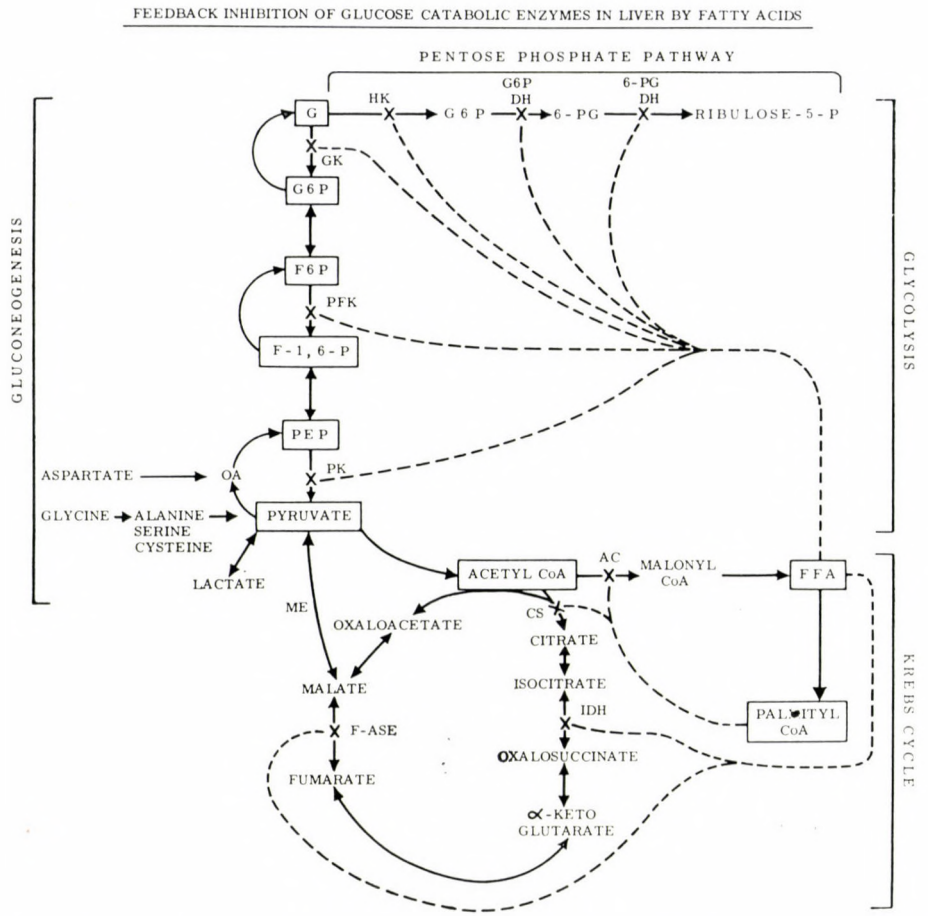


Fig. 3. Feedback inhibition of glucose-catabolizing enzymes in liver by free fatty acids. GK, glucokinase; PFK, phosphofructokinase; PK, pyruvate kinase; HK, hexokinase; G6P DH, glucose 6-phosphate dehydrogenase; 6-PG DH, 6-phosphogluconate dehydrogenase; IDH, isocitrate dehydrogenase; F-ASE, fumarase; ME, malic enzyme; AC, acetyl CoA carboxylase; CS, citrate synthase. The following enzymes are not inhibited: glucose 6-phosphatase, fructose 1,6-diphosphatase, phosphohexose isomerase, lactate dehydrogenase, the malic enzyme and malic dehydrogenase. The free fatty acids may be generated in the liver or may arrive from the periphery in gluconeogenic conditions, when insulin drops or when there is a preponderance of the lipolytic hormones such as glucocorticoids, growth hormone, or glucagon. WEBER *et al.* [25]

acetyl CoA carboxylase, citrate synthase, and the enzymes of the pentose phosphate pathway [26—33].

Our further work showed that in addition to octanoate other fatty acids such as oleic, lauric and myristic acids were able to act as inhibitors of key gluconeogenic and shunt pathway enzymes [2]. In recent studies we demonstrated that the FFA exerted a dose-dependent inhibition of lactate production in a suitably fortified supernatant fluid system [2].

The specificity of FFA inhibition was further indicated by the specific protection phenomena observed. The inhibition of glucokinase by octanoate can be prevented by glucose and the effect was dependent on the glucose concentration. In contrast, phosphofructokinase was not protected by glucose but was protected by its own substrate, fructose 6-phosphate. Neither glucose nor fructose 6-phosphate protected pyruvate kinase and the substrate of this enzyme, phosphoenolpyruvate, was also ineffective as a protecting agent [2, 25].

The feedback inhibition and the selective action of FFA on glucose catabolizing enzymes in liver are illustrated in Fig. 3. The consequences of the selective inhibition of the key enzymes are outlined in Table 2.

Reciprocal control of pyruvate kinase by fructose 1,6-diphosphate and ATP. Recent investigations in this laboratory showed that liver pyruvate kinase was activated by fructose 1,6-diphosphate and inhibited by ATP [2] (Fig. 4). The role of fructose 1,6-diphosphate and ATP appears important, since both of these compounds are effective in concentrations that occur physiologically in the liver cell. The liver pyruvate kinase is approximately 100 times more sensitive to fructose 1,6-diphosphate [2] than is the yeast enzyme where HESS *et al.* [34] described a half-maximal activation at 1.5×10^{-4} M FDP. The activation of pyruvate kinase by the glycolytic precursor, FDP, may represent the mechanism for which the term "positive feed-forward" control was suggested [34].

Hepatic pyruvate kinase activity is the subject of a number of regulating molecules acting on regulatory receptor sites on this enzyme. Previous work from this laboratory indicated that NADH inhibits this enzyme [4]; current work showed that cysteine, EDTA, glucose 6-phosphate and bovine serum albumin can activate liver pyruvate kinase.

It is assumed that when the concentrations of ATP and NADH and octanoate fall, pyruvate kinase becomes sensitive for the activating effect of FDP. The presence or absence of these inhibitory molecules, especially ATP and FFA, may provide the "information" to pyruvate kinase whether the fructose 1,6-diphosphate arises from gluconeogenesis or from glycolysis. When it comes from gluconeogenesis there will also be a concomitant increase in FFA and in NADH concentrations which in turn inhibit pyruvate kinase activity. Preliminary experimental evidence shows that the pyruvate kinase inhibited by octanoate or ATP is not sensitive to activation by fructose 1,6-diphosphate. Thus, in the absence of these inhibitors, fructose 1,6-diphosphate, coming from glucose, activates the enzyme, "informing" pyruvate kinase that glycolysis is proceeding.

Since ATP may be considered one of the products of the pyruvate kinase reaction, the inhibition by this nucleotide which also arises from the functioning of the Krebs cycle may be considered a product or end product inhibition.

Table 2

Selectivity and functions of FFA action

$K_i \times 10^{-3}$	Enzymes inhibited	Metabolic function of inhibition	Enzymes not affected	Function of lack of inhibition
	Key glycolytic enzymes		*Key gluconeogenic enzymes*	
5.8	Glucokinase	(1) Decreases glucose utilization	Glucose-6-phosphatase	Permits gluconeogenesis to proceed
4.5	Hexokinase	(2) Decreases FDP production (FDP is an activator of PK)	Fructose-1,6-diphosphatase	
13.0	Phosphofructokinase	(3) Inhibits PK; this permits the channelling of PEP to glucose preventing recycling		
2.5	Pyruvate kinase (PK)			
	Pentose phosphate enzymes		*Bifunctional enzymes*	
1.0	G-6-P dehydrogenase	(1) Decreases supply of NADPH; thus decreases FFA formation	Phosphohexoseisomerase	Permits reversal of glycolysis
4.4	6-PG dehydrogenase		Lactate dehydrogenase	Provides NADH to block PK. This makes possible the reversal of glycolysis
	Krebs cycle enzymes			
	Citrate synthase (Palmityl CoA)	(1) Blocks entry of oxaloacetate into Krebs cycle	Malate dehydrogenase	Permits malate to oxaloacetate
		(2) Blocks entry of acetyl CoA into Krebs cycle	Malic enzyme	Permits malate → pyruvate
		(3) Promotes oxaloacetate → phosphoenolpyruvate		
		(4) Acetyl CoA activates pyruvate carboxylase		
	Fumarase	Does not permit malate to leak back into Krebs cycle		
0.8	Isocitrate dehydrogenase	Decreases supply of NADPH		

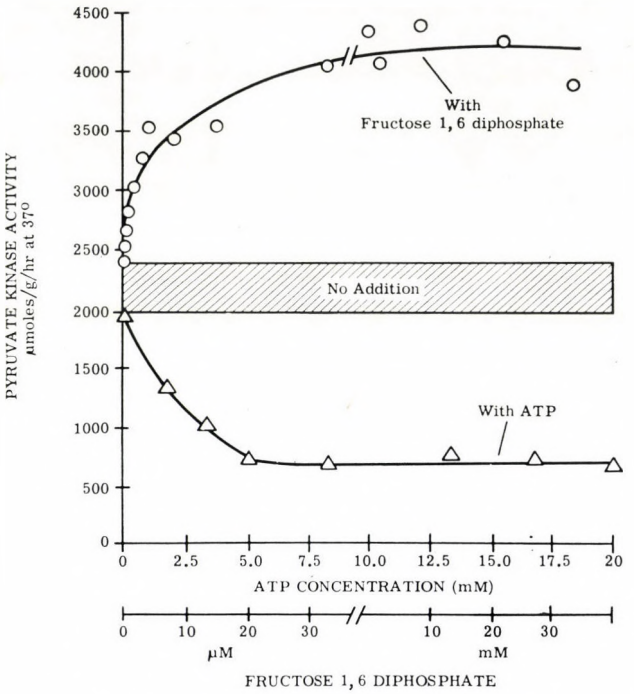


Fig. 4. Reciprocal control of liver pyruvate kinase by addition of fructose 1,6-diphosphate or ATP to the assay reaction mixture for the enzyme. WEBER *et al.* [2]

Acute and chronic regulatory mechanisms of homeostasis at the enzyme level. The various control mechanisms involved in the effects of hormones and signal molecules are illustrated in Fig. 5. Nutritional influences provide precursors for enzyme biosynthesis, supply key metabolites, and regulatory signal molecules such as FFA. Nutritional circumstances influence blood glucose concentrations which then may affect the levels of certain hormones, such as insulin, epinephrine and glucagon. These hormones regulate the levels of metabolites and precursors some of which may function as regulatory signals, such as FDP. The hormones also control the levels of free amino acids, thus affecting protein biosynthesis. Hormones may exert an influence on the manifestations of genes, controlling the biosynthetic rate of specific catalytic proteins, the enzymes. As Fig. 5 shows in *acute adaptation* feedback regulatory action, conformational changes, saturation and stabilization of the enzymes, play an important role. On the other hand, the enzyme biosynthesis may be affected by the action of various hormones which thus operate in *chronic adaptation* [2].

The study of nutritional and hormonal influences at the enzyme level revealed regulatory control in physiological and pathological conditions.

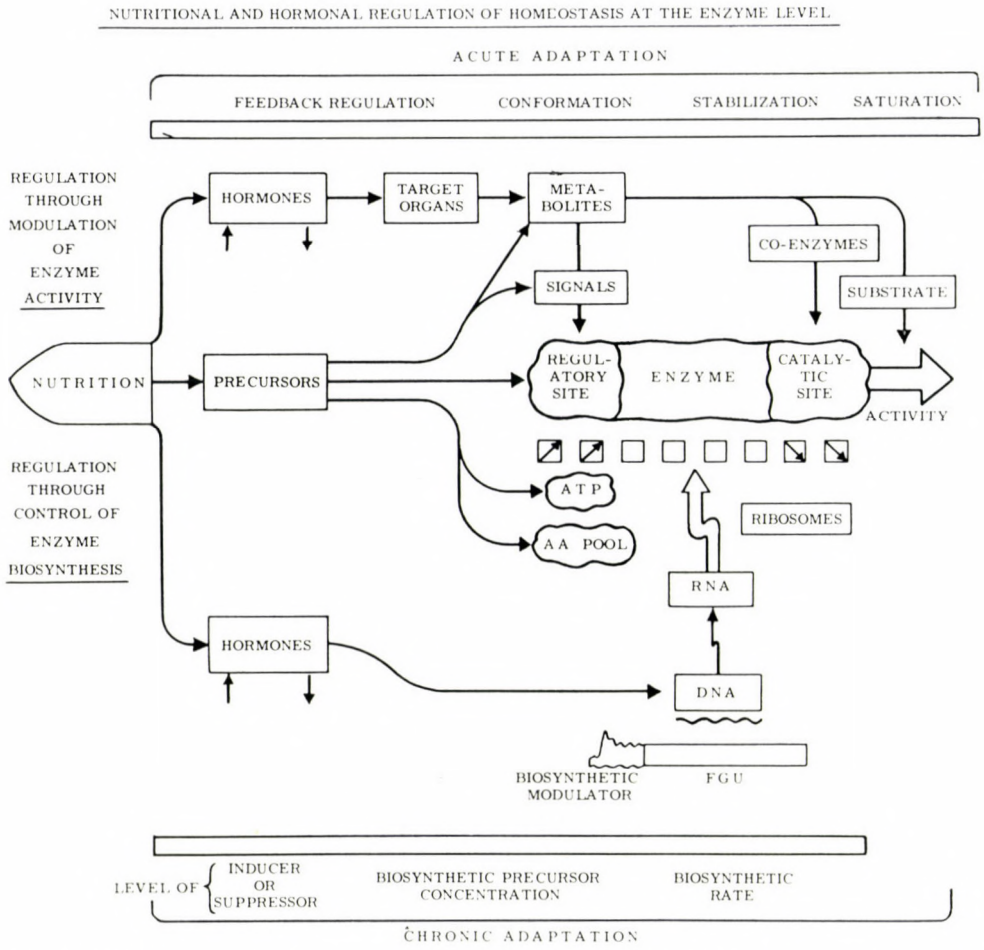


Fig. 5. Regulatory mechanisms in acute and chronic adaptation induced by nutritional and hormonal disturbances of homeostasis. WEBER *et al.* [2]

An understanding of such regulatory processes provides an attacking point for the design of drugs; through such enzyme pharmacology it is hoped that the various metabolic diseases, including cancer, may become a subject for rational chemotherapy.

Acknowledgements

The advice of Sir Hans A. KREBS in the interpretation of the action of free fatty acids and his suggestion to study the effects of ATP are gratefully acknowledged. Discussions with Drs. James ASHMORE and J. R. WILLIAMSON were helpful in this research work. The sodium octanoate was presented to me by Dr. Sidney WEINHOUSE whose valuable, stimulating comments were important in this investigation.

My appreciation is due to my associates and assistants who contributed to different aspects of this investigation. Drs. Hazel J. HIRD CONVERY, Elizabeth A. FISHER, Michael A. LEA and Radhey L. SINGHAL and Nancy B. STAMM collaborated in various aspects of this work, the details of which are published elsewhere. Excellent technical assistance was provided by Mrs. Freida JONES, Delores CAMERON and Patricia JENKINS.

The research work reported in this paper was supported by grants from U. S. Public Health Service, Grant CA—05034—07 of the National Cancer Institute, the American Cancer Society and the Damon Runyon Memorial Fund.

REFERENCES

1. KREBS, H. A.: (1954) Bull. Johns Hopkins Hosp. **95,** 19. — 2. WEBER, G., LEA, M. A., CONVERY, H. J. HIRD, STAMM, N. B.: (1967) *In:* Advances in Enzyme Regulation, Vol. 5, Ed. G. Weber, Pergamon, Oxford. In Press. — 3. WEBER, G., SINGHAL, R. L., SRIVASTAVA, S. K.: (1965) *In:* Advances in Enzyme Regulation. Vol. 3, Ed. G. Weber, Pergamon, Oxford, P. 43. — 4. WEBER, G., SINGHAL, R. L., STAMM, N. B., SRIVASTAVA, S. K.: (1965) Fed. Proc. **24,** 745. — 5. WEBER, G., LEA, M. A., FISHER, E. A., STAMM, N. B.: (1966) Enzym. Biol. clin. **7,** 11. — 6. MORGAN, C. R., LAZAROW, A.: (1963) Diabetes, **12,** 115. — 7. MORGAN, C. R., LAZAROW, A.: (1965) Diabetes **14,** 669. — 8. WEBER, G., SINGHAL, R. L., SRIVASTAVA, S. K.: (1965) Proc. nat. Acad. Sci. (Wash.) **53,** 96. — 9. WEBER, G., SINGHAL, R. L., STAMM, N. B., LEA, M. A., FISHER, E. A.: (1966) *In:* Advances in Enzyme Regulation, Vol. 4. Ed. G. Weber, Pergamon, Oxford. P. 59. — 10. WEBER, G., STAMM, N. B., FISHER, E. A.: (1965) Science **149,** 65. — 11. WEBER, G., SINGHAL, R. L.: (1965) Life Sci. **4,** 1993. — 12. WEBER, G.: (1962) *In:* Advances in Enzyme Regulation, Vol. 1. Ed. G. Weber, Pergamon, Oxford. P. 322. — 13. WEBER, G., LEA, M. A.: (1966) *In:* Advances in Enzyme Regulation, Vol. 4. Ed. G. Weber, Pergamon, Oxford. P. 115. — 14. WEBER, G.: (1966) Gann Monograph **1,** 151. — 15. WEBER, G., LEA, M. A.: (1967) *In:* Methods in Cancer Research, Vol. 2. Ed. H. Busch, Academic Press, New York. In Press. — 16. WEBER, G., MORRIS, H. P.: (1963) Cancer Res. **23,** 987. — 17. SWEENEY, M. J., ASHMORE, J., MORRIS, H. P., WEBER, G.: (1963) Cancer Res. **23,** 995. — 18. KREBS, H. A.: (1966) *In:* Advances in Enzyme Regulation. Vol. 4. Ed. G. Weber, Pergamon, Oxford. P. 339. — 19. KREBS, H. A., SPEAKE, R. N., HEMS, R.: (1965) Biochem. J. **94,** 712. — 20. HAYNES, R. C., JR.: (1965) *In:* Advances in Enzyme Regulation, Vol. 3. Ed. G. Weber, Pergamon, Oxford. P. 111. — 21. HERRERA, M. G., KAMM, D., RUDERMAN, N., CAHILL, G. F., JR.: (1966) *In:* Advances in Enzyme Regulation, Vol. 4. Ed. G. Weber, Pergamon, Oxford. P. 225. — 22. STRUCK, E., ASHMORE, J., WIELAND, O.: (1966) *In:* Advances in Enzyme Regulation. Vol. 4. Ed. G. Weber, Pergamon, Oxford. P. 219. — 23. WILLIAMSON, J. R., KREISBERG, R. A., FELTS, W. P.: (1966) Proc. nat. Acad. Sci. (Wash.) **56,** 247. — 24. FRIEDMANN, B., GOODMAN, E. H., WEINHOUSE, S.: (1966) Fed. Proc. **25,** 347. — 25. WEBER, G., HIRD CONVERY, R. J., LEA, M. A., STAMM, N. B.: (1966) Science **154,** 1357. — 26. BORTZ, W. M., LYNEN, F.: (1963) Biochem. Z. **339,** 77. — 27. TUBBS, P. K., GARLAND, P. B.: (1964) Biochem. J. **93,** 550. — 28. WIELAND, O., WEISS, L.: (1963) Biochem. biophys. Res. Commun. **10,** 333. — 29. WIELAND, O., WEISS, L.: (1963) Biochem. biophys. Res. Commun. **13,** 26. — 30. TUBBS, P. K.: (1963) Biochim. biophys. Acta (Amst.) **70,** 608. — 31. WIELAND, O.: (1966) *In:* Advances in Enzyme Regulation. Vol. 4. Ed. G. Weber, Pergamon, Oxford. P. 281. — 32. WIELAND, O., WEISS, L., EGER-NEUFELDT, I.: (1964) *In:* Advances in Enzyme Regulation. Vol. 2. Ed. G. Weber, Pergamon, Oxford. P. 85. — 33. BORTZ, W. M., LYNEN, F.: (1963) Biochem. Z. **337,** 505. — 34. HESS, B., HAECKEL, R., BRAND, K.: (1966) Biochem. biophys. Res. Commun. **24,** 824.

ÜBER DIE REGELUNG DER BIOSYNTHESE UND DER AKTIVITÄT DER FÜR DIE GLYKONEOGENESE UND GLYKOLYSE VERANTWORTLICHEN ENZYMEN

G. WEBER

1. Die jüngsten Fortschritte in der Erforschung der Aktivitäts- und Biosynthesensteuerung der Enzyme werden besprochen.

2. Alimentäre und hormonale Faktoren bewirken häufig chronische Adaptationsänderungen, die die Biosynthese der Enzyme beeinflussen. Die Reaktionen der Biosynthese von Enzymgruppen des Kohlenhydratmetabolismus während Glukoneogenese (Hunger, Diabetes,

nach Steroidinjektionen usw.) werden mitgeteilt. Die beschriebenen synchronischen Verhaltensarten stimmen mit den Voraussetzungen der Theorie über die genische Funktionseinheit überein.

3. Im Gegensatz zu der Reaktivität des Enzymsynthesensystems auf hormonale und alimentäre Stimulation wurde in Leberkrebszellen das Fehlen einer solchen Reaktivität beobachtet. Die Korrelation zwischen den wichtigsten Enzymen des Kohlenhydratmetabolismus und der Wachstumsrate von Hepatomen ermöglicht es, die Veränderungen der allgemeinen Stoffwechselwege auf molekulärer Stufe zu erfassen.

4. Die akute Adaptation bedeutet, daß die regulativen Signalmoleküle auf das Enzym selbst einwirken. Die selektive Aktivität von freien Fettsäuren in der Kontrolle des Kohlenhydratstoffwechsels wurde auf Enzymaktivitätsstufe nachgewiesen. Freie Fettsäuren wirken als feedback-Inhibitoren der wichtigsten Glykolysenenzyme, steuern die allgemeinen Stoffwechselwege und den Krebs-Zyklus. In weiteren Experimenten wurde die wechselseitige Kontrolle der Pyruvatkinasen-Aktivität der Leber durch Fruktose-1,6-diphosphat, und ATP untersucht.

5. Die Hauptmechanismen der akuten und chronischen Adaptation unter verschiedenen alimentären und hormonalen Einflüssen werden besprochen.

РЕГУЛИРОВАНИЕ АКТИВНОСТИ И БИОСИНТЕЗА ЭНЗИМОВ, ОТВЕТСТВЕННЫХ ЗА ГЛИКОНЕОГЕНЕЗ И ГЛИКОЛИЗ

Г. ВЕБЕР

1. Обсуждаются новые результаты в области исследования регулирования активности и биосинтеза энзимов.

2. Алиментарные и гормональные факторы часто вызывают хронические изменения адаптации, влекущие за собой изменение биосинтеза энзимов. Сообщаются изменения биосинтеза групп энзимов углеводного обмена в условиях гликонеогенеза, как напр., при голодании, диабете, после введения стероидов и т. д. Описанные синхронные способы поведения соответствуют предположениям теории о функциональном единстве генов.

3. В противоположность реактивности синтезирующих ферменты систем, на алиментарную или гормональную стимуляцию в раковых клетках печени наблюдается отсутствие реактивности. Корреляция между активностью важнейших энзимов углеводного обмена и ростом гепатом способствует выяснению изменения общих путей метаболизма на молекулярном уровне.

4. Острая адаптация означает, что регуляционные сигнализационные молекулы действуют на самый энзим. Избирательное действие свободных жирных кислот в области контроля углеводного обмена было выявлено на уровне активности энзимов. Свободные жирные кислоты действуют в качестве ингибиторов механизма обратного включения важнейших энзимов гликолиза, они управляют окислением и циклом Кребса. В дальнейших работах был описан взаимный контроль между активностью пируватной киназы печени, фруктозы, 1,6-дифосфата и АТФ.

5. Обсуждаются важнейшие механизмы, действующие при острой и хронической адаптации в различных алиментарных и гормональных условиях.

Dr. G. Weber; Dept. of Pharm., Indiana Univ. School of Medicine, Indianapolis, Indiana, U.S.A.

Acta morphologica Acad. Sci. hung. **16** *(1), p. 145, 1968*

RECENSIO

Antti U. Arstila:

Electron Microscopic Studies on the Structure and Histochemistry of the Pineal Gland of the Rat

Supplement to "Neuroendocrinology" Vol. 2. S. Karger, Basel—New York, 1967., 101 Pd., 72 Figs

This little monograph presents an excellent summary of present knowledge on the pineal body. The author's electron microscopic and histochemical studies have clarified numerous controversial problems arisen in context of this endocrine gland.

The text, on a total 67 pages, includes seven chapters. Chapter 1 is a short introduction, summarizing the main structural and functional aspects of the pineal body. Chapter 2 describes the author's electron microscopic findings; this is perhaps the most valuable part of his work. Correlating his own results with well-selected references, he concludes that the pineal body contains three different cell types, i.e. dark and light main cells and interstitial cells. An unequivocal and persuasive differentiation of the three cell types is presented, based on their submicroscopic features. Inconsistenly with the majority of authors, Arstila states that the interstitial cells are special parenchymal cells, definitely differing from the glial elements of the central nervous system. Chapter 3, dealing with histochemistry, discusses the hydrolytic enzymes of the pineal body. The main cells of this gland exhibit acid phosphatase, arylsulphatase and thioacetic acid esterase activities, but do not give positive alkali phosphatase and cholinesterase reactions. In Chapter 4 is described the ultrastructure of pineal nerve fibres. A short experimental part appended to this chapter discusses the submicroscopic changes taking place in the nerve fibres under the influences of various experimental interventions such as bilateral cervical sympathectomy, treatment with reserpine or oxypertine. In Chapter 5 are presented the conclusions drawn by the author. Chapter 6 contains a concise description of the contents, whereas Chapter 7 is a compilation of carefully selected references, including more than 150 papers.

The demonstration material (Chapter 8) includes 72 figures arranged in 18 plates and occupies 37 pages. Except for 3 schematic drawings, the whole material consists of original electron micrographs and light and electron microscopic pictures of histochemical reactions.

B. Mess

ANNOUNCEMENT

The First National Congress of the Hungarian Society for Anatomy, Histology and Embryology will be held in Budapest, October 21—23, 1968. Guests from abroad are kindly invited for participation. The general topic of the Congress is "Correlations between Structure and Function". For further details, please contact the Secretary General of the Society. (Institute of Anatomy, University Medical School, Budapest IX. Tűzoltó u. 58. Hungary.)

B. Csillik, M. D.
Secretary General
Department of Anatomy,
University Medical School,
Kossuth L. s-út 40.
Szeged, Hungary

Prof. I. Törő, M. D.
President
Department of Histology,
University Medical School
IX., Tűzoltó u. 58.
Budapest, Hungary

INDEX

Printed in Hungary

A kiadásért felelős az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Farkas Sándor

A kézirat nyomdába érkezett: 1968. III. 13. — Terjedelem: 12,75 (A/5) ív 74 ábra (8 színes), 1 melléklet

68.65266 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

The Acta Morphologica publish papers on experimental medical subjects in English, German, French and Russian.

The Acta Morphologica appear in parts of varying size, making up volumes.

Manuscripts should be addressed to:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Correspondence with the editors and publishers should be sent to the same address.

The rate of subscription to the Acta Morphologica is 165 forints a volume. Orders may be placed with "Kultúra" Foreign Trade Company for Books and Newspapers (Budapest I., Fő utca 32. Account No. 43-790-057-181) or with representatives abroad.

Les Acta Morphologica paraissent en français, allemand, anglais et russe et publient des travaux du domaine des sciences médicales expérimentales.

Les Acta Morphologica sont publiés sous forme de fascicules qui seront réunis en volumes.

On est prié d'envoyer les manuscrits destinés à l'adresse suivante:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Toute correspondence doit être envoyée à cette même adresse.

Le prix de l'abonnement est de 165 forints par volume.

On peut s'abonner à l'Entreprise du Commerce Extérieur de Livres et Journaux «Kultúra» (Budapest I., Fő utca 32. Compte-courant No. 43-790-057-181) ou à l'étranger chez tous les représentants ou dépositaires.

«Acta Morphologica» публикуют трактаты из области экспериментальных медицинских наук на русском, немецком, английском и французском языках.

«Acta Morphologica» выходят отдельными выпусками разного объема. Несколько выпусков составляют один том.

Предназначенные для публикации авторские рукописи следует направлять по адресу:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

По этому же адресу направлять всякую корреспонденцию для редакции и администрации.

Подписная цена «Acta Morphologica» — 165 форинтов за том. Заказы принимает предприятие по внешней торговле книг и газет «Kultúra» (Budapest I., Fő utca 32. Текущий счет № 43-790-057-181) или его заграничные представительства и уполномоченные.

57,— Ft

Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

ALBANIA
Ndermarja Shtetnore e Botimeve
Tirana

AUSTRALIA
A. Keesing
Box 4886, GPO
Sydney

AUSTRIA
Globus Buchvertrieb
Salzgries 16
Wien I

BELGIUM
Office International de Librairie
30, Avenue Marnix
Bruxelles 5
Du Monde Entier
5, Place St. Jean
Bruxelles

BULGARIA
Raznoiznos
1, Tzar Assen
Sofia

CANADA
Pannonia Books
2, Spadina Road
Toronto 4, Ont.

CHINA
Waiwen Shudian
Peking
P. O. B. 88

CZECHOSLOVAKIA
Artia
Ve Směčkách 30
Praha 2
Poštovná Novinová Služba
Dovoz tisku
Vinohradská 46
Praha 2
Mad'arská Kultura
Václavské nám. 2
Praha I
Poštová Novinová Služba
Dovoz tlače
Leningradská 14
Bratislava

DENMARK
Ejnar Munksgaard
Nörregade 6
Copenhagen

FINLAND
Akateeminen Kirjkauppa
Keskuskatu 2
Helsinki

FRANCE
Office International de Documentation et Librairie
48, rue Gay Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC
Deutscher Buch-Export und Import
Leninstraße 16
Leipzig 701
Zeitungsvertriebsamt
Clara Zetkin Straße 62
Berlin N. W.

GERMAN FEDERAL REPUBLIC
Kunst und Wissen
Erich Bieber
Postfach 46
7 Stuttgart S.

GREAT BRITAIN
Collet's Holdings Ltd.
Dennington Estate
London Rd.
Wellingborough. Northants.
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
Oxford

HOLLAND
Swetz and Zeitlinger
Keizersgracht 471—487
Amsterdam C
Martinus Nijhof
Lange Voorhout 9
The Hague

INDIA
Current Technical Literature
Co Private Ltd.
India House OPP
GPO Post Box 1374
Bombay I

ITALY
Santo Vanasia
Via M. Macchi 71
Milano
Libreria Commissionaria Sansoni
Via La Marmora 45
Firenze

JAPAN
Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
Tokyo
Maruzen and Co. Ltd.
P. O. Box 605
Tokyo-Central
Far Eastern Booksellers
Kanda P. O. Box 72
Tokyo

KOREA
Chulpanmul
Phenjan

NORWAY
Johan Grundt Tanum
Karl Johansgatan 43
Oslo

POLAND
RUCH
ul. Wronia 23
Warszawa

ROUMANIA
Cartimex
Str. Aristide Briand 14—18
Bucureşti

SOVIET UNION
Mezhdunarodnaya Kniga
Moscow G—200

SWEDEN
Almquist and Wiksell
Gamla Brogatan 26
Stockholm

USA
Stechert Hafner Inc.
31, East 10th Street
New York, N. Y. 10003
Walter J. Johnson
111, Fifth Avenue
New York, N. Y. 10003

VIETNAM
Xunhasaba
19, Tran Quoc Toan
Hanoi

YUGOSLAVIA
Forum
Vojvode Mišića broj 1
Novi Sad
Jugoslovenska Knjiga
Terazije 27
Beograd

25. VI. 1968 Index: 26.017

Acta Morphologica

Academiae Scientiarum Hungaricae

TOMUS XVI * FASCICULUS 2

Akadémiai Kiadó Budapest

1968

ACTA MORPHOLOGICA

A MAGYAR TUDOMÁNYOS AKADÉMIA ORVOSTUDOMÁNYI KÖZLEMÉNYEI

SZERKESZTŐSÉG ÉS KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY U. 21.

Technikai szerkesztő:
Dr. Somogyi Endre

Az Acta Morphologica német, angol, francia és orosz nyelven közöl értekezéseket a kísérletes orvostudomány tárgyköréből.

Az Acta Morphologica változó terjedelmű füzetekben jelenik meg. Több füzet alkot egy kötetet.

A közlésre szánt kéziratok a következő címre küldendők:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Ugyanerre a címre küldendő minden szerkesztőségi és kiadóhivatali levelezés.

Az Acta Morphologica előfizetési ára kötetenként belföldre 120, külföldre 165 Ft. Megrendelhető a belföld számára az Akadémiai Kiadónál (Budapest V., Alkotmány utca 21. Bankszámla 05-915-111-46), a külföld számára pedig a „Kultúra" Könyv- és Hírlap Külkereskedelmi Vállalatnál (Budapest I., Fő utca 32. Bankszámla: 43-790-057-181) vagy annak külföldi képviseleteinél és bizományosainál.

Die Acta Morphologica veröffentlichen Abhandlungen aus dem Bereiche der experimental-medizinischen Wissenschaften in deutscher, englischer, französischer und russischer Sprache.

Die Acta Morphologica erscheinen in Heften wechselnden Umfages. Mehrere Hefte bilden einen Band.

Die zur Veröffentlichung bestimmten Manuskripte sind an die folgende Adresse zu senden:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

An die gleiche Anschrift ist auch jede für die Schriftleitung und den Verlag bestimmte Korrespondenz zu richten.

Abonnementspreis pro Band: 165 Forint. Bestellbar bei dem Buch- und Zeitungs-Außenhandels-Unternehmen »Kultúra« (Budapest I., Fő utca 32. Bankkonto Nr. 43-790-057-181) oder bei seinen Auslandsvertretungen und Kommissionären.

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 147—156, 1968*

Department of Anatomy, Histology and Embryology (Director: Prof. I. KROMPECHER), University Medical School, Debrecen, and Central Research Institute (Director: Dr. S. HOLLÁN) of the National Blood Bank, Budapest

STUDY OF EXPERIMENTAL FIBRILLOGENESIS IN TUNICA MUSCULARIS OF THE RAT URETER

P. LADÁNYI and GY. LELKES

(Received July 25, 1966)

The development of collagenous and elastic fibres was studied in the muscular layer of the hydro-ureter developed above a ligature applied on the ureter of adult male rats. Based on both light- and electron-microscopic examinations, it is suggested that the elastic and collagenous fibres are produced by the smooth muscle cells of the tunica muscularis. This function of smooth muscles is considered an activation by mechanical factors of atavistic fibroelastoblastic capacities, *viz.* a special case of qualitative adaptation.

Introduction

Numerous recent examinations have shown that the media of muscular, as well as elastic type arteries contains a single cell type, the smooth muscle cell or its modified form [8, 9, 19, 20, 21, 22, 23, 25, 27]. These smooth muscle cells should be considered to produce the extracellular substances in the media. Our previous studies on the comparative histogenesis of elastic fibres in the aorta have suggested that the intracellular matrix is produced by cells which later differentiate into smooth muscle cells and continue matrix production in the phase when they already develop contractile filaments [19]. SEIFERT [25] regards the smooth muscle cells of the media as a particular kind of muscle cell with fibroblast functions. A similar function has been considered by KARRER [9] to be a distinctive feature between medial and "common" visceral smooth muscle cells. The problem is whether or not this distinction is justified, or, in other words, whether or not the "common" mesenchymal smooth muscle had in fact lost its capacity of producing extracellular matrices. Recent works dealing with the morphology and function of smooth muscle [5, 6, 7] do not mention any fibroblast-like function. In contrast, the textbook by STÖHR, MÖLLENDORFF and GOERTTLER [28], presents the following sentence: ». . . . . der Schluß liegt nahe, daß die glatten Muskelzellen ebenso wie die Bindegewebszellen die Fähigkeit besitzen, Intrazellularsubstanz zu bilden . . .« (". . . . the conclusion is at hand that smooth muscle cells, similarly to connective tissue cells, are capable of producing intracellular substance . . . .").

In the present study it has been examined on the basis of LANGE's [16] results, whether or not the smooth muscle cells of a visceral smooth muscle tissue were able to produce extracellular matrix.

Materials and methods

A total of 15 adult male rats was used. Laparotomy was performed under ether anaesthesia and the right ureter was ligated with a fine silk thread transperitoneally at the border between its middle and lower thirds and then the abdominal wall was closed. The animals were killed 3, 4, 7, 9, 14 and 21 days later and both ureters were prepared for light and electron microscopic studies.

For light microscopy, Susa fixation was followed by embedding in paraffin and staining with haematoxylin chromotrope, Goldner, or resorcin-funchsin van Gieson methods.

For electron microscopy, the specimens were fixed in 6% glutaraldehyde in Millonig buffer. After rinsing in buffer, the specimens were treated with 1% OsO_4. Following embedding in Durcupan, ultrathin sections were prepared by a Porter-Blum MT-1 ultramicrotome. For staining, lead citrate or uranyl acetate was used. Examinations were performed with a JEM 6 C electron microscope.

Results

In the muscular layer of untreated rat ureters, smooth muscle cells formed compact coherent bundles. Neither collagenous, nor elastic fibres were seen between the neighbouring smooth muscle cells (Fig. 1).

Fig. 1. Detail of tunica muscularis from a control ureter. The smooth muscle cells are close to each other, neither elastic fibres, nor connective tissue cells are present. Resorcin-fuchsin van Gieson's stain

Fig. 2. Hydro-ureter 21 days after ligation. The smooth muscle cells of the tunica muscularis are practically all surrounded by elastic fibres. Resorcin-fuchsin van Gieson's stain

In electron micrographs, the surface of smooth muscle cells appeared to be covered by a 400—500 Å thick basal membrane. No other structured extracellular component was detectable. The myofilaments of the smooth muscle cells were clearly distinguishable; they filled practically the whole cytoplasm. Cellular organelles were exceptional. The presence of numerous pinocytotic

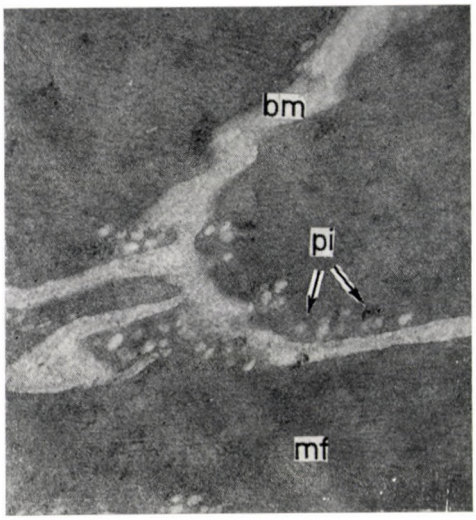


Fig. 3. Control ureter. In the extracellular space between the apically coherent smooth muscle cells no structured extracellular component is detectable except for the basal membrane. Magnification. × 25 000

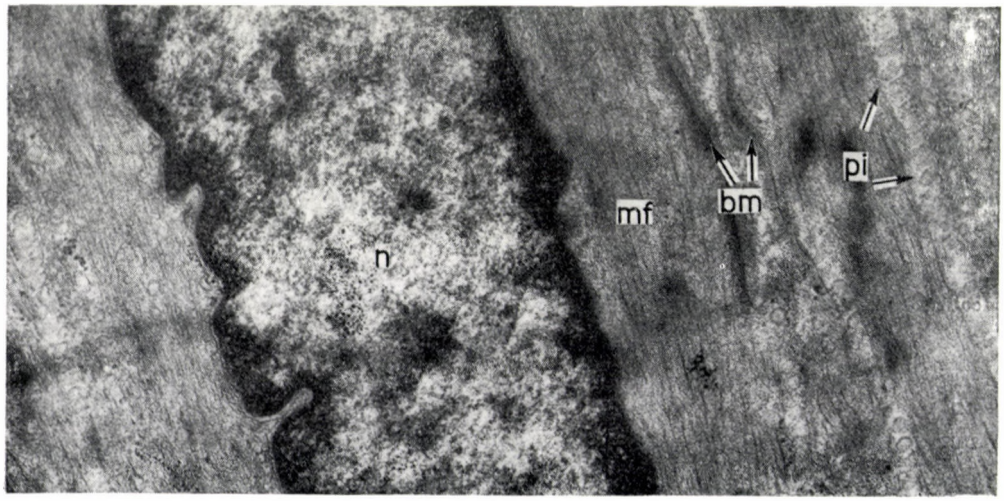


Fig. 4. Control ureter. The cytoplasm of laterally coherent smooth muscle cells is filled with myofilaments. Close to the cell surface, pinocytotic activity is visible. The extracellular space is restricted and exhibits contents identical to that shown in Fig. 3. Magnification, × 26 500

vesicles close to the membrane completed the well-known electron microscopic picture of the normal smooth muscle cell (Figs 3 and 4).

Next to the ligature a hydro-ureter developed and this was followed by hydronephrosis. The smooth muscle cells of the tunica muscularis were

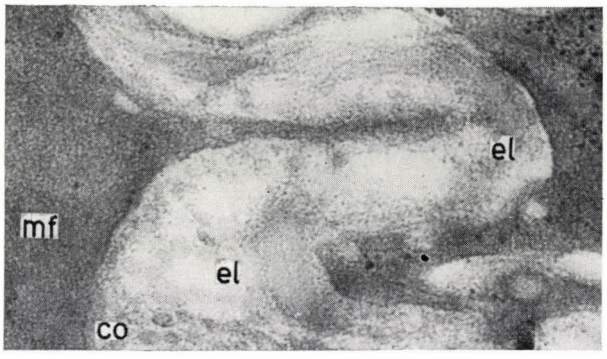


Fig. 5. Hydro-ureter 9 days after ligation. In the distended extracellular space, between two neighbouring smooth muscle cells collagenous and elastic fibres are seen. Magnification, × 42 000

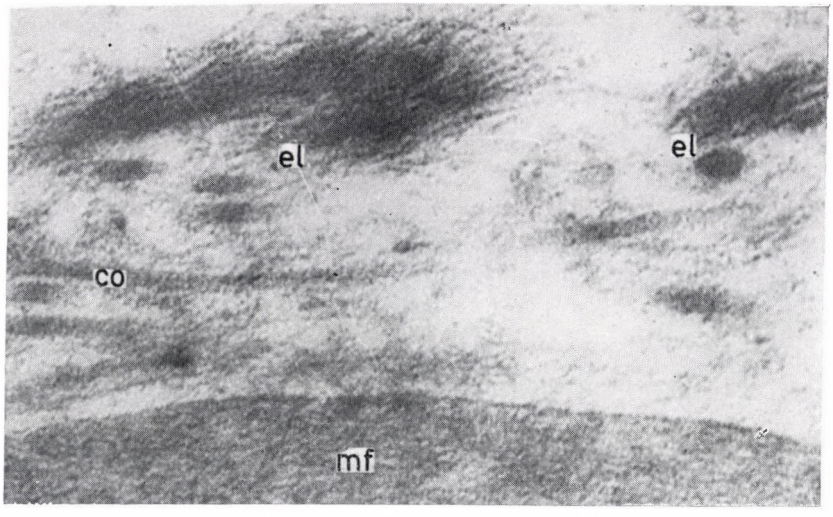


Fig. 6. Hydro-ureter 21 days after ligation. In the markedly distended extracellular space, collagenous and elastic fibres are seen. The smooth muscle cell in the lower sixth of the picture contains numerous myofilaments. Magnification, × 75 000

hypertrophic and elongated, the nuclei short and broad. At the same tissue, an increasing amount of extracellular matrix appeared between the smooth muscle cells, causing separation of the usually compact structure.

This process began as early as 3—4 days after ligation. In the newly formed extracellular matrix, both elastic and collagenous fibres were detectable. In the tunica muscularis, the elastic fibres appeared in close contact with the smooth muscle cells, seemingly ensheathing the individual cells. In later

phases, the number of elastic and collagenous fibres increased constantly. Their close association with the smooth muscle cells was, however, unequivocal even in 21-day specimens (Fig. 2). Apart from smooth muscle cells, no other cells were present in the tunica muscularis, where excessive numbers of connective tissue fibres were seen to develop.

The electron microscopic pictures supported the light microscopic observations, showing distended extracellular spaces in which collagenous and elastic

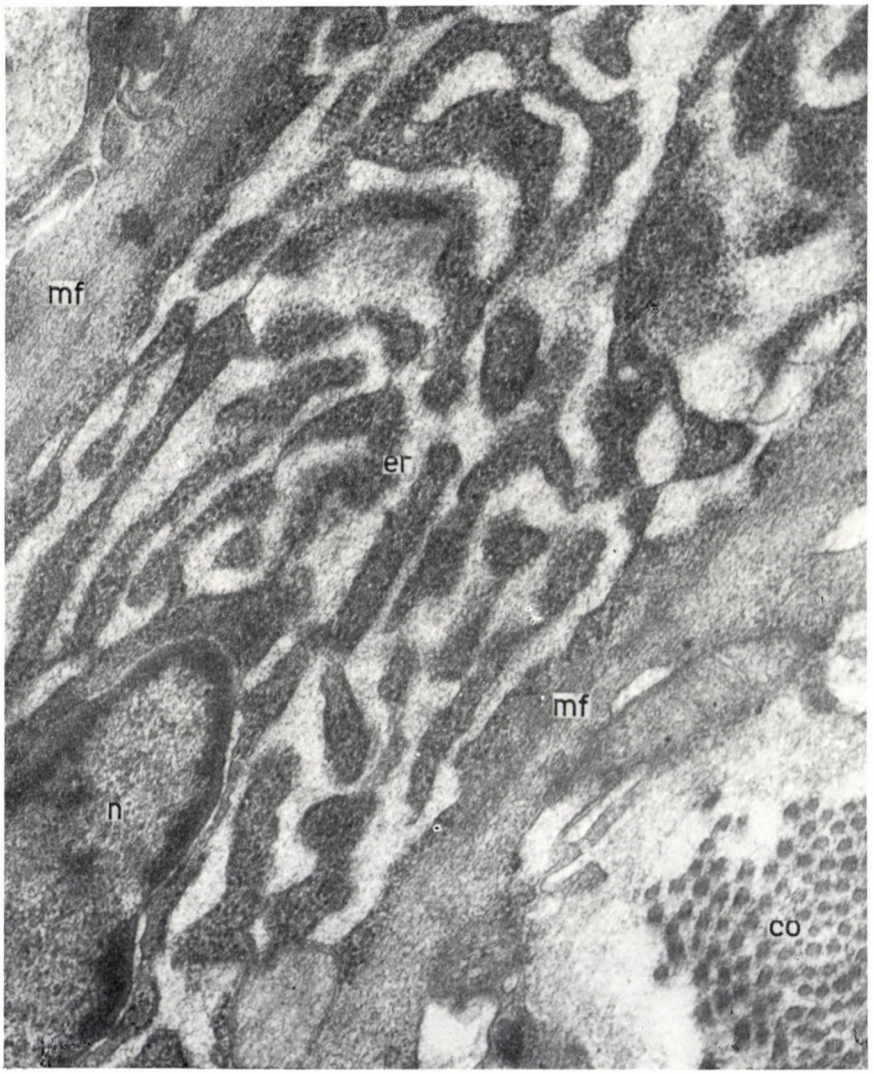


Fig. 7. Hydro-ureter 21 days after ligation. The central area of the smooth muscle cell's cytoplasm is filled with ergastoplasm consisting of dilated cisterns. At the periphery at both sides, the maintained myofilamentary structure is clearly recognizable. Magnification, × 3000

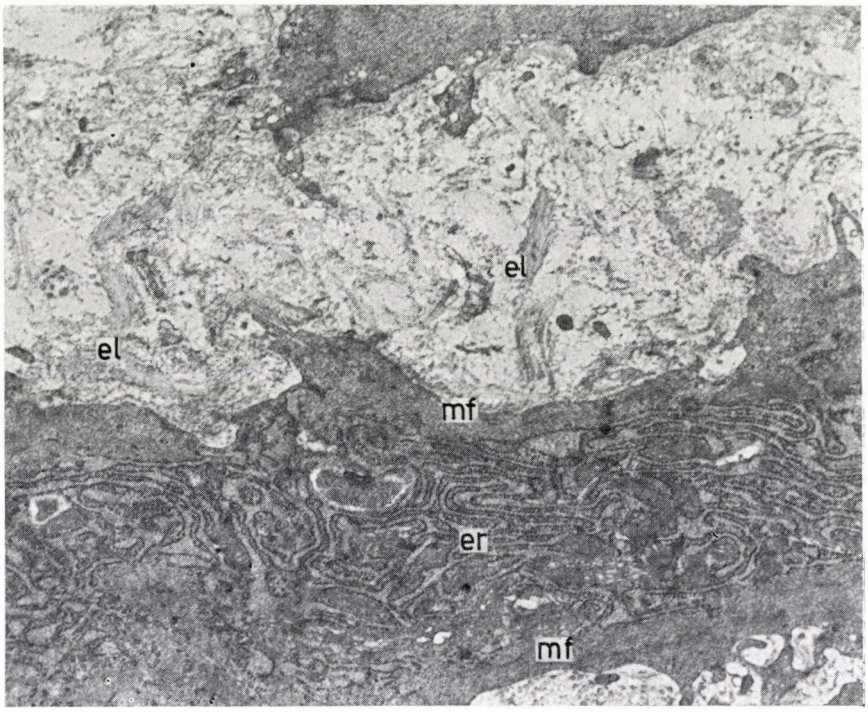


Fig. 8. Hydro-ureter 21 days after ligation. The altered structure of the smooth muscle cell is identical with that shown in Fig. 7. In the extracellular space, elastic fibres are predominating. Magnification, × 11 400

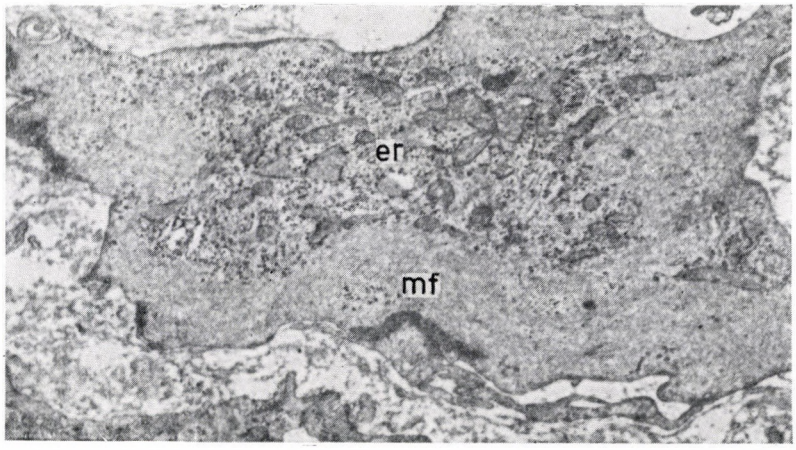


Fig. 9. A media cell from the aorta of a 35 mm sheep embryo. Magnification, × 13 500
Explanations to the Figures: *n* = nucleus, *er* = ergastoplasm, *mf* = myofilament, *pi* = pinocytotic vesicle, *bm* = basal membrane, *co* = collagenous fibre, *el* = elastic fibre

elements made their appearance (Figs 5, 6). Simultaneously, the smooth muscle cells exhibited considerable structural changes. The number of organelles increased at both ends of the nuclei. This was particularly conspicuous with the rough-surfaced endoplasmic reticulum, which exhibited cistern-like enlargements filled with intermediary electron-dense substance. The appearance and accumulation of endoplasmic reticulum caused the smooth muscle cells to resemble fibroblasts. An important differentiating feature was, however, the presence at the periphery of smooth muscle cells of an about 1 μ wide zone filled with myofilaments (Figs 7 and 8).

Discussion

The nature and structure of the intercellular matrix as well as the ultrastructure of the smooth muscle cells in the control ureters was essentially identical with that known from literature [16, 17].

In the wall of the hydro-ureter which had developed above the ligature, the appearance of elastic fibres agreed with the observations of LANGE [16] in specimens with stricture. In the rat, stricturing of the ureter as experimental method can hardly be carried out; it would involve namely considerable technical difficulties, particularly if standardization of the method would be required. Proximal to the stricture an intermittent increase of pressure takes place which is clearly the cause of all lesions in the retrostrictural part of the ureter and of the appearance of intercellular elastic fibres as well. The importance of mechanical factors in the formation and development of elastic fibres was emphasized by KROMPECHER in 1928 and again in 1940 [10, 14]. BLOOM [3] stated that the contractile force is not an absolute requirement of the development of elastic fibres, nevertheless in tissue cultures the most intensive fibre formation was seen to occur in regions where such a force had been at play. LELKES and KARMAZSIN [18] observed the formation of new elastic elements in aorta cultures which remained in contact with the beating heart. In contrast, explants of pure aortic tissue failed to give rise to elastic elements, and the residual fibres disintegrated into granular debris.

In the ligated ureter, the filling of the proximal part of the organ results in a highly increased intra-ureteral pressure and above a certain level absorption starts through the wall of the pelvis. Subsequently the actual tension depends on the ratio of excretion and absorption [1]. An increase of tension elevates the frequency and speed of peristaltic waves [4, 26] and frequent anti-peristaltic waves also seem to appear. The results have shown that *the altered mechanical environment produced by the ligature was an effective stimulus for the formation of connective tissue fibres.*

The experimentally induced fibrillogenesis in the wall of the ureter proves that production of connective tissue fibres is not bound to certain specific

cells but seems to be common to various cell types. The media cells of embryonic vessels produce overwhelmingly elastic fibres in the early phase of evolution. According to KROMPECHER, these cells may make their appearance in certain pathologic conditions and may be connected with the large amount of newly formed elastic fibres ["elastoblast"; KROMPECHER 1928; 10, 11, 12, 14]. The elastic fibres of elastic cartilages are produced by cartilage cells ["elastochondroblast"; KROMPECHER 1928; 11, 13]. In his paper on elastogenesis in tissue culture, SCHWARTZ [24] stated that fibroblasts producing elastic fibres did not differ from common fibroblasts. The present experiments have shown unequivocally that *the smooth muscle cells of the rat ureter are capable of producing connective tissue fibres.*

In the muscular layer of the hydro-ureter developing proximal to the ligation, parallel with the transformation of the smooth muscle cell ultrastructure the development of collagenous and elastic fibres in the intercellular space has been observed. The ergastoplasmic accumulation in the smooth muscle cells of the tunica muscularis suggested the secretory activity of these cells. Details of the phenomenon are not quite clear. It may be supposed that in this case a special reaction to the altered environment of a preformed membrane system normally masked by the myofilaments takes place rather than a de-differentiation of smooth muscle cells into fibroblasts. The problem requires further study. The possibility of de-differentiation has been contradicted by our finding that 41 days after the operation peristaltic movements could still be elicited in the hydro-ureter of the anaesthetized animal.

Comparison of these experimental results with pictures of the developing aorta revealed a striking similarity. Fig. 7 shows the aortic media of a 35 mm sheep embryo. Here, too, the ergastoplasm occupies the central part of the cells, whereas myofilaments are present at their periphery.

Our results appear to permit the conclusion that *the smooth muscle cells are not definitely differentiated for a single function, but are reacting dynamically to environmental stimuli. This may result in their outstanding role in the production and maintenance of intercellular matrix*, a function which under normal conditions is best seen in the vessel wall.

The pluripotent mesenchymal cells developing into the media cells of arteries live under conditions inducing and maintaining their capacity to produce extracellular connective matrices. In the absence of adequate mechanical impulses, the undifferentiated mesenchymal cells develop into common smooth muscle. Under experimentally induced changes in the mechanical conditions such as a lasting increase of pressure with intermittently changing tension, the latent primary fibro-elastoblastic capacities of these cells are activated. The phenomenon may be considered a special case of qualitative adaptation [15].

REFERENCES

1. Babics, A., Rényi-Vámos, F.: (1950) A vese üregrendszerének patofiziológiája és műtétei. Akadémiai Kiadó, Budapest. — 2. Benninghoff, A.: (1930) Blutgefäße und Herz. W. Möllendorff: Handbuch der mikroskopischen Anatomie, Vol. 6, part 1. Springer, Berlin. — 3. Bloom, W.: (1930) Studies on Fibers in Tissue Culture. II. The Development of Elastic Fibers in Cultures of Embryonic Heart and Aorta. Arch. exp. Zellforsch. **9,** 6. — 4. Enhörning, G. and Weaver, R. G.: (1964) Intraureteric Pressure above and below an Area of Compression. Amer. J. Obst. Gynec. **90,** 1332. — 5. Gansler, H.: (1960) Phasenkontrast- und elektronenmikroskopische Untersuchungen zur Morphologie und Funktion der glatten Muskulatur. Z. Zellforsch. **52,** 60. — 6. Gansler, H.: (1961) Struktur und Funktion der glatten Muskulatur II. Licht- und elektronmikroskopische Befunde an Hohlorganen von Ratte, Meerschweinchen und Mensch. Z. Zellforsch. **55,** 724. — 7. Häggqvist, G.: (1956) Gewebe und Systeme der Muskulatur. In: W. Möllendorff: Handbuch der mikroskopischen Anatomie. Vol. 2. Part 4, Springer, Berlin. — 8. Karrer, H. E.: (1960) Electron Microscope Study of Developing Chick Embryo Aorta. J. Ultrastruct. Res. **4,** 420. — 9. Karrer, H. E.: (1961) An Electron Microscope Study of the Aorta in Young and in Ageing Mice. J. Ultrastruct. Res. **5,** 1. — 10. Krompecher, St.: (1928) Die Entwicklung der elastischen Elemente der Arteriewand. Z. Anat. Entwickl.-Gesch. **85,** 704. — 11. Krompecher, St.: (1928) A rugalmas elemek fejlődése. Magy. Orv. Arch. **6,** 3. — 12. Krompecher, St.: (1930) Teleangiostenose, die morphologische Grundlage der »juvenilen« oder »spontanen« Gangränen (Endarteritis obl., Arteriitis obl., Thromboangiitis obl., (Arteriolosclerosis renum und Scleroderma). Beitr. path. Anat. **85,** 647. — 13. Krompecher St.: (1931) Die Entwicklung des elastischen Knorpels. Verh. anat. Ges., Anat. Anz. **71,** 42. — 14. Krompecher, St.: (1940) Die Gefässwandentwicklung in kausalhistogenetischer und vergleichend-funktioneller Darstellung. Z. anat. Entwickl.-Gesch. **110,** 423. — 15. Krompecher, St.: (1958) Die qualitative Adaptation der Gewebe. Z. mikr.-anat. Forsch. **64,** 59. — 16. Lange, K. H.: (1938) Über Anpassungsvorgänge am Ureter, Ductus deferens und Uterushorn der weissen Ratte. Verh. anat. Ges., Anat. Anz. **87,** 379. — 17. Leeson, Th.S. and Leeson, C. R.: (1965) The Rat Ureter. Fine Structural Changes during its Development. Acta anat. (Basel) **62,** 60. — 18. Lelkes, Gy. and Karmazsin, L.: (1955) Development of Elastic Elements in Tissue Cultures. Acta morph. Acad. Sci. hung. **5,** 149. — 19. Lelkes, Gy., Ladányi, P.: (1964) Az érfal elastica képződésének elektronmikroszkópos vizsgálata. Magyar angiológiai sectio, Debrecen. — 20. Paule, W. J.: (1963) Electron Microscopy of the Newborn Rat Aorta. J. Ultrastruct. Res. **8,** 219. — 21. Parker, F.: (1958) An Electron Microscope Study of Coronary Arteries. Amer. J. Anat. **103,** 247. — 22. Pease, D. C. and Molinari, S.: (1960) Electron Microscopy of Muscular Arteries; Pial Vessels of the Cat and Monkey. J. Ultrastruct. Res. **3,** 447. — 23. Pease, D. C., Paule, W. J.: (1960) Electron Microscopy of Elastic Arteries of the Thoracic Aorta of the Rat. J. Ultrastruct. Res. **3,** 469. — 24. Schwartz, W.: (1964) Elektronmikroskopische Untersuchungen über die Bildung elastischer Fasern in der Gewebekultur. Z. Zellforsch. **63,** 636. — 25. Seifert, K.: (1963) Elektronenmikroskopische Untersuchungen der Aorta des Kaninchens. Z. Zellforsch. **60,** 293. — 26. Sleator, W. jr., Butcher, H. R. jr.: (1955) Action Potentials and Pressure Changes in Ureteral Peristaltic Waves. Amer. J. Physiol. **180,** 261. — 27. Smith, Chr., Seitner, M. M., Huan-Pao Wang: (1951) Ageing Changes in the Tunica Media of the Aorta. Anat. Rec. **109,** 13. — 28. Stöhr, W., v. Möllendorff, W., Goerttler, K.: (1955) Lehrbuch der Histologie. 27. ed. Fischer, Jena.

UNTERSUCHUNG DER ELASTOGENESE DER URETERMUSKULATUR BEI RATTEN

P. LADÁNYI und GY. LELKES

Bei erwachsenen Ratten ließ sich in der Muskelschicht des Hydroureters, oberhalb der Ureterligatur die Entwicklung elastischer Fasern beobachten. Es wird angenommen, daß die elastischen und begleitenden kollagenen Fasern durch die Glattmuskelzellen der muskulären Schicht produziert wurden. Diese Funktion der Glattmuskelzellen wird als ein spezieller Fall der qualitativen Adaptation betrachtet und zwar dürfte es sich annehmbar um eine auf Wirkung mechanischer Faktoren entstandene Aktivierung des latenten fibro- bzw. elastoblastischen Potentials handeln.

ИССЛЕДОВАНИЕ ЭКСПЕРИМЕНТАЛЬНОГО ФИБРИНОГЕНЕЗА В МЫШЕЧНОЙ ОБОЛОЧКЕ МОЧЕТОЧНИКА КРЫСЫ

П. ЛАДАНЬИ и Д. ЛЕЛКЕШ

Авторы наблюдали возникновение коллагенных и эластичных волокон в мышечном слое гидроуретера, возникшего над лигатурой мочеточника у взрослых крыс. На основе исследований под световым и электронным микроскопами кажется вероятным, что возникшие эластичные волокна и сопровождающие их коллагенные волокна производятся клетками гладких мышц мышечной оболочки. Эту функцию гладкомышечных клеток авторы рассматривают как активацию первобытного, существующего в скрытом состоянии фибро- или эластобластического потенциала под влиянием механических факторов, как специфический случай качественной адаптации.

Dr. Pál Ladányi; Debrecen 12, Hungary
Dr. György Lelkes; Budapest XI., Daróczi út 24, Hungary

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 157—163, 1968*

Institute of Anatomical Pathology (Director: Prof. L. HARANGHY), University Medical School, Budapest

EXPERIMENTAL INJURY OF MUSCULAR-TYPE BLOOD VESSELS BY CHEMICAL AGENTS

KLÁRA SZEMENYEI, A. KÓCZÉ and H. JELLINEK

(Received April 20, 1967)

The regeneration of blood vessels has been studied since the end of the last century (PICK [31], PEKELHARING [29]; SOKOLOFF [37]; and others). As early as 1885 PICK pointed out that proliferative cells probably originate from smooth muscle or connective tissue cells and the main problem of pertinent investigations is still the origin of cells involved in regeneration. We have studied the problem on large elastic-type and small vessels [3, 14, 15, 17, 18, 22, 39, 40, 45]. In the present paper we shall describe investigations into the effect of different agents on large muscular-type vessels and into the origin of cellular elements of intimal proliferation occurring in regeneration.

Materials and methods

Thirty-one 2 to 3-year-old rabbits of both sexes weighing 4 to 5 kg were used. A 3 cm part of the femoral artery on each leg was exposed in ether anaesthesia at the same site in all animals. Then the vessel was deprived of the adventitia and painted with 33% and 8% hydrochloric acid and iodine. The animals were sacrificed by bleeding at intervals between 5 minutes and 352 days after treatment. The untreated part of the femoral artery of the same animal was used as control. Histological examination was performed after fixation in neutral formalin and staining with haematoxylin-eosin, Azan, Mallory's phosphotungstic acid haematoxylin, resorcin-fuchsin and van Gieson, and optical polarization methods. Because of their high potassium content muscle cells and erythrocytes stained selectively yellow with Siena-Orange (S—O) recommended by CARRERE and COOMS.

Results

The three agents caused almost identical changes in the arterial wall. The necrotizing effect of 33% hydrochloric acid was more definite than that of the other solutions. There was no difference between the agents in the time of the development of the lesion and in regeneration. The degree of intimal proliferation and subsequent fibrosis was also similar with the three agents. The findings, therefore, are not discussed separately.

A few minutes after treatment the vascular wall became necrosed, homogeneous and thin and stained evenly; parts remaining relatively intact contained vacuolized smooth muscle cells characterized by weak staining. The internal elastic membrane lost its usual wavy form and became elongated (Fig. 1). In 5 out of 32 vessels thrombosis was observed. From the 5th to 9th days onward the internal elastic membrane broke up or disintegrated (Fig. 2). Through these parts an intimal proliferation grew and narrowed the lumen gradually.

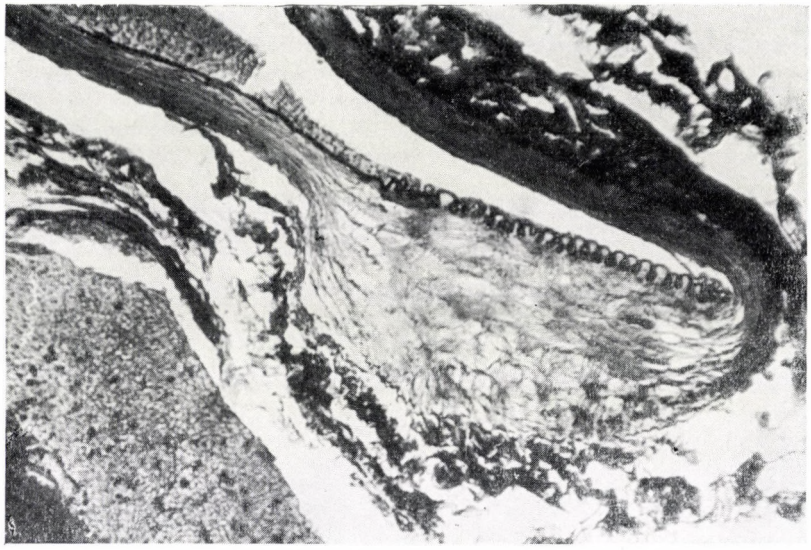


Fig. 1. 15 minutes after HCl treatment the necrosed part of the artery is thinner, smooth muscle cells are replaced by homogeneous material. Vacuolized muscle cells in the relatively intact area. Azan stain

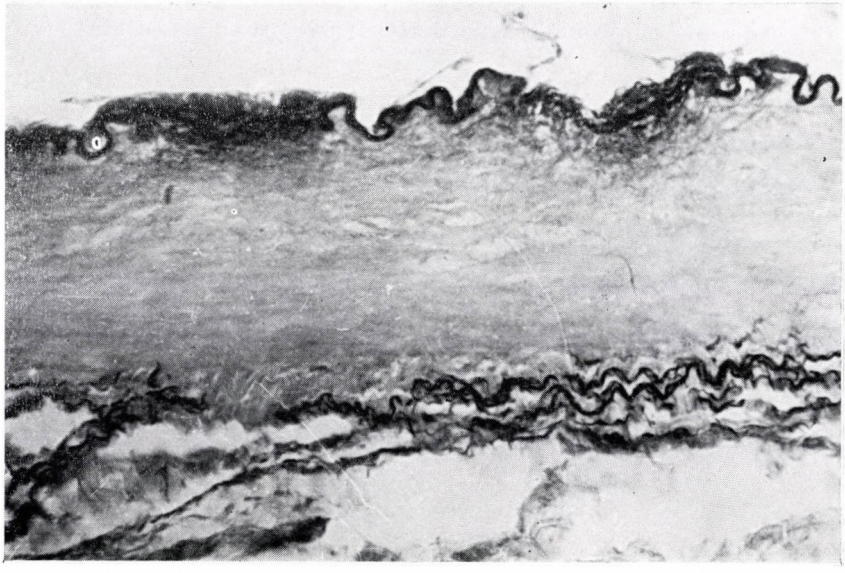


Fig. 2. 9 days after HCl treatment disintegration and disruption in the internal elastic membrane. Resorcin-fuchsin stain

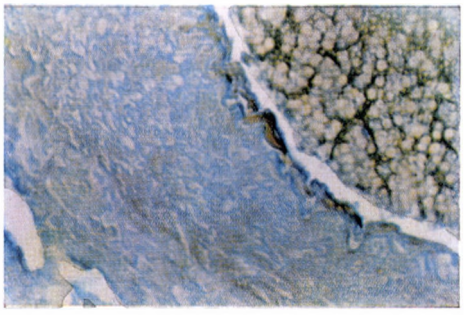


Fig. 3. 5 days after HCl treatment, smooth muscle cells staining yellow with Siena-Orange in the new intima; they are well distinguishable from the blue internal elastic membrane and from endothelial cells

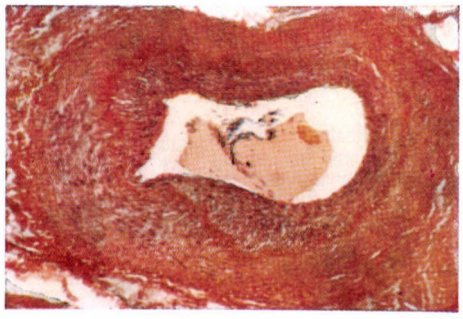


Fig. 4. 120 days after HCl treatment, cells of the thick intimal proliferation are similar in staining and arrangement to smooth muscle cells of the original vascular wall. Mallory's triple dye

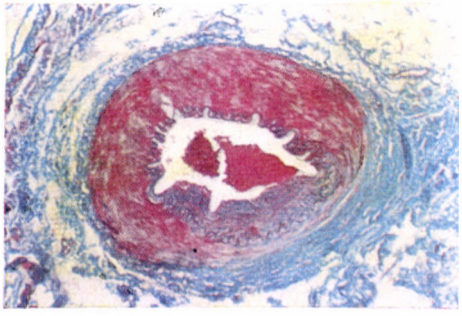


Fig. 7. 144 days after HCl treatment, thick intimal proliferation narrowing the lumen. Many collagenous fibres in the media, adventitia, and in the intimal proliferation facing the lumen. Azan stain

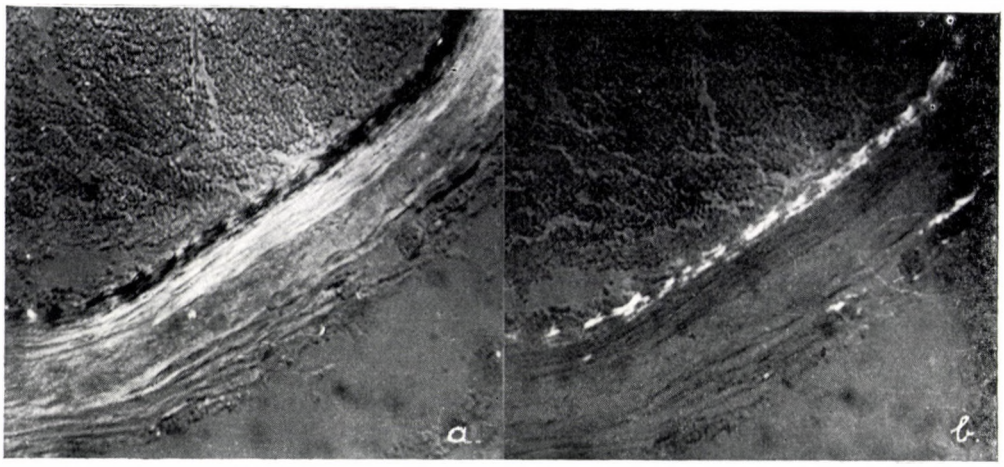


Fig. 5. 40 days after HCl treatment: *(a)* polarization micrograph showing doubly refractive smooth muscle cells in the newly formed intima; *(b)* the same with reversed compensation. Aniline reaction

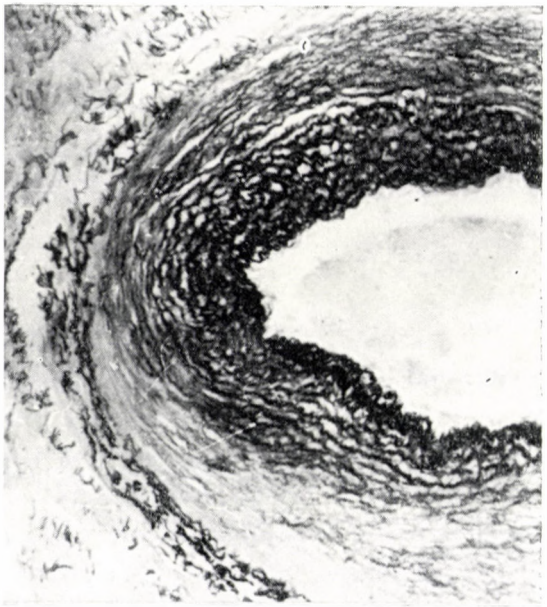


Fig. 6. 40 days after HCl treatment, large numbers of wavy elastic fibres in intimal proliferation. The new internal elastic membrane is clearly distinguishable. Resorcin-fuchsin stain

The cells originated partly from the media situated at the border between the normal and pathological areas, partly from intact smooth muscle cells of the necrotic media situated under the internal elastic membrane. In the stomata and surface of the membrane S—O staining revealed cells reacting as smooth muscle cells (Fig. 3).

After 20 to 30 days there was a thick intimal proliferation which narrowed the lumen after 60 days (Fig. 4). By Mallory and S—O staining the cells were identified as smooth muscle cells. Polarization microscopy after aniline reaction also showed them to be positive doubly refractive smooth muscle cells (Fig. 5). On the surface of proliferation facing the lumen a new internal elastic membrane had developed. In the adventitia there was a fibrous change. After longer periods fine wavy elastic fibres developed at the site of the necrotic media and in the intimal proliferation. The fibres stained well with resorcin-fuchsin (Fig. 6). After 100 to 120 days, starting from the intimal proliferation facing the lumen, a fibrous connective tissue developed which gradually replaced the smooth muscle cells. The process spread to the media and joined the earlier fibrous change in the adventitia (Fig. 7).

Discussion

SOLOVYEV [38] in a review described that for regeneration studies vascular wall lesions were brought about by compression (MALKOFF and D'ANNA) or ligation of the vessel (JORES), removing the adventitia (SCHILLING), thermocautery and silver nitrate (ANDRIEVICH), turpentine (SMINKOVA), zinc chloride (FORMACHIDIS) and thermocautery (SOLOVYEV). TRENCKMAN [44] applied paraffin blockade and JAFFE et al., [16] electric current for inducing intimal proliferation. For the same purpose THORBAN [43] brought about a partial injury of the sciatic, tibial and fibular nerves.

Intimal proliferation cells are regarded by some authors as of connective tissue, by others as of endothelial origin. According to KARRER [21] it is fibroblasts, according to PETRI and HEBERER [30] and SINAPIUS [35], it is fibrocytes which develop into endothelial or smooth muscle cells. MAXIMOV [26] stated that fibrocytes may be produced from endothelial cells. This finding was confirmed by MARUYAMA [25] in tissue cultures. Opinions also disagree as to the regeneration capacity of endothelial cells. EFSKIND [6] and LINZBACH [24] denied the possibility, while FLOREY et al. [7] stressed the importance, of endothelial cell regeneration. In the opinion of EFSKIND [6], SINAPIUS [35] and others, giant cells situated under the endothelium play an important role in regeneration. ALTSCHUL and FAUL-BOEHMLER [1], HAUST [12] and RODBARD et al. [33] supposed that pluripotent intermediary cells may differentiate into endothelial, connective tissue and smooth muscle cells.

Our findings indicated that the cells of intimal proliferation are smooth muscle cells originating from the media. This was shown by the fact that Mallory and S—O staining revealed reactions characteristic of smooth muscle cells. Growth of these elements through the fenestrated internal elastic membrane was also demonstrated. The smooth muscle origin of these cells was advocated by DIXON [5], JOHNSON et al. [19], SOLOVYEV [38], MOHRATRA [27],

RAAB [32], SINAPIUS [35], CSILLAG [3], JELLINEK et al. [17, 18], FLOREY et al. [7] and HAUST [12] and confirmed by electronmicroscopic examinations [4, 8, 22, 45].

In the development of intimal proliferation neural factors may also be involved. GUTSTEIN et al. [10] showed subendothelial cell proliferation and fibrosis of the elastic tissue in the aorta of rats after excitation of the splanchnic nerve. THORBAN [43] attributed an important role to the injury of vegetative nerve fibres in intimal proliferation elicited by a partial damage of the sciatic, tibial and fibular nerves; the condition led to permeability disorders and intimal oedema and in the final stage to vascular fibrosclerosis. Similar factors may have had a role in our experiments, since the chemical agents used must have injured the nerves situated in the neighbourhood of the artery. In chronic lesions fine wavy elastic fibres were present in the intimal proliferation and necrotic media. Similar observations were made by several authors [7, 8, 9, 12, 21, 28]. As to the origin of these fibres there are several hypotheses. PEASE and PAULE [28], GEER [9], FRENCH et al. [8] and KARRER [21] supposed that smooth muscle cells were responsible for their formation. According to HAUST [12] they originate from decomposing elastic fibres. FRENCH rejected this conception and thought likely that they developed from connective tissue cells. LAITINEN [23] supposed an extracellular origin. KÁDÁR et al. [22] called the attention to a close connection between the fibres and smooth muscle cells. The observations of THORBAN [43] indicated that vascular fibrosis was associated with permeability disorders. SEIFERT [34] explained this observation as follows. The rabbit's aorta, which contains no vasa vasorum, is nourished by diffusion from the adventitia and intima. Under normal conditions the loose structure of the adventitia ensures this process through the tissue fluids. Nutrition of the femoral artery might take place in a similar manner. Fibrosis spreading to the intima and media in advanced injury may be the consequence of adventitial fibrosis ensuring in the early stage, as the accumulation of fibres leads to a decrease in permeability and thus to a disturbance in the vessel's nutrition.

REFERENCES

1. ALTSCHUL, R., FAUL-BOEHMLER, E.: (1963) Endothelium in Contracted Arteries. Virchows Arch. path. Anat. **336,** 383. — 2. BAUMGARTEN, P.: (1876) Über die sogenannte Organisation des Thrombus. Zbl. med. Wiss. **14,** 593. — 3. CSILLAG, I., JELLINEK, H.: (1960) Regeneration of the Wall of Large Veins with Different Grafts. Acta chir. Acad. Sci. hung. **1,** 375. — 4. DAVID, H., HACKENSELLNER, H. A., WOLF, W.: (1963) Submikroskopische Untersuchungen an der Neointima in Kunststoffprothesen beim Hund. Frankfurt. Z. Path. **72,** 548. — 5. DIXON, O. J.: (1934) Experimental Studies in Vascular Repair. J. Amer. med. Ass. **103,** 1129. — 6. EFSKIND, L.: (1941) Die Regenerationsverhältnisse im Intimaepithel nach Gefäßsutur. Acta chir. scand. **89,** 283. — 7. FLOREY, H. W., GREER, S. J., POOLE, J. C. F., WERTHESSEN, N. T.: (1961) The Pseudointima Lining Fabric Grafts of the Aorta. Brit. J. exp. Path. **42,** 236. — 8. FRENCH, J. E., JENNINGS, M. A., POOLE, J. C. F., ROBINSON, D. S., FLOREY, H.: (1963) Initial Changes in the Arteries of Ageing Survive. Proc. roy. Soc. B. **158,** 24. — 9. GEER, J. C.: (1965) Fine Structure of Human Aortic Intimal Thickening and Fatty Streaks.

Lab. Invest. **14,** 1764. — 10. GUTSTEIN, W. H., LATAILLADE, J. N., LEWIS, L.: (1962) The Role of Neuroexcitation in Arteriosclerosis. Metabolismus Parietis Vasorum. P. 307. Comptes Rendus du IV^{e} Congrès International d'Angiologie, Praha. Septembre 1961. Státni zdravotnické nakladatelství. — 11. HAUST, M. D., MORE, R. H., MOVAT, H. Z.: (1960) The Role of Smooth Muscle Cells in the Fibrogenesis of Arteriosclerosis. Amer. J. Path. **37,** 377. — 12. HAUST, M. D.: (1965) Fine Fibrils of Extracellular Space (Microfibrils). Their Structure and Role in Connective Tissue Organization. Amer. J. Path. **47,** 1113. — 13. HOLLE, G.: (1959) Die Bedeutung der Nierengefäße für den Ablauf der chronischen Pyelonephritis und die Steinpyelonephrose. Virchows Arch. path. Anat. **332,** 494. — 14. HÜTTNER, I., JELLINEK, H., KERÉNYI, T., SZEMENYEI, K.: (1965) Savecseteléssel létrehozott fibrinoid érfalnecrosis kialakulása. Morph. és Ig. Orv. Szle **5,** 91. — 15. HÜTTNER, I., JELLINEK, H., KERÉNYI, T., SZEMENYEI, K.: (1966) Fibrinoid Necrosis of the Vascular Wall Induced by Painting with Acid. Acta morph. Acad. Sci. hung. **14,** 169. — 16. JAFFE, R. H., WILLIS, D., BACHEM, A.: (1929) Über die nach elektrischen Gefäßwandschädigungen auftretenden Heilungsvorgänge. Zbl. allg. Path. **44,** 1241. — 17. JELLINEK, H., SZEMENYEI, K., KERÉNYI, T., HÜTTNER, I.: (1965) Muscularis típusú kisarteriák savecsetelés után észlelt elváltozása. Morph. és Ig. Orv. Szemle **5,** 88. — 18. JELLINEK, H., SZEMENYEI, K., KERÉNYI, T., HÜTTNER, I.: (1966) Effect of Painting with Acid on Muscular Type Small Arteries. Acta morph. Acad. Sci. hung. **14,** 165. — 19. JOHNSON, J., KIRBY, C. K., GREIFENSTEIN, F. E., CASTILLO, A.: (1949) The Experimental and Clinical Use of Vein Grafts to Replace Defects of Large Arteries. Surgery **26,** 945. — 20. JORES, L.: (1898) Über die Neubildung elastischer Fasern in der Intima bei Endarteritis. Zieglers Beitr. path. Anat. **24,** 458. — 21. KARRER, H. E.: (1961) An Electron Microscope Study of the Aorta in Young and Ageing Mice. J. Ultrastruct. Res. **5,** 1. — 22. KÁDÁR, A., VERESS, B., JELLINEK, H.: (1966) Fibre Formation in Experimentally Induced Intimal Proliferation. Acta morph. Acad. Sci. hung. Suppl. **14,** 355. — 23. LAITINEN, E.: (1963) Changes in the Elemental Structures of the Aorta in Human and Experimental Atherosclerosis. Acta path. microbiol. scand. Suppl. 167. — 24. LINZBACH, A. J.: (1951) Untersuchungen über die Grenzschicht zwischen Blut und Gefäßwand. Verh. dtsch. Ges. Path. **34,** 252. — 25. MARUYAMA, J.: (1963) The Human Endothelial Cell in Tissue Culture. Z. Zellforsch. **60,** 69. — 26. MAXIMOV, A.: (1927) Bindegewebe und blutbildende Gewebe. Arch. exp. Zellforsch. **4,** 14. — 27. MOHRATRA, R. M. L.: (1953) An Experimental Study of Changes which Occur in Ligated Arteries and Veins. J. Path. Bact. **65,** 307. — 28. PEASE, D. C., PAULE, W. J.: (1960) Electron Microscopy of Elastic Arteries, the Thoracic Aorta of the Rat. J. Ultrastruct. Res. **3,** 469. — 29. PEKELHARING, C. A.: (1890) Über Endothelwucherung in Arterien. Beitr. path. Anat. **8,** 245. — 30. PETRI, G., HEBERER, G.: (1957) Neubildung der Gefäßwand auf Grundlage synthetischer Arterienprothesen. Lagenbecks Arch. klin. Chir. **286,** 249. — 31. PICK, E.: (1885) Über die Rolle der Endothelien bei der Endarteritis post ligaturam. Z. Heilk. **6,** 459. — 32. RAAB, F.: (1879) Über die Entwicklung der Narbe im Blutgefäße nach der Unterbildung. Arch. klin. Chir. **23,** 156. — 33. RODBARD, S., LEHOCZKY, J. M., TERNER, J. Y., MCCANDLESS, E. L.: (1962) Mechanical Factors Affecting Conversion of Arterial Smooth Muscle into Fibroblasts or Cartilage. Metabolismus Parietis Vasorum. P. 696. Comptes Rendus du IV^{e} Congrès International d'Angiologie, Praha. Septembre 1961. Státni zdravotnické nakladatelství. — 34. SEIFERT, K.: (1963) Elektronenmikroskopische Untersuchungen der Aorta des Kaninchens. Z. Zellforsch. **60,** 293. — 35. SINAPIUS, D.: (1952) Über das Aortenendothel. Virchows Arch. path. Anat. **322,** 662. — 36. SMINKOVA, P.: (1903) Ein Beitrag zur Genese der Arteriosklerose. Zieglers Beitr. path. Anat. **34,** 242. — 37. SOKOLOFF, A.: (1893) Über die Bedingungen der Bindegewebsneubildung in der Intima doppelt unterbundener Arterien. Beitr. path. Anat. **14,** 10. — 38. SOLOVYEV, A.: (1929—30) Experimentelle Untersuchungen über die Heilungsvorgänge in der Arterienwunde. Zieglers Beitr. path. Anat. **83,** 485. — 39. SZEMENYEI, K., NAGY, L., JELLINEK, H., CSILLAG, I.: (1962) Submikroskopische und histochemische Untersuchungen der Aortenwand-Regeneration bei experimenteller Nekrose. Metabolismus Parietis Vasorum. P. 257. Comptes Rendus du IV^{e} Congrès International d'Angiologie, Praha, Septembre 1961. Státni zdravotnické nakladatelstvi. — 40. SZEMENYEI, K., JELLINEK, H., NAGY, L.: (1963) Vascular Changes Subsequent to Acid Treatment. Acta morph. Acad. Sci. hung. Suppl. **12,** 21. — 41. SZEMENYEI, K., KÓCZÉ, A., JELLINEK, H.: (1966) Lesion Provoked by Various Agents on Different Types of Blood Vessels and their Regeneration. Acta biol. Acad. Sci. hung. Suppl. **17,** 394. — 42. THOMA, R.: (1883) Über die Abhängigkeit der Bindegewebsneubildung in der Arterienintima von den mechanischen Bedingungen des Blutlaufes. Virchows Arch. path. Anat. **93,** 443. — 43. THORBAN, W.: (1962) Gefäßveränderungen beim experimentellen Sudeck-Syndrom. Metabolismus Parietis Vasorum. P. 579. Comptes Rendus du IV^{e} Congrès International d'Angiologie, Praha, Septembre 1961. Státni zdravotnické nakladatelství. — 44. TRENCKMANN, H.: (1962) Beitrag zur Lokalisation von Gefäßwandprozessen. Metabolismus Parietis Vasorum. P. 1005. Comptes Rendus du IV^{e} Congrès Internatio-

nal d'Angiologie, Praha, Septembre 1961. Státni zdravotnické nakladatelstvi. — 45. Veress, B., Kádár, A., Jellinek, H.: (1966) Ultrastructure of the Cells of Experimentally Induced Intimal Proliferation. Acta morph. Acad. Sci. hung. Suppl. **14**, 356.

VON VERSCHIEDENEN SCHÄDIGENDEN FAKTOREN ZUSTANDEGEBRACHTE EXPERIMENTELLE VERÄNDERUNGEN DER GEFÄSSE MUSKULÄREN TYPS

KLÁRA SZEMENYEI, A. KÓCZÉ und H. JELLINEK

Die großen Gefäße muskulären Typs reagieren auf verschiedene schädigende Einwirkungen gleichmäßig. Vom 5—9. Tag an ist über der Membrana elastica interna eine Zellvermehrung sichtbar, die später ausgeprägter wird und das Gefäßlumen verengt. Verfasser sind der Ansicht, daß die Proliferationszellen aus den Glattmuskelelementen der Media stammen. In der neugebildeten dicken Intima und auch an der Stelle der nekrotischen Media entwickelt sich ein feines elastisches Netz; dadurch werden die Gefäße muskulären Typs den elastischen Gefäßen ähnlich. In der Spätphase vermehren sich auf dem geschädigten Gebiet in allen 3 Gefäßwandschichten die Kollagenfasern, was zur Fibrose dieses Gebietes führt.

ЭКСПЕРИМЕНТАЛЬНЫЕ ИЗМЕНЕНИЯ ВОЗНИКАЮЩИЕ НА СОСУДАХ МЫШЕЧНОГО ТИПА ПОД ВЛИЯНИЕМ РАЗЛИЧНЫХ ВРЕДНЫХ ФАКТОРОВ

К. СЕМЕНЬЕИ, А. КОЦЕ и Х. ЙЕЛЛИНЕК

Крупные сосуды мышечного типа дают под влиянием различных вредных факторов одинаковые реакции. Начиная с 5—9-го дня над membrana elastica interna появляется пролиферация клеток, которая впоследствии все более утолщается, вызывая сужение просвета сосуда. Клетки пролиферации — по мнению авторов — происходят из элементов гладкой мышцы средней оболочки сосуда. В возобновляющейся толстой внутренней оболочке сосудов и на месте некротической средней оболочки сосудов возникает тонкая эластичная волокнистость, и в результате этого сосуды мышечного типа становятся похожими на эластичные сосуды. В поздней стадии в поврежденной области во всех трех слоях сосудистой стенки накопляется все больше коллагенных волокон, обуславливая фиброз данной области.

Dr. Klára Szemenyei
Dr. Antal Kóczé
Dr. Harry Jellinek
II. Kórbonctani Intézet, Budapest IX., Üllői út 93, Hungary

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 165—171, 1968*

First Department of Surgery (Director: Prof. P. RUBÁNYI), Institute of Anatomy (Director: Prof. J. SZENTÁGOTHAI), University Medical School, Budapest and Institute of Anatomy, Histology and Embryology (Director: Prof. I. KROMPECHER), University Medical School, Debrecen

EFFECT OF A SINGLE NEONATAL DOSE OF CORTISOL ON THE SERUM AND TISSUE MUCOPOLYSACCHARIDES IN THE RAT

K. VALLENT, M. PALKOVITS and B. MÁNDI

(Received June 15, 1967)

An attempt was made to evaluate the effect of a single neonatal dose of cortisol on the serum and tissue mucopolysaccharides, four weeks after the cortisol administration. Cortisol treatment of newborn rats induced a significant decrease in the serum hexosamine, glycoprotein and tissue mucopolysaccharide. There was a significant decrease in the lymphocyte and medullary basophil (mast cell) count four weeks after treatment.

The effects exerted by postneonatal thymectomy on the body growth, on the morphology of the endocrine and lymphoid organs, and on the white blood cells of mice and rats have been described in the literature (MILLER 1961; GOOD et al. 1962; SHERMAN and DAMESHEK 1963; FACHET et al. 1965).

The thymus contained high number of mast cells (FREEMAN et al. 1956; ARNESEN 1958; MICHELS 1963; CSABA et al. 1963); PAS positiv substance (ARNESEN 1958; CSABA et al. 1960; BURNET 1965) and heparine (CHARLES and SCOTT 1933).

It has been shown that the immunological reactions are associated with an increase in the basophil, mast cell count (WINQIST 1963; KELLER 1966) and the tissue and blood mucopolysaccharide (MPS) level (SONNET 1955; BASTEINIE 1958; GERŐ et al. 1959; FEHÉR et al. 1966). It is well known that glycocorticoids induce a significant decrease in the MPS content of tissues and blood, and the immunological reactions (DAVIDSON 1964; LORENZEN and ZACHARIAE 1966; FEHÉR et al. 1966).

SCHLESINGER and MARK (1963) observed fatal cachexia in mice following one single injection of cortisol. FACHET et al. (1966) have shown that a single dose of cortisol administered in the neonatal period caused lasting thymo-lymphatic system involution and reduced the number of circulating lymphocytes in the rat. The cachexia and thymo-lymphatic system involution thus induced closely resembled the "wasting-syndrome". SCHAPIRO (1965) observed similar changes following repeated neonatal injections of cortisol.

The finding by DOUGHERTY and WHITE (1943) that in the adult animal regeneration begins as early as 24 to 48 hours after the injection of cortisol,

induced us in the present series of experiments to use newborn rats and administer one single dose of the hormone. In the present experiments an attempt was made to evaluate the effect of a single neonatal dose of cortisol on the serum and tissue mucopolysaccharides, four weeks after the cortisol administration.

Material and methods

To newborn Wistar rats of 8 ± 2 g weight, one 1 mg/10 g body weight of cortisol*, in 0.1 ml volume was injected i.p. on the first day of life. The controls were treated similarly with 0.9% NaCl solution. Under identical conditions the hormone-treated animals lagged in development, in comparison to the controls. All animals were killed by decapitation 4 weeks after delivery. The serum hexosamine level was determined according to ELSON and MORGAN (1933) as modified by SZABOLCS and TANKÓ (1958), by using a standard solution of glucosamine-hydrochloric acid. The serum glycoprotein level was determined according to WINZLER (1955), using a 1 : 1 mixture of galactose-mannose. The samples were fixed in 4% neutral formalin, embedded in paraffin, cut into 6 μ slices, and stained with haematoxylin—eosin and PAS—alcian blue. The bone marrow was stained according to May-Grünwald-Giemsa and the basophil count number was determined for 5000 nucleate cells. Mathematical analysis was performed with Student's t-test.

Results

Animals treated with cortisol presented at 4 weeks, in comparison with the controls, a lag of development, a sparser fur, thinner skin, and less subcutaneous fatty tissue (Fig. 1).

In addition, there was a significant drop in the number of circulating lymphocytes ($p < 0.001$) and of basophilic (mast cells) cells in the bone marrow

Fig. 1. Effect of a single neonatal dose of cortisol upon growth, four weeks after administration, A = Cortisol 1.0 mg/10 g body weight intraperitoneally. B = 0.9% NaCl

* Cortisol = Hydrocortisone acetate (Kőbánya Pharmaceutical Factory of Budapest).

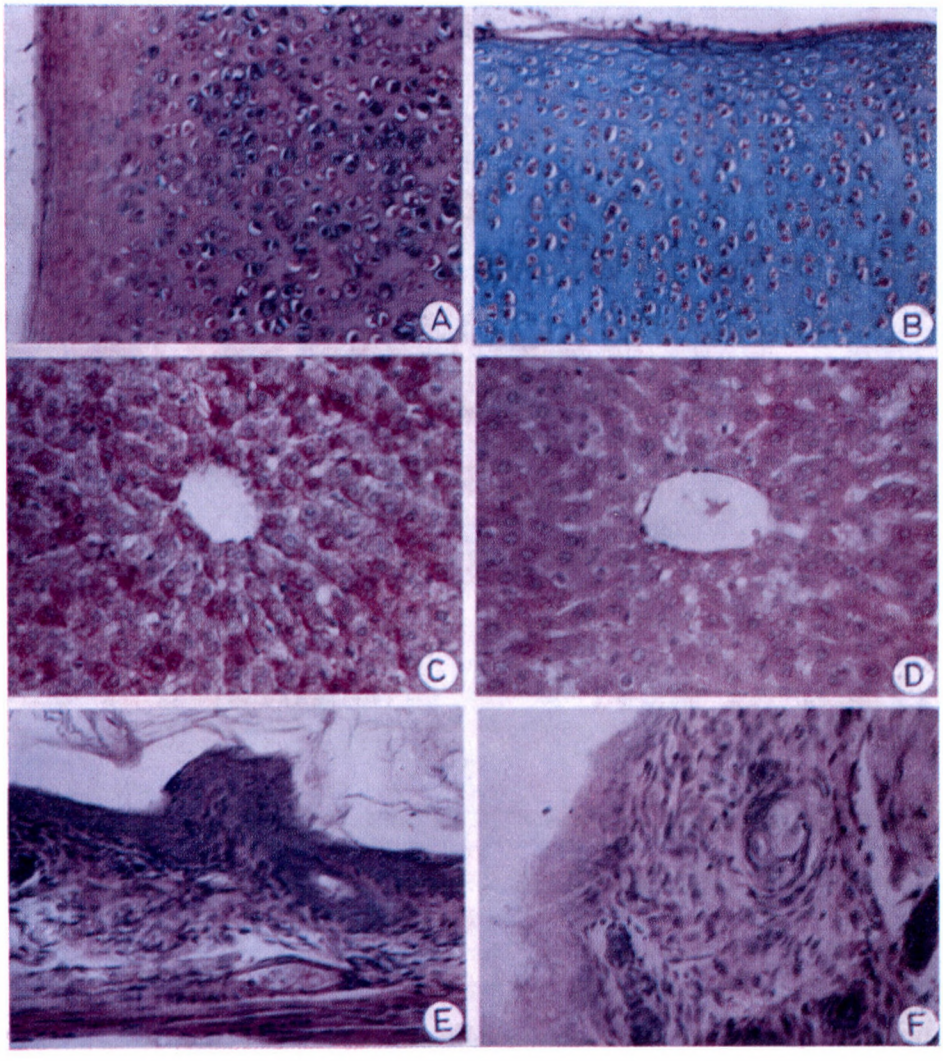


Fig. 2. Effect of a single neonatal dose of cortisol upon the acid and neutral mucopolysaccharide level of cartilage, liver and skin. A = cartilage, NaCl 0.9%. B = cartilage, Cortisol. C = liver, NaCl 0.9%. D = liver, Cortisol. E = skin, NaCl 0.9%. F = skin, Cortisol

($p < 0.01$) (Table I), with a significant decrease of both the serum hexosamine (A) and glycoprotein (B) levels ($p < 0.001$) (Fig. 3).

PAS positivity was restricted to the cartilage membrane (A); the cartilage stained more intensivity with alcian-blue (B); the structure of the liver appeared looser in the treated animals and showed less PAS positivity (D); the alcian-blue and PAS positivity of the skin was somewhat reduced (F) (Fig. 2).

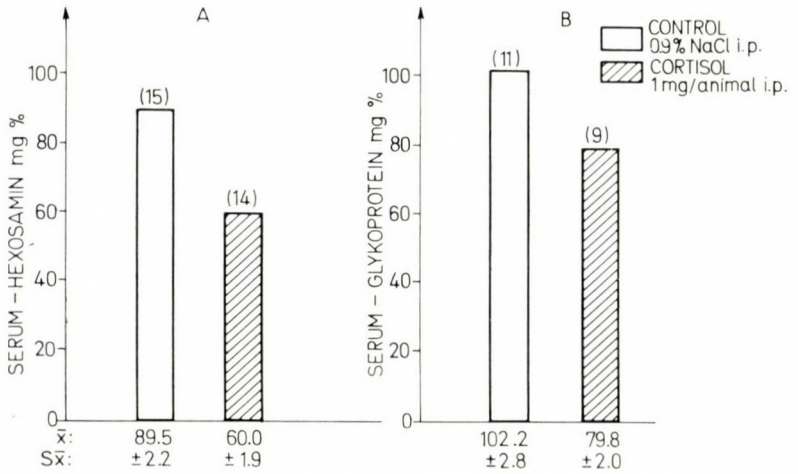


Fig. 3. Effect of a single neonatal dose of cortisol upon the serum hexosamine and glycoprotei levels. A = hexosamine mg per 100 g. B = glycoprotein mg per 100 g

Discussion

The metabolism and regulation of mucopolysaccharides is still a debated question. Studies by KROMPECHER (1960) have pointed to the significant part of hypoxia in the regulation of MPS metabolism. The latter according to some authors (KROMPECHER et al. 1961; JÓZSA et al. 1964; VALLENT et al. 1966) depends on thyroid function, while according to others (MONKHOUSE 1956; DAVIDSON 1964; LORENZEN and ZACHARIAE 1966; VALLENT et al. 1966) on adrenocortical function.

Connective tissue is known to abound in MPS (MEYER and RAPPORT 1950; YEANLOZ and FORCHIELLI 1950; MARBET and WINTERSTEIN 1952; MUIR 1958; JACKSON 1961; BALÓ and BANGA 1961; NÉMETH-CSÓKA 1965) and to represent the site of most antigen—antibody reactions (BASTENIE et al. 1958). It has been shown, that the basophil, fibroblast and mast cells abounding in MPS and especially those involved in MPS production, play a part in anaphylactic and inflammatory reactions (BRAUNSTEINER 1959; RILEY 1963; PADAWER 1963; MICHELS 1963; WINQUIST 1963; BURNET 1965; SELYE 1965; KELLER 1966). BURNET (1965) assumed that the thymocytes can turn into mast cells

Table I

Effect of a single neonatal dose of cortisol upon the bone marrow basophil and the qualitative blood count

Indicated are the average values ($\bar{X}$), their scatter ($S_{\bar{x}}$) and rate of significance (p)

Controls 0.9% NaCl	Juvenile	Rod	Segmented	Eosinophil	Basophil	Lymphocyte	Monocyte	WBC	Bone marrow basophil
	Per cents								
1.	—	2	22	—	—	72	4	8700.0	3
2.	—	1	19	1	—	76	3	9500.0	2
3.	—	—	24	—	—	71	5	8900.0	4
4.	—	1	15	—	—	80	4	9200.0	3
5.	—	—	19	1	—	78	2	8100.0	2
6.	—	1	14	1	—	81	3	9500.0	3
7.	—	2	17	—	—	76	5	8900.0	3
8.	—	—	15	—	—	82	3	7400.0	2
9.	—	1	17	2	—	78	2	9300.0	4
10.	—	1	15	—	—	80	4	8200.0	3
$\bar{X}$:	0	0.9	17.7	0.5	0	77.4	3.5	8770.0	2.9
$S_{\bar{x}}$:	—	—	—	—	—	±1.2	—	±220.0	±0.2

Cortisol 1.0 mg/animal	Juvenile	Rod	Segmented	Eosinophil	Basophil	Lymphocyte	Monocyte	WBC	Bone marrow basophil
	Per cents								
1.	—	2	28	—	—	67	3	8,200.0	—
2.	—	3	32	—	—	61	4	7,700.0	—
3.	1	3	34	—	—	59	3	8,300.0	—
4.	1	4	27	—	—	65	3	8,900.0	—
5.	—	5	29	—	—	62	4	11,100.0	—
6.	—	2	33	—	—	63	2	6,400.0	—
7.	2	5	28	—	—	62	3	7,500.0	—
8.	1	4	31	—	—	60	5	10,500.0	—
9.	—	4	26	—	—	68	2	9,700.0	—
10.	—	3	31	—	—	62	4	9,200.0	—
11.	—	2	18	—	—	77	3	9,800.0	—
12.	—	4	27	—	—	66	3	11,000.0	—
$\bar{X}$:	0.4	3.4	28.7	0	0	64.3	3.2	9 030.0	0
$S_{\bar{x}}$:	—	—	—	—	—	±1.4	—	±420.0	—
						$p < 0.001$		$p > 0.60$	$p < 0.01$

and ACASU and WEST (1960) demonstrated the capacity of mast cells to store, and also to produce antibodies. These data suggest the role of MPS substances in immunological reactions.

The present observations have confirmed the results of SCHLESINGER and MARK (1963) and of FACHET et al. (1966) who found an inhibition of, and a reduction in, the number of circulating lymphocytes, after cortisol treatment in the newborn period. In addition, a pronounced decrease in the serum hexosamine and glycoprotein levels in the neutral MPS content of the tissues and the number of basophils in the bone marrow, has been registered. Simultaneously an increased alcian-blue positivity, pointing to the presence of acid MPS in cartilage, beside a decrease of PAS positivity was present. The opposite change in the content of acid and neutral MPS has been observed in our experiments (VALLENT et al. 1966), in accordance with observations described in the literature (BOAS 1956; KROMPECHER et al. 1961; JÓZSA et al. 1964).

Particular attention should be paid to the fact that the reduction of hexosamine and glycoprotein levels was observed 4 weeks after one single dose of cortisol, i.e. at a time when elevated blood corticoid values could not any longer prevail. Based upon our data it is suggested that a single dose of cortisol, administered neonatally produces a long lasting inhibition in the production of blast cells (fibroblasts, basophils, mast cells), which might play a significant role in MPS production. This suggestion has gained further support, when it was shown that a similar treatment of adult animals failed to elicit any effect (VALLENT 1967).

*

Acknowledgement. We are indebted to J. Fachet of the Medical Research Institute of the Hungarian Academy of Sciences, Budapest, for the interpretation of thymo-lymphatic system results.

REFERENCES

1. ACASU, A., WEST, G. B.: (1960) Anaphylaxis in the Dog. Int. Arch. Allergy, **16,** 326. — 2. ARNESEN—KRISTEN: (1958) The Secretory Apparatus in the Thymus of Mice. Acta path. microbiol. Scand. **43,** 339. — 3. BALÓ, J., BANGA, I.: (1961) Az elastase és kollagenmucoproteinase kutatás területén elért eredmények. Orvosképzés, **36,** 161. — 4. BASTENIE, P. A., CONRAD, V., OMS, H., VERBIEST, M.: (1958) Variations des mucopolysaccharides et des mucoproteides du sérum au cours des affections dégénératives vasculaires. Influence du foie et des corticosurrénales. Acta med. scand. **160,** 205. — 5. BOAS, N. P.: (1956) Low Molecular Weight Hexosamine Containing Compounds in Urine. Proc. Soc. exp. Biol. (N. Y.) **92,** 122. — 6. BRAUNSTEINER, A.: (1959) Physiologie und Physiopathologie der weissen Blutzellen. Thieme, Stuttgart. — 7. BURNET, F. M.: (1965) Mast Cells in the Thymus of NZB Mice. J. Path. Bact. **89,** 271. — 8. CHARLES, A. F., SCOTT, D. A.: (1933) Studies on Heparin II. Heparin in Various Tissues. J. biol. Chem. **102,** 431. — 9. CSABA, G., TÖRŐ, I., BODOKY, M.: (1963) Über die Bildung von Mastzellen in Thymus und den lymphatischen Organen. Z. mikr. anat. Forsch. **70,** 242. — 10. CSABA, G., TÖRŐ, I., ÁCS, T., KISS, F. J.: (1960) The Behaviour of the Thymus in Conditions Associated with Tissue Proliferation. Acta morph. Acad. Sci. hung. **9,** 187. — 11. DAVIDSON, E. A.: (1964) Hormonal Control of Connective Tissue Metabolism. Proc. Soc. Intern. Congress of Endocrinology, London. — 12. DOUGHERTY, T. F., WHITE, A.: (1943) Influence of Adrenal Secretion on Blood Elements. Science, **98,** 367. — 13. ELSON, L. A., MORGAN, W. T. J.: (1933)

Colorimetric Method for Determination of Glucosamine and Chondrosamine. Canad. J. Biochem. **27,** 1824. — 14. FACHET, J., PALKOVITS, M., VALLENT, K.: (1965) Effect of Neonatal Thymectomy on Endocrine and Lymphatic Organs, Reticular Elements and Blood Count II. Findings in Rats with Wasting-Syndrome. Acta med. Acad. Sci. hung. **21,** 305. — 15. FACHET, J., PALKOVITS, M., VALLENT, K., STARK, E.: (1966) Effect of a Single Glycocorticoid Injection on the First Day of Life in Rats. Acta endocr. **51,** 71. — 16. FEHÉR, J., JAKAB, L., GIMPL, F.: (1966) Untersuchungen der Serum-Mukopolysaccharide bei experimentellen immunologischen Prozessen. Z. inn. Med. **21,** 143. — 17. FREEMAN, MARX L., MARX, E.: (1956) Tissue Heparin and Mast Cells in Rats and Rabbits. Fed. Proc. **15,** 255. — 18. GERŐ, S., GERGELY, J., JAKAB, L., SZÉKELY, J., VIRÁGH, S., FARKAS, K., CZUPPON, A.: (1959) Inhibition of Cholesterol Atherosclerosis by Immunization with Beta-Lipoprotein. Lancet, **2,** 6. — 19. GOOD, R. A., DALMASSO, A. P., MARTINEZ, C., ARCHER, O. K., PIERCE, J. O., PAPIERMASTER, B. W.: (1962) The Role of Thymus in Development of Immunological Capacity in Rabbits and Mice. J. exp. Med. **116,** 773. — 20. HAMMERSTROM, R. N., ADAMS, F. H., BUSSMAN, J., LILLEHEI, W. E.: (1952) Normal Mucoprotein Values in the Dog: Response to Distemper Vaccine and to Endocarditis. Proc. Soc. exp. Biol. (N. Y.) **29,** 527. — 21. JACKSON, S. F.: (1961) Some Biochemical Characteristics of Connective Tissue. In: Mills, L. C., and Mayer, J. H.: Inflammation and Diseases of Connective Tissue. Saunders, Philadelphia. — 22. JANKOVICH, B. D., WAKSMANN, B. H., ARNASON, B. G.: (1962) Role of the Thymus in Immune Reactions in Rats. I. J. exp. Med. **116,** 159. — 23. JÓZSA, L., SZEDERKÉNYI, G., PERNECZKY, M., LUSZTIG, G.: (1964) Einfluss des Thyreotropins auf den Mucopolysacharidgehalt der Aorta. Hoppe-Seyler's Z. Physiol. Chem. **337,** 172. — 24. KELLER, R.: (1966) Mast Cells in the Immune Reactions. Karger, Basel. — 25. KROMPECHER, ST., HADHÁZY, CS., BERENCSI, G., OLÁH, E. H., LÁSZLÓ, M. B.: (1961) Experimental Data Concerning the Connexion between Thyroid Function and Mucopolysaccharide (Hexosamine)-Level. Nature, **189,** 670. — 26. KROMPECHER, ST.: (1960) Hypoxibiose und Mukopolysaccharidbildung in der Differenzierung und Pathologie der Gewebe sowie über den Zusammenhang zwischen Schilddrüsenfunktion und Mukopolysacchariden. Barth, Leipzig. — 27. LORENZEN, J., ZACHARIAE, H.: (1966) Effect of Glucorcorticoids upon the Mucopolysaccharide Content of Human Skin. Acta med. scand. **179,** 337. — 28. MARBET, R., WINTERSTEIN, A.: (1952) Zur Kenntnis der Mukopolysaccharide, insbesonders des Heparins. Experientia **8,** 41. — 29. MEYER, K., RAPPORT, M.: (1950) Hydrolysis of Chondroitin Sulfate by Testicular Hyaluronidase. Arch. Biochem. **27,** 297. — 30. MICHELS, N. A.: (1963) The Mast Cells. Ann. N. Y. Acad. Sci. **103,** 235. — 31. MILLER, J. F. A.: (1961) Immunological Function of the Thymus. Lancet, **2,** 748. — 32. MONKHOUSE, G. C.: (1956) The Extractable Heparin in Different Animal Tissue. Canad. J. Biochem. **34,** 757. — 33. MUIR, H.: (1958) The Nature of the Link between Protein and Carbohydrate of a Chondroitin Sulphate Complex from Hyaline Cartilage. Biochem. J. **69,** 195. — 34. NÉMETH-CSÓKA, M.: (1965) Adatok a kötőszöveti állomány szerkezetéhez. Morph. és Ig. Orv. Szemle, **5,** 19. — 35. PADAWER, J.: (1963) Quantitative Studies with Mast Cells. Ann. N. Y. Acad. Sci. **103,** 87. — 36. PARROTT, D. M. W., EAST, J.: (1962) Role of the Thymus in Neonatal Life. Nature, **195,** 347. — 37. RILEY, J. F.: (1963) Functional Significance of Histamine and Heparin in Tissue Mast Cells. Ann. N. Y. Acad. Sci. **103,** 51. — 38. SCHAPIRO, S.: (1965) Neonatal Cortisol Administration: Effect on Growth, the Adrenal Gland and Pituitary-Adrenal Response to Stress. Proc. Soc. exp. Biol. (N. Y.) **120,** 771. — 39. SCHLESINGER, M., MARK, R.: (1963) Wasting Disease Induced in Young Mice by Administration of Cortisol Acetate. Science **143,** 965. — 40. SELYE, H.: (1965) The Mast Cells. Butterworths, Washington. — 41. SHERMAN, J. S., DAMESHEK: (1963) Wasting Disease Following Thymectomy in the Hamster. Nature, **197,** 469. — 42. SONNET, J.: (1955) Les glycoproteines sériques à l'état normal et pathologique. Vol. I. Broux, Paris. — 43. SZABOLCS, M., TANKÓ, B.: (1958) Serum hexosamin sclerodermában. Kísérl. Orvostud. **10,** 212. — 44. VALLENT K., FACHET, J., STARK, E: (1966) Quantitative Changes in the Serum Heparin Level after Thymectomy, Hypophysectomy, Adrenalectomy, or Corticoid Treatment in the Rat. Acta med. Acad. Sci. hung. **22,** 212. — 45. VALLENT, K.: (1967) Effect of a Single Dose of Cortisol on the Tissue Mucopolysaccharides in the Adult Rat. (Personal Communication) — 46. WINQIST, G.: (1963) Electronmicroscopy of the Basophilic Granulocytes Ann. N. Y. Sci. **103,** 352. — 47. WINZLER, R. J.: (1955) Methods of Biochemical Analysis. Interscience Publ. New York. — 48. YEANLOZ, P., FORCHIELLI, E.: (1950) Studies on Hyaluronic Acid and Related Substances. J. biol. Chem. **186,** 495.

WIRKUNG EINMALIGER, NEONATAL VERABFOLGTER HYDROCORTISONINJEKTION AUF DEN SERUM- UND GEWEBEMUKOPOLYSACCHARIDGEHALT BEI RATTEN

K. VALLENT, M. PALKOVITS und B. MÁNDI

Anläßlich der Untersuchung der auf den Serum- und Gewebemukopolysaccharidgehalt entfaltenen Wirkung einer einmaligen, neonatal verabfolgten Hydrocortisoninjektion ließen sich nach vier Wochen folgende Ergebnisse registrieren: Abnahme des Se-Hexosamin-, -Glykoprotein-, sowie neutralen Gewebemukopolysaccharidgehalts, signifikante Verminderung der Knochenmark-Basophilenzahl (Mastzellen) und der zirkulierenden Lymphozyten. Der neutrale und saure Gewebemukopolysaccharidgehalt zeigten einander entgegengesetzte Veränderungen.

ДЕЙСТВИЕ РАЗОВОГО ВВЕДЕНИЯ ИНЪЕКЦИИ ГИДРОКОРТИЗОНА НОВОРОЖДЕННЫМ КРЫСАМ НА СОДЕРЖАНИЕ МУКОПОЛИСАХАРИДОВ В СЫВОРОТКЕ И ТКАНЯХ

К. ВАЛЛЕНТ, М. ПАЛКОВИЧ и Б. МАНДИ

Авторы исследовали в опытах на крысах действие однократного введения новорожденным крысам инъекции гидрокортизона на содержания мукополисахаридов в сыворотке и тканях, через 4 недели после введения гидрокортизона.

Разовое введение инъекции гидрокортизона новорожденным крысам вызвало уменьшение содержания гексозамина и гликопротеина в сыворотке и нейтральных мукополисахаридов в тканях. Достоверно понизилось число базофилов (тучных клеток) в костном мозге и число циркулирующих лимфоцитов. В тканях содержание нейтральных и кислых мукополисахаридов показало изменения противоположного направления.

Dr. Károly VALLENT; I. Sebészeti Klinika, Budapest, Hungary
Dr. Miklós PALKOVITS; Anatómiai Intézet, Budapest IX., Tűzoltó u. 58, Hungary
Dr. Barnabás MÁNDI; Anatómiai Intézet, Debrecen, Hungary

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 173—181, 1968*

THE CHROMOSOMES OF THE RAT'S SOMATIC CELLS*

First Institute of Pathological Anatomy and Experimental Cancer Research
(Director: Prof. J. Baló), University Medical School, Budapest

M. Sellyei, Mária Héjjas and E. László

(Received July 20, 1967)

The chromosomes have been studied in the bone marrow of a non-inbred Wistar albino rat strain. The diploid set was found to consist of 42 chromosomes. Discernible individually in the karyotype were the chromosome pairs 1, 2, 3, 13, 14, 19, 20 as well as the Y chromosome. Undistinguishable were the individual pairs within the groups X, 4—10, 11—12 and 15—18. As to shape, the chromosomes could be classified into telocentric: pairs 2, 3, 4—10 and 19 as well as X and Y; subtelocentric: pairs 1 and 14; submediocentric: pair 13; mediocentric: pairs 11, 12, 15—18 and 20. Pairs 3 and 19 could be identified individually by their permanent satellites. The Y chromosome was a well discernible marker of male cells. Polymorphism within the same sex was not observed in the karyotypes of the tested 20 animals.

Whenever genetic, radiobiologic, oncologic and tissue culture experiments are performed with animals, there is need for a thorough knowledge of the chromosomes in order to make a proper assessment of the changes they undergo in pathological conditions or in response to external influences.

Earlier investigators have uniformly established that the somatic cells of the rat *(Rattus norvegicus)*, tested in tissues and tissue cultures both of embryos and of adult animals, contain 42 chromosomes [5, 7, 9, 15, 17, 19] but, owing to a poor technique, the shapes of individual chromosomes and the position of the centromere have not been clearly discernible. Then the method of treating cells with a hypotonic solution [10, 21] has furnished the possibility to isolate them in the metaphase stage so that they could precisely be counted and individually examined. Later authors have confirmed the chromosome number 42 as characteristic for the somatic cells of the rat; karyotypes (meaning a classification of chromosome pairs according to length and shape) have also been set up [3, 6, 10, 14, 20, 22, 23] but the data differed in more than one respect. Particular importance attaches to the statement Nowell et al. [12], as well as Hungerford and Nowell [6] made about chromosomal polymorphism, when they found that the sex chromosome X of the rat occurred in two different forms, *viz.* 1. with the centromere situated at the ends of the chromatids; 2. with the centromere in subterminal location and consequently with a pronounced short arm of the X chromosome. Examining the inbred BN rat strain, they found subterminal X chromosomes in all animals, while in the

* Based on a paper read at the 7th Congress of the Hungarian Biologic Society, Pécs, 1966.

inbred Lewis strain and the non-inbred Wistar (albino) strain the two types of X chromosome occurred in various combinations, though among the cells of one and the same animal there has never been any difference. A similar kind of polymorphism was observed by YOSIDA and AMANO [23] in the 3rd autosomal pair.

Prior to our own investigations of the chromosomal changes presenting themselves in pathological conditions, we deemed it advisable to determine the karyotypes of the rats to be studied.

Material and method

The chromosomes in the bone marrow cells of 20 adult Wistar rats from our own breed were examined. Intraperitoneally was given colchicine in solution in an amount of 1.5 mg per kg of body weight 4 to 12 hours before killing. After decapitating the conical animals, femures were removed, the epiphyses cut off and the marrow washed into a centrifuge tube, with physiological salt solution. After centrifuging the cell suspension at 500 r.p.m. for 3 minutes the supernatant was discarded and 4 ml of a 0.8% sodium citrate solution was layered on the cells, which were then cautiously resuspended and kept for 20 minutes at 37° C. After repeated centrifugation some of the supernatant was again removed, leaving roughly as much hypotonic solution in the tube as was the volume of the sedimented cells; then under mild shaking 2—3 ml of an 1 : 3 mixture of acetic acid and absolute alcohol was added. Thirty minutes later the mixture was centrifuged, the supernatant discarded, acetic alcohol was added to the cells and the mixture was left overnight at +4° C. Next day the suspension was again centrifuged and the solution was then substituted first by a 45% and subsequently by a 60% solution of acetic acid; after 5 minutes, one third volume of methyl alcohol was added. The cell suspension was then dropped on a slide and the fluid set alight. This method, recommended by SAKSELA and MOORHEAD [18] for the demonstration of secondary constrictions, is preferable to slow drying [11, 16] and has proved best to prevent the chromosomes of a cell moving too far apart on the slide, and to establish their actual number. Then the preparation was hydrolyzed in 1 N hydrochloric acid at 60° C for 6 minutes and stained with ammoniacal Giemsa solution [4].

In the specimens from each animal the number of chromosomes were counted in 100 dividing cells and pictures were taken on ORWO Dokumenten Film (DK 3), under oil immersion and in phase contrast. From the negatives, linear 3.5 to 4.5 enlargements were made. The enlarged pictures of well spread metaphases were cut up, the chromosomes selected and paired in order to set up the karyotypes.

The chromosomes of the same serial number in various dividing cells were found to vary in length within wide limits. This seems to be in relationship with the despiralization of the chromatin substance, which is more pronounced in the prophase and subsequently (the chromosomes being thinner and longer) than in the later stage of the metaphase. Therefore the length of each chromosome was expressed in percentage of the total length of all chromosomes in the given cell; furthermore, to characterize the chromosome shape (the position of the centromere), the size of the long arm was expressed in percentage of the full length of the given chromosome (see Table I and Fig. 3). Measurements were taken of the cells of 6 female and 12 male animals.

Results

Between 70 and 90% of the dividing bone marrow cells were found to contain 42 chromosomes. This is the diploid value (2n). Diverging counts were mostly below 42. Between 0.5 and 1% of the cells were polyploid, and most of these euploid, containing an exact multiple (usually 4n or 8n) of the basic haploid number of chromosomes (n=21) (Fig. 2.) Hyperdiploid cells with more than 42 chromosomes were infrequent.

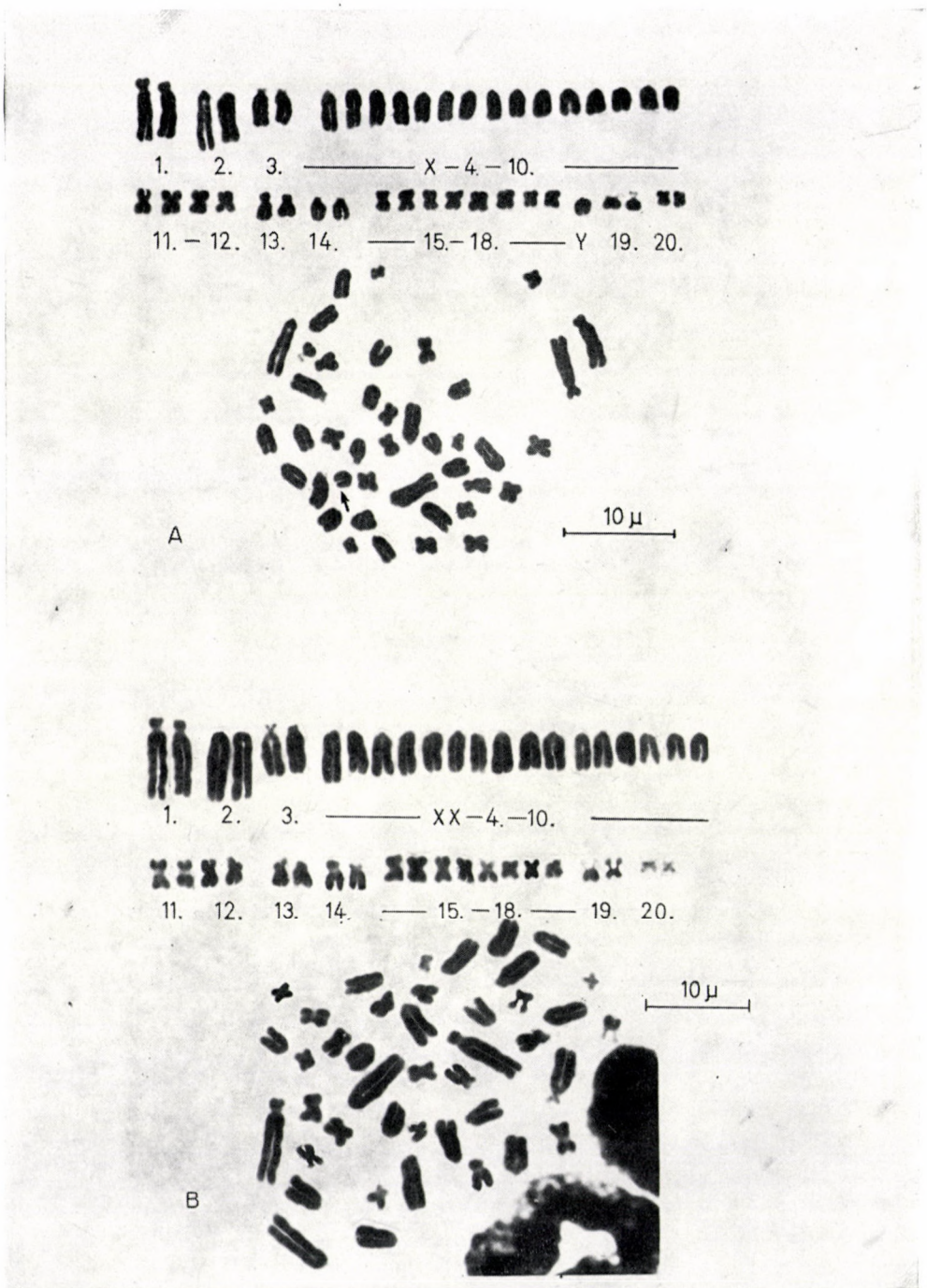


Fig. 1. A) Dividing male cell and its karyotype. The arrow (↗) indicates the Y chromosome. B) Dividing female cell and its karyotype

The 42 chromosomes of the rat-karyotype may be divided into the main groups of long and short chromosomes (Fig. 1). The long ones are telocentric and are marked by the absence of a short arm, since the centromere is situated at the end of the long arm, except for the longest pair (the first one) which is subtelocentric and therefore easy to identify. Well distinguishable is the telocentric 2nd pair with its size being almost as long as that of the first. Telocentric but much shorter are the chromosomes of the 3rd pair, individually identifiable by

Fig. 2. Octoploid rat bone marrow cell with well distinguishable satellites of the chromosomes 19 (↗). × 600

the DNA-deficient, faintly staining, negatively heteropycnotic satellites located near the centromere (Fig. 1). The telocentric chromosomes are uniformly shorter from pair 4 to 10 and individual pairs are hard to differentiate within this group where the number of chromosomes is 15 in the male and 16 in the female cells. To this group belongs the sex chromosome X. The subtelocentric X chromosome found by HUNGERFORD and NOWELL [6] in every animal of the inbred BN strain and in some more specimens showed the longest size in groups 4 to 10. In our studies, though we failed to find subtelocentric X chromosomes in any animal, we felt justified on the basis of the above to regard the first chromosome (in male cells) and the first and second chromosomes (in female cells) of the group X—4—10, as X chromosomes.

Most of the short chromosomes were mediocentric (see Table I and Figs 1, 3); so were those of pairs 11 and 12 which could not be differentiated reliably from each other as they showed a nearly equal total length and a nearly equal ratio

between the long and the short arms. Similar in size were the pairs 13 and 14; but 13 consisted of submediocentric chromosomes with a smaller ratio, while 14 rather of subtelocentric chromosomes with a larger ratio of their long arms (Table I). Smaller than in 11—12 were the mediocentric chromosomes in pairs 15—18, with a uniform decrease in size. Individual pairs within these groups were undiscernible. The pair 19 appeared as small telocentric chromosomes with attached satellites (Figs 1, 2). Pair 20 again was found to consist of mediocentric chromosomes which, on account of their small size, could be differentiated from groups 15—18. An unpaired telocentric chromosome, equal in size to those of group 15—18, occurred in male animals, and corresponded to the characteristic male chromosome Y.

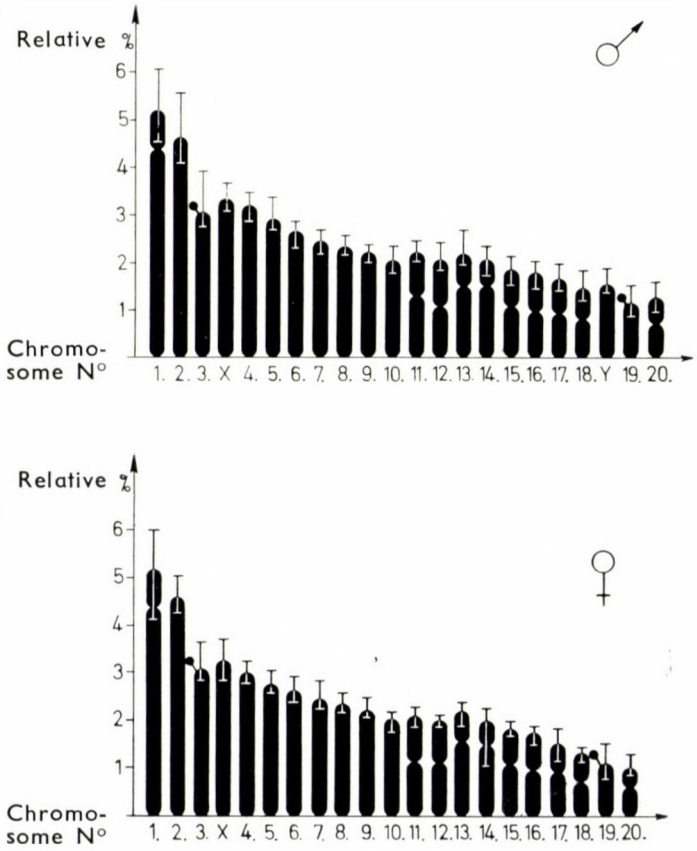


Fig. 3. Diagram of the rat karyotype, based on the average size of six female (♀) and 12 male (♂) cells. Bold lines indicate average chromosome length, as expressed in percentage of the whole length of the diploid set. Thin lines indicate ranges. Indications of the centromeres (constrictions on the bold lines) correspond to the average values. The appendages at the upper chromosome ends 3 and 19 stand for the satellites

Table I

Relative length of rat chromosomes and ratio of chromosome arms, in 6 female and 12 male cells

Serial number of chromosome	Average length of individual chromosome in percentage of the total length of the diploid karyotype*+		Average ratio of long arm to the total chromosome length in percentage*	
	female	male	female	male
1.	5.13 (4.09—5.91)	5.20 (4.56—6.06)	84.83 (82—91)	83.62 (80—88)
2.	4.56 (4.27—4.98)	4.64 (4.05—5.55)		
3.+	3.06 (2.51—3.60)	3.05 (2.69—3.89)		
X	3.25 (2.89—3.66)	3.31 (3.04—3.64)		
4.	2.97 (2.76—3.19)	3.15 (2.84—3.43)		
5.	2.75 (2.61—2.98)	2.88 (2.68—3.32)		
6.	2.59 (2.34—2.87)	2.63 (2.27—2.82)		
7.	2.43 (2.25—2.81)	2.44 (2.14—2.66)		
8.	2.30 (2.18—2.54)	2.30 (2.13—2.50)		
9.	2.18 (2.06—2.44)	2.15 (1.94—2.32)		
10.	1.99 (1.71—2.13)	2.00 (1.71—2.29)		
11.	2.07 (1.89—2.23)	2.16 (1.96—2.40)	56.00 (50—63)	56.29 (50—62)
12.	2.01 (1.87—2.06)	2.00 (1.80—2.35)	54.75 (51—63)	55.62 (50—63)
13.	2.13 (1.84—2.33)	2.10 (1.89—2.60)	71.08 (65—76)	68.29 (57—82)
14.	2.00 (1.18—2.20)	2.00 (1.61—2.28)	74.16 (67—86)	74.67 (64—93)
15.	1.83 (1.71—1.94)	1.78 (1.42—2.08)	57.33 (51—68)	57.00 (51—66)
16.	1.70 (1.49—1.84)	1.74 (1.38—1.98)	57.75 (50—66)	54.25 (50—66)
17.	1.49 (1.32—1.78)	1.59 (1.33—1.89)	59.08 (50—65)	54.50 (50—65)
18.	1.29 (1.11—1.40)	1.39 (1.12—1.76)	56.75 (50—66)	54.75 (50—63)
Y	—	1.48 (1.29—1.80)		
19.+	1.10 (0.77—1.51)	1.07 (0.80—1.45)		
20.	1.02 (0.84—1.28)	1.21 (0.87—1.52)	57.46 (52—65)	55.42 (50—63)

* Extreme values given in brackets.
+ Karyotype and chromosome sizes do not include the length of the satellites.

Discussion

The number of chromosomes in some cells was found to differ from the diploid value (42). While the occurrence of tetraploid and octoploid cells may have been related with the division of megakaryocytes, the assumption cannot be excluded that the colchicine by inhibiting the formation of mitotic spindle had arrested the mitosis and the polyploid cells arose from repeated division, endomitosis.

Hypodiploid values result either from abnormal division (non-disjunction, multipolar division) or are artefacts. In the former case, about the same number of cells ought to have presented itself with more as with less than 42 chromosomes. Hyperdiploid cells, however, were quite rarely encountered. Multipolar division in the normal bone marrow is so infrequent that it could not have accounted for the relative multitude of hypodiploid cells. More probable is a loss of chromosomes in the course of preparation, with the conclusion that the hypodiploid values usually are artefacts.

The karyotypes from animals of the same sex displayed no differencesc The absence of subtelocentric X chromosomes in our animals confirmed thi claim [1, 3, 8, 22, 23] that even noninbred rats contained none but telocentre X chromosomes. This seems to indicate the cytogenetical homogeneity of our animals, so far as ascertainable under the light microscope.

The permanent satellites make it possible to identify the individual telocentric chromosomes in pairs 3 and 19. These SAT-chromosomes (*sine acido* thymonucleinico) play a part in the process of nucleolar organization [13]. Most authors [3, 20, 22, 23] had regarded the rat chromosome satellites as short arms, only HUNGERFORD and NOWELL [6] became aware of their real nature. The satellites can only be demonstrated in impeccable preparations and are sometimes well observable even in polyploid cells (Fig. 2). In human cells the SAT-chromosomes do not occur as invariably as we found them in the rat [2]. Only YOSIDA and AMANO [23] reported on individual rats or rat strains lacking a satellite in one or both chromosomes of the 3rd pair.

HUNGERFORD and NOWELL [6] in males of the BN strain discovered a telocentric Y chromosome which could not be differentiated from the shortest members of the groups X—4—10. The telocentric Y chromosome observed in our animals was short and little practice was needed to identify it even without setting up a karyotype (Fig. 1a). It suits itself well as a marker chromosome for the identification of dividing cells of the male rat, allowing their differentiation from female cells [24].

REFERENCES

1. Dowd, G., Dunn, K., Moloney, W. C.: (1964) Chromosome Studies in Norma and Leukemic Rats. Blood, **23,** 564. — 2. Eggen, R. R.: (1963) Cytogenetics. Amer. J. clin. Path. **39,** 3. — 3. Fitzgerald, P. H.: (1961) Cytological Identification of Sex in Somatic Cells of the Rat, Rattus norvegicus. Exp. Cell Res. **25,** 191. — 4. Genest, P., Auger, C.: (1963) Observations on the Technique for the Study of Human Chromosomes by the Culture of Leukocytes from Peripheral Blood. Canad. med. Ass. J. **88,** 302. — 5. Gläss, E.: (1956) Die Identifizierung der Chromosomen im Karyotyp der Rattenleber. Chromosoma (Berl.) **7,** 655. — 6. Hungerford, D. A., Nowell, P. C.: (1963) Sex Chromosome Polymorphism and the Normal Karyotype in Three Strains of Laboratory Rat. J. Morphol. **113,** 275. — 7. Koller, P. C., Darlington, C. D.: (1934) The Genetical and Mechanical Properties of the Sex Chromosomes. J. Genet. **29,** 159. — 8. Krooth, R. S., Shaw, M. W., Campbell, B. K.: (1964) A Persistent Strain of Diploid Fibroblasts. J. nat. Cancer Inst. **32,** 1031. — 9. Makino, S.: (1943) Studies on the Murine Chromosomes. III. A Comparative Study of the Chromosomes in Five Species of Rattus. J. Fac. Sci. Hokkaido Imp. Univ. Sec. VI. **9,** 19. — 10. Makino, S., Hsu, T. C.: (1954) Mammalian Chromosomes in vitro. V. The Somatic Complement of the Norway Rat (Rattus norvegicus). Cytologia (Tokyo) **19,** 23. — 11. Nagy, S.: (1964) Chromosoma vizsgálat kísérleti állatoknál. Kísérl. Orvostud. **16,** 561. — 12. Nowell, P. C., Ferry, S., Hungerford, D. A.: (1963) Chromosomes of Primary Granulocytic Leukemia (chloroleukemia) in the Rat. J. nat. Cancer Inst. **30,** 687. — 13. Ohno, S., Kaplan, W. D., Kinosita, R.: (1959) The Centromeric and Nucleolus-associated Heterochromatin of Rattus norvegicus. Exp. Cell Res. **16,** 348. — 14. Ohno, S., Kinosita, R.: (1955) The Primary and Secondary Constrictions on the Chromosomes of the Rat Lymphoblast. Exp. Cell Res. **8,** 558. — 15. Painter, T. S.: (1928) A Comparison of Chromosomes of the Rat and Mouse with Special Reference to the Question of Chromosome Homology in Mammals. Genetics **13,** 180. — 16. Rothfels, K. H., Siminovitch, L.: (1958) An Air-drying Technique for Flattening Chromosomes in Mammalian Cells Grown in vitro. Stain Technol. **33,** 73. — 17. Sachs, L.: (1953) Simple Methods for Mammalian Chromosomes. Stain Technol. **28,** 169. — 18. Saksela, E., Moorhead, P. S.: (1962) Enhancement of Secondary Constrictions and the Heterochromatic X in Human Cells. Cytogenetics **1,** 225.— 19. Tanaka, T.: (1953) A Study of the Somatic Chromosomes of Rats. Cytologia (Tokyo) **18,** 343. — 20. Tjio, J. H., Levan, A.: (1956) Comparative Idiogram Analysis of the Rat and the Yoshida Rat Sarcoma. Hereditas (Lund) **42,** 218. — 21. Tjio, J. H., Whang, J.: (1962) Chromosome Preparations of Bone Marrow Cells without Prior in vitro Culture or in vivo Colchicine Administration. Stain Technol. **37,** 17. — 22. Vrba, M., Donner, L.: (1965)Summary Finding on the Rat Karyotype and on Chromosomal Characteristics of Rat Tumours Induced by Rous Sarcoma Virus in vitro. Neoplasma **12,** 265. — 23. Yosida, T. H., Amano, K.: (1965) Autosomal Polymorphism in Laboratory Bred and Wild Norway Rats, Rattus norvegicus, Found in Misima. Chromosoma (Berl.) **16,** 658. — 24. Zieverink, W. D., Moloney, W. C.: (1965) Use of the Y Chromosome in the Wistar (Furth) Rat as a Cellular Marker. Proc. Soc. exp. Biol. N. Y. **119,** 370.

CHROMOSOMEN DER SOMATISCHEN ZELLEN BEI RATTEN

M. SELLYEI, MÁRIA HÉJJAS und E. LÁSZLÓ

Die Chromosomen der Knochenmark wurden bei Wistar-Albinoratten (keine Inzucht) nach Colchicinbehandlung untersucht. Der Diploidvorrat bestand aus 42 Chromosomen. Im Karyotyp waren die 1., 2., 3., 13., 14., 19. und 20. Chromosompaare und das Y-Chromosom individuell erkennbar. Innerhalb der X—4—10., 11—12. und 15—18. Gruppen ließen sich die einzelnen Paare nicht differenzieren. Der Form entsprechend können die Chromosomen folgendermaßen klassifiziert werden: telozentrische Chromosomenpaare: 2., 3., 4—10. und 19.; subtelozentrische Paare: 1. und 14.; submediozentrisches Paar: 13.; mediozentrische Paare: 11—12., 15—18. und 20. Die individuelle Erkennung der 3. und 19. Paare ermöglichen die stets anwesenden Satelliten. Das charakteristische Merkmal (»marker«) der männlichen Zellen ist das Y-Chromosom. Der Karyotyp der untersuchten 20 Ratten zeigte keine intrasexuelle Polymorphie.

ХРОМОСОМЫ СОМАТИЧЕСКИХ КЛЕТОК КРЫСЫ

М. ШЕЙЕИ, М. ХЕЙЯШ и Э. ЛАСЛО

Авторы в костном мозге не инбредных белых крыс штамма Вистар после введения кольхицина исследовали хромосомы. Диплоидный набор состоит из 42 хромосом. В кариотипе индивидуально распознаваемы 1-, 2-, 3-, 13-, 14-, 19- и 20-ая пары хромосом и хромосома У. В пределах групп Х—4—10, 11—12 и 15—18 нельзя обособлять отдельных пар хромосом. Судя по форме, хромосомы можно классифицировать по следующему: пары 2, 3, 4—10 и 19, Х и У телоцентрические; пары 1 и 14 субтелоцентрические; пара 13 — субмедиоцентрическая, пары 11, 12, 15—18 и 20 — медиоцентрические. Индивидуальное распознавание пар 3 и 19 возможно на основе постоянного наличия сателлитов. Хромосома У представляет собой легко распознаваемый «маркер» мужских клеток. Кариотипы исследовавшихся 20 крыс в пределах одинакового пола не проявляли полиморфизма.

Dr. Mihály Sellyei
Dr. Mária Héjjas
Dr. Előd László

I. Kórbonctani Intézet, Budapest
VIII., Üllői út 26, Hungary

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 183—203, 1968*

Department of Anatomy, University Medical School, Budapest

CELLULAR AND SYNAPTIC ORGANIZATION OF THE DORSO-MEDIAL THALAMIC NUCLEUS

Teréz TÖMBÖL

(Received July 29, 1967)

The cell types and synaptic architecture in the nucleus dorso-medialis of the thalamus have been studied on the basis of classical Golgi and perfusion Kopsch preparations in the cat. Four cell types are described: (1) the thalamo-cortical relay cells; (2) Golgi II type interneurons; (3) fusiform cells, having a non-specific dendritic arborization pattern; (4) a medium size cell, resembling Type 2 but with one longer axon in addition to many short branches. Four different kinds of afferent fibre arborizations can be distinguished; their exact origin, however, is not sufficiently known. The mutual interrelations between various cell types by means of axon collaterals or short axons are described in detail. The functional implications of neuropil architecture are briefly discussed.

In spite of considerable differences in technical approach and general outlook — according to their emphasis predominantly on architectural, systematic, developmental or also on functional aspects — three main types of neurons are usually mentioned to occur in the specific, particularly in the sensory relay nuclei of the thalamus. There can be little doubt that the thalamo-cortical relay neurons (Type 1) correspond to cells with a relatively large body and so-called "tufted" dendritic arborization pattern (RAMON-MOLINER 1962; TÖMBÖL 1967) and the dendritic tree occupying a fairly regular sphere not more than 150 μ in radius. Small cells (Type 2) that with respect to their short but profusely arborizing axons may be considered Golgi II type neurons, have been described first by CAJAL (1911) and repeatedly mentioned by various authors. From the less uniform group of medium size cells two different cell types appear to emerge: (Type 3) fusiform cells with relatively few straight and long dendrites having a rather indistinct or indetermined arborization pattern and (Type 4) a medium size cell resembling in shape and arborization pattern of the dendrites to the Golgi II neurons but for their axons that, although branching profusely in the close neighbourhood of the body, have at least one main branch that can be traced farther. Type 3 obviously corresponds to the reticular formation type cells described in most specific thalamic nuclei by LEONTOVICH and ZHUKOVA (1963), and might be identical with the type of neuron described by SCHEIBEL and SCHEIBEL (1966a) as an integrator neuron.

Classification of cell types on purely descriptive basis, however, leads not very far if it does not — or at least does not attempt to — carry some

functional meaning or idea. The difficulties of meaningful classification may also be different with the various nuclei. In the lateral geniculate body (LGB), for example, the true relay cells are showing a much larger variability, as seen from the papers of SHKOLNIK-YARROS (1955) and GUILLERY (1959), and only few and probably not the most important among them correspond to the fairly uniform type [1] of the thalamic relay nuclei. In spite of striking similarities of the main cell types in many of the specific thalamic nuclei it would be misleading to make sweeping generalizations both in classification of cells as well as in the functional significance attributed to the various types. There is nothing to exempt us from a careful structural analysis of all nuclear subdivisions of the thalamus, using as criteria for the classification of neurons not only cell size and dendritic arborization patterns but also all the relevant feature of the axon and its ramification. Of equal, or from the functional point of view even higher, importance is a detailed study of the terminal ramification pattern of various axons both afferent to the nucleus under consideration or of local source. The positions of various cell types in the terminal axonal feltwork and their synaptic relations are obviously more significant features than minor or even than major variations of cell size and of the dendritic tree. Such studies have to rely heavily on Golgi procedures, as beautifully shown by the recent studies of SCHEIBEL and SCHEIBEL (1966b, c) on the reticular nucleus as well as the nucleus ventralis anterior. But they are still only preliminaries to further studies using degeneration methods for the distant connexions and electron microscopic investigations for the finer details of synaptic structure.

The present paper is the first of a series devoted to the analysis of the nucleus dorsalis medialis (DM); it will be followed by electron microscopic and degeneration studies on the same nucleus.

Material and methods

This study is mainly based on Golgi material of the cat, however, also Nauta series of brains of animals carrying lesions of various regions or pathways contributing afferent fibres to the DM have been examined for tentative identification of various characteristic terminal axon ramifications.

The Golgi procedure has been applied in young kittens (1—2 days) in the form of the classical rapid (osmic acid) procedure, repeated generally according to the suggestions by VALVERDE (1962). This kind of material and these procedures are essential for reliable information on the course of axons and neuron connectivity on larger scale.

Unfortunately relatively little confidence can be placed into information gained from immature brains concerning dendritic and terminal axonal ramifications as well as their mutual relationships and synaptic arrangements particularly if quantitative relations come into question: i.e. sizes and shapes of territories embraced or covered by dendritic and axonal arborizations of various kinds. These quantitative parameters of neuronal interrelations are of crucial importance whenever functional considerations are entering into the evaluation of the structure, and since they are grossly changed during the process of maturation of the brain, it is important to gain insight into the Golgi architecture of the mature or nearly mature thalamus or other centres of delayed maturation. Also, physiological investigations are made generally on mature animals. With these considerations in mind it was the endeavour of this group to develop modifications of the Golgi procedure giving satisfactory results for the thalamus

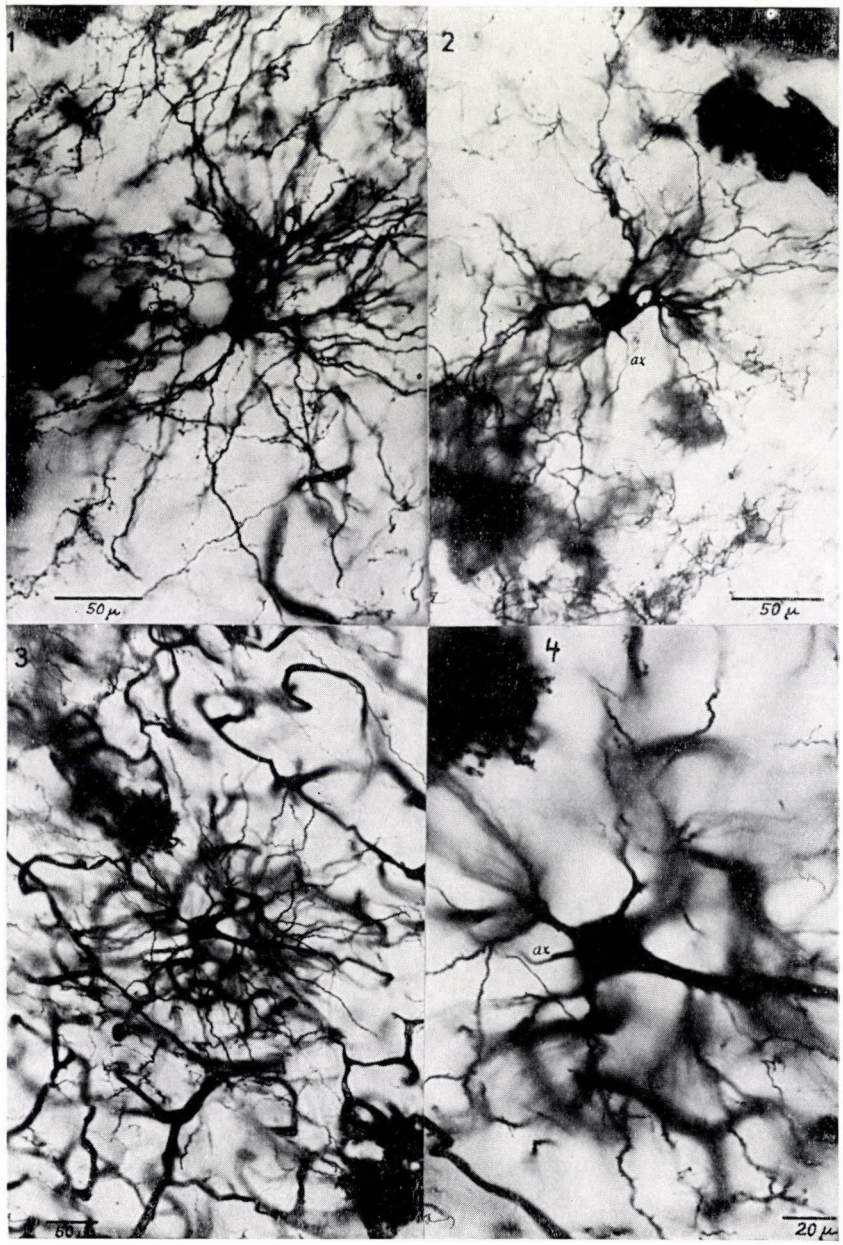
and upper brainstem of adult or near adult animals. A perfusion Kopsch procedure (SZENTÁGOTHAI 1963; TÖMBÖL 1967) using — after rapid perfusion with physiological saline of the deeply anaesthetized animal as perfusion fluid — a freshly prepared mixture of four parts of 5% potassium dichromate solution and one part of 40% formol (or 5% glutaraldehyde) gives fairly reliable results in the adult cat or even better in 2—3-month-old cats that from the point of view of maturation of the thalamus (in the Golgi picture) can safely be considered to have an essentially adult pattern and adult quantitative parameters. The brain cut into 3 mm thick slices parallel to one of the Horsley-Clarke planes is kept further 24 hours in the same fluid, and then is transferred into 3.5% potassium dichromate solution for further 4 days. After treatment in the usual 0.75% silver nitrate solution for 4 days the last two steps of the procedure are routinely repeated once or, if razor blade sections of one or two unimportant regions of one block or another do not show satisfactory staining, even twice. A rapid embedding into celloidin — up to 8% concentration — secures good cutting qualities (50—100 μ) of complete slices of the brain, without shredding or breaking of the tissue and with practically no loss of the staining. Almost uninterrupted series with the loss of one or two sections only at the surfaces of slices of whole brains can be made without difficulty.

Although various cell types of the thalamus (and other upper brain stem nuclei — more rarely of the lower brain stem from the pons downwards — cerebral and cerebellar cortex) and particularly terminal axon ramification are generally well stained, larger axons — already myelinated — do not stain with this procedure. This is a considerable drawback of Golgi work on the adult brain and has to be taken seriously into consideration. In order to get the complete information one has, therefore, to recourse always to newborn material stained with the classical rapid procedure, which supplies the decisive information on the neuronal interrelations on a somewhat larger scale by showing the trajectory of the main axons as well as their preterminal arborizations. The crucial importance of this kind of information cannot be emphasized enough as spectacularly illustrated in the recent studies of SCHEIBEL and SCHEIBEL (1966a, b, c). Our point of view in dealing with various regions of the thalamus and geniculate bodies (SZENTÁGOTHAI 1963; MAJOROSSY et al. 1965; SZENTÁGOTHAI et al. 1966; TÖMBÖL 1967) differs slightly from that of SCHEIBEL and SCHEIBEL by being shifted somewhat towards the structural relations on a more minute scale, justified also by the use of the electron microscope and its combination with degeneration studies. It is obvious that neither of these approaches presents the full picture, which is complete only if the information from both lines of study can be combined and matched.

Results

1. Cell types of the medial dorsal nucleus

From the usual cell types of the thalamus mentioned in the introduction all the four types appear, although with varying incidence. In the order of frequency in which they appear in the perfusion Kopsch preparations these are: Type 1 is obviously the thalamocortical projective neuron; Type 2 a short axon local cell closely resembling Golgi II type neurons; Type 3 a medium size "reticular" or "isodendritic" neuron and Type 4 a medium size cell resembling Type 2 but for having a longer axon, nevertheless with abundant local arborizations. Particularly in the primate thalamus authors often speak of a medial or rostro-medial magnocellular and a lateral parvicellular part. However, as also stated by KUHLENBECK (1954) this differentiation is by no means well defined and very little if anything of such a difference shows up in Golgi material. Cell density appears to be greater in more medial parts of the nucleus gradually to decrease in lateral direction. Such differences are not conspicuous with techniques so highly selective as the Golgi procedures. The differences may also be caused by more fibres entering and starting to arborize in or simply running through the lateral part of the nucleus.

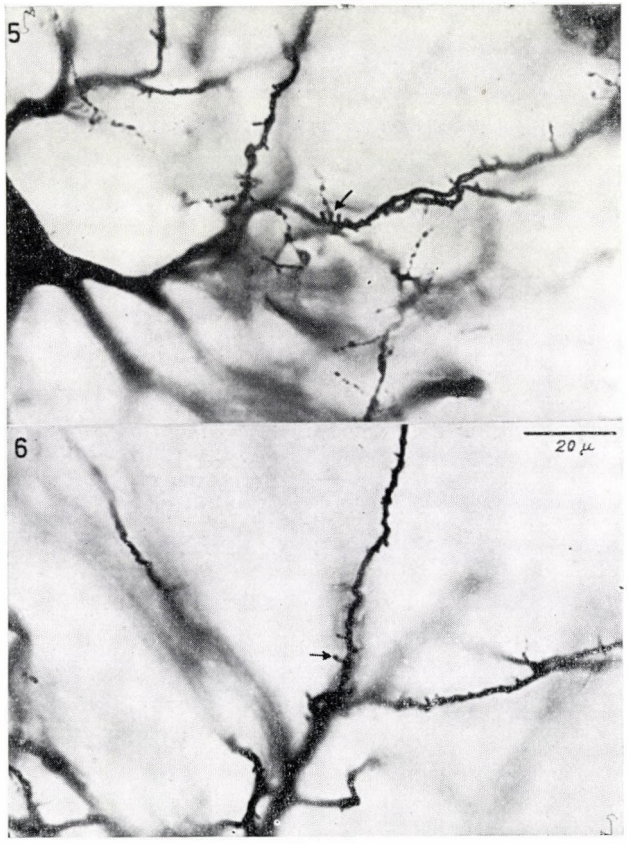


Figs 1—4. Thalamo-cortical relay cells (Type 1) of the DM nucleus with characteristic "tufted" dendritic arborization pattern. The axons of the cells are seen in Figs 2 and 4. Note characteristic spheric dendrite tree of this cell type in Fig. 3, having a radius of not more than 150 μ. From 2—3-month-old cats, perfusion Kopsch procedure

Type 1 : the large thalamo-cortical projective neurons. This cell type, shown in Figs 1—4, has a large multangular body and about 25—30 μ in diameter. 5—10 coarse principal dendrites originate from the cell body in radial direction. At a relatively short usually less than 50 μ distance from the cell body these main dendrites break off suddenly into brushes or tufts of secondary branches, resembling the branchings of the flower-stems of umbrelliferous plants. Larger secondary branches may occasionally show tertiary branchings in similar fashion, although further branching is relatively poor and of simple dichotomous character. The dendritic tree occupies a fairly regular sphere with a radius of about 150 μ as clearly seen in Fig. 3; few of the terminal dendritic branches go beyond the shell of 150 μ radius. This dendritic arborization pattern has been called "tufted" by RAMON-MOLINER (1962) and it is indeed most characteristic for nearly all specific thalamic nuclei. As the sudden "explosion" into the brush- or broom-shaped tufts occurs at distances less than 50 μ from the cell body, each of these Type 1 cells is surrounded by a shell with a radius of approximately 40—80 μ, in which there is a maximum density of secondary dendritic branches immediately distal to the tufts, whereas the density, for obvious geometrical reasons, soon has to decrease in outer zones due to sparse further branching of the dendrites. The importance of this for synaptic connexions has been mentioned previously (TÖMBÖL 1967) and a hypothesis on the modes and geometry of synaptic linkage has recently been based on it by SZENTÁGOTHAI (1967). Although — as we shall show in forthcoming papers on the synaptic ultrastructure of this nucleus — the whole distal part of the dendrites is covered with synaptic contacts, it is the region of the secondary branches in the dense parts of the tufts that has the largest number of postsynaptic differentiations in the form of short spine-like processes. Characteristic examples of these are shown in Figs 5 and 6. The nature of these short blunt side-branches in various thalamic or geniculate nuclei has been discussed repeatedly (SZENTÁGOTHAI 1963; MAJOROSSY et al. 1965; TÖMBÖL 1967; ECCLES et al. 1967). It has been shown that the short blunt outgrowths demonstrated in Fig. 5 are characteristic postsynaptic structures. They have generally a group of mitochondria or at least a single mitochondrion in their ends and have been termed by SZENTÁGOTHAI (1963) for distinction from dendritic spines "dendritic protrusions", or "digits" (in the cerebellum) by FOX et al. (1967). Somewhat more distally these protrusions become larger and more slender, so that they are more closely resembling spines. However, as true spines are rarely found in the EM picture of dendrites believed to belong to Type 1 cells, it is probable that they are still "protrusions" or "digits". It is obvious that in the dense parts of the tufts the postsynaptic surface is considerably increased by these outgrowths.

The axon of the Type 1 neurons is a thick smooth process originating from a distinct cone of the cell body (Figs 2 and 4). After a tortuous initial course it

leaves the thalamus through the anterior peduncle. In perfusion Kopsch preparations of adult brains the axon can rarely be traced very far, obviously due to its myelin sheath, which surrounds it at 50—100 μ distance from its origin. In the classical rapid Golgi method carried out according to Valverde's modi-



Figs 5 and 6. Short blunt side branches of Type 1 cell dendrites (arrows). These branches are not spines but rather so-called protrusions. Thorn-like structures on more distal parts of the dendrites as incompletely stained terminal knobs. Same material as Figs 1—4

fication, in newborn or very young kittens but sometimes also in the perfusion Kopsch preparations (Fig. 7) one can recognize an initial collateral — already described in the sensory relay nuclei (TÖMBÖL 1967) — which terminates in the close neighbourhood of the cell.

Type 2: the small Golgi II interneurons. This cell type is somewhat less frequent than Type 1 in perfusion Kopsch preparations of the adult, but still common in most of our material. Although quantitative estimates should not be made with such highly selective methods as the Golgi procedure, one has the feeling that the number of these small interneurons is somewhat larger than

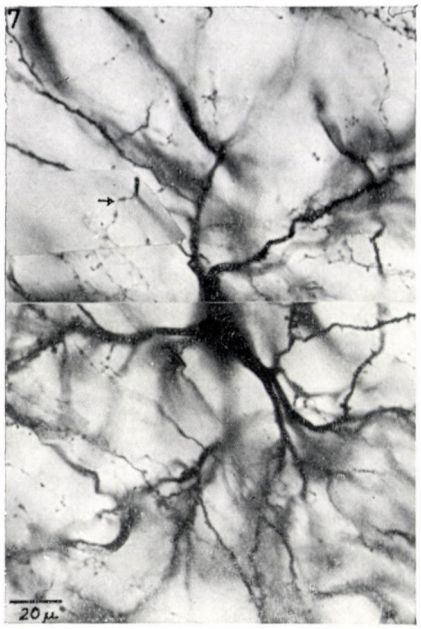


Fig. 7. Type 1 thalamo-cortical relay neuron. Arrow points to delicate initial collateral of the axon. Incompletely stained thorn-shaped boutons terminaux are well seen on lower dendrites. Material as Figs 1—4

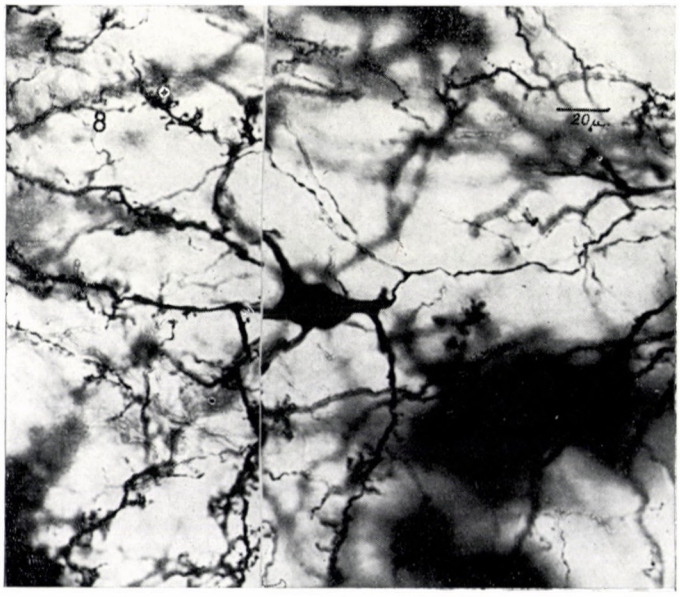


Fig. 8. Golgi II type internuncial cell with axon that begins immediately to arborize (right upper quadrant). Dendrites of this cell are "wavy" and have an irregular branching pattern. Numerous drumstick shaped spines are seen on the dendrites; they become increasingly numerous towards the periphery of the dendrites. Same material as Figs 1—4

in the sensory nuclei VPL — VPM, where according to the estimate of McLardy (1963) on Nissl staining the interneurons would attain ratios of 1 : 3 —1 : 5 as compared to the thalamo-cortical relay (TCR) cells.

The cell bodies of these cells are of a rounded or ovoid shape, less than 20 μ in average diameter. Few (3—5) main dendrites take origin from the cell bodies. Instead of the consequently radial direction of the dendrites in Type 1 here the dendrites have a wavy course from the very beginning (Fig. 9), or sometimes simply irregular (Fig. 8) with secondary branches given at right angles. In the beginning the dendrites are smooth, but soon small spine-like processes appear, having the characteristic drum-stick shape (Figs 8 and 9).

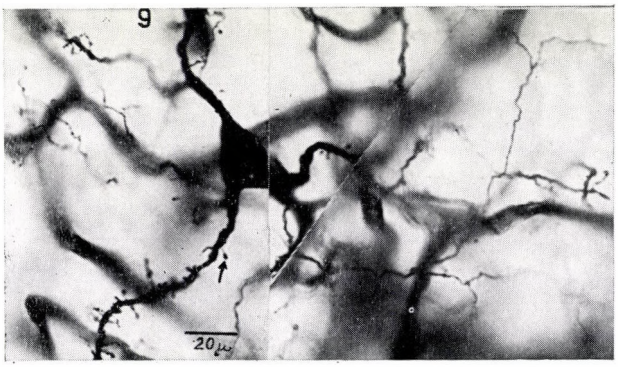
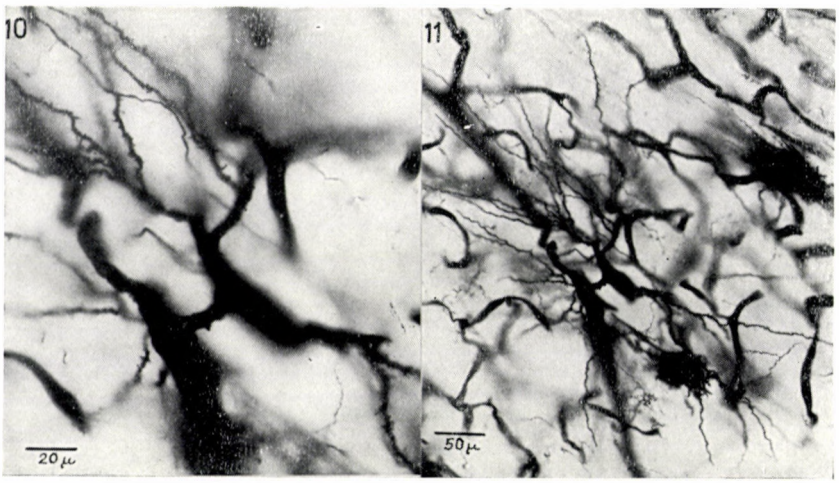


Fig. 9. Same Type 2 cell as in Fig. 8. Arrow points to characteristic spine. Profuse arborization of axon can be seen at right. Two-month-old cat, perfusion Kopsch procedure

They are sparse and shorter in proximal parts of the dendrites and are becoming increasingly numerous, longer and of more elaborate pattern in distal parts of the dendrites (Tömböl 1967). These processes closely resemble true spines, but in the EM picture the number of true axon spine synapses — so characteristic of electron micrographs of the cerebral cortex or the molecular layer of the cerebellar cortex, — is unexpectedly small (Tömböl 1967). One has, of course, to be cautious in making quantitative estimates by simply looking at electron micrographs as one is easily led astray by not being able to predict from the selective Golgi pictures how large a fraction of the whole tissue space appearing in the EM ought to be occupied by the "spiny branchlets" of the Type 2 cells in the thalamus. These branchlets are obvious enough in the Golgi picture, but they may constitute such a tiny fraction of the whole tissue space that the spine synapses disappear among larger elements. Thus we have not been able to decide whether or not to consider these processes true spines. The dendrites are considerably longer than the Type 1 neurons; they reach often 400 μ or more. The dendritic arborization pattern is "wavy" as termed by Ramon-Moliner (1962).

The axon readily stains with the perfusion Kopsch procedure and is thus obviously unmyelinated (Figs 8, 9). It arises generally from the proximal part of one of the dendrites, seldom from the cell body itself. Not far from the cell body it begins to arborize and all of its branches arborize repeatedly. Although the axon branches are not confined to so rigorously limited spaces than the cerebral cortex often is, and the branching does not exhibit any regular pattern, no branch of the axons appears to leave the territory of the nucleus. On the contrary the dense part of the arborization reaches less far than most of the dendrites do. There is, therefore, sufficient justification for considering this cell type a Golgi II type neuron.

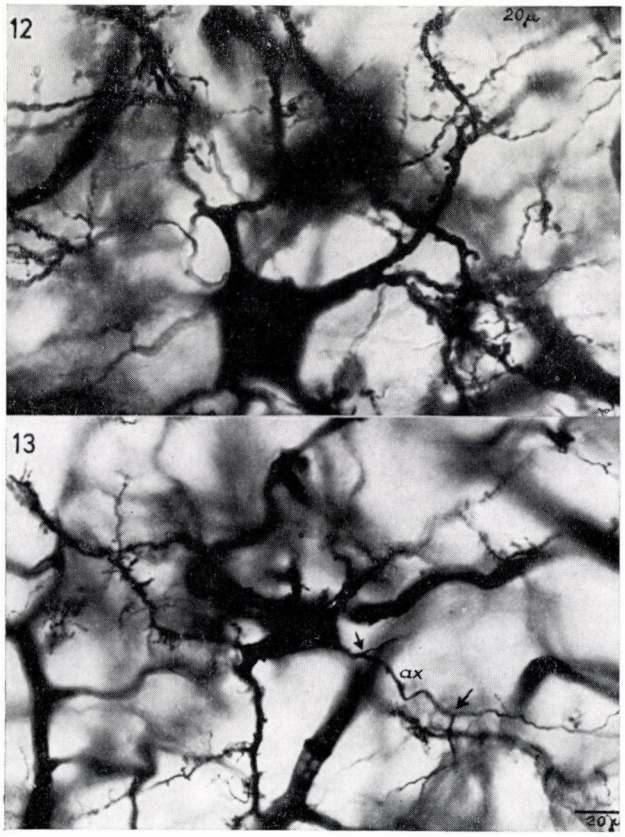


Figs 10 and 11. Fusiform medium size cells (Type 3) with faintly determined dendritic arborization pattern. Incompletely stained boutons terminaux on all dendrites

Type 3: fusiform cells. They are mostly medium in size, although their diameter varies considerably, few dendrites originating predominantly on the opposite ends of their fusiform cell body (Fig. 10). These main dendrites arborize by dichotomy and give rise to straight secondary and to few tertiary branches of irregular radiate course. The dendritic arborization is sparse and the cells resemble the generalized cell type of the reticular formation. As described by LEONTOVICH and ZHUKOVA (1963) the majority of the cells in the so-called non-specific nuclei of the thalamus is of this type, however, they invade also the specific nuclei. They are found sporadically also in the DM. Their coarse axon arises from the cell body or from one of the principal dendrites, gives off numerous collaterals, but has always a larger main branch which leaves the nucleus. It has not been possible to trace any axon from this type of neuron to its final destination. The same kind of cell has been described recently by

SCHEIBEL and SCHEIBEL (1966a) in various nuclei of the thalamus, particularly in the VPL, and has been labelled "integrator cell".

The spine-like structures that cover the surface particularly of the secondary dendrites have no drum-stick heads but taper off into a pointed tip. Such "pseudo-spines" that occur in large numbers on Type 1 neurons have



Figs 12 and 13. Medium size Type 4 cell. Axon seen in Fig. 13 can be traced to longer distance. Profusely branching initial collaterals indicated by arrows. Same material as Figs 1—4

been interpreted by our group as incompletely stained axo-dendritic boutons. This explanation is in accord with the EM picture.

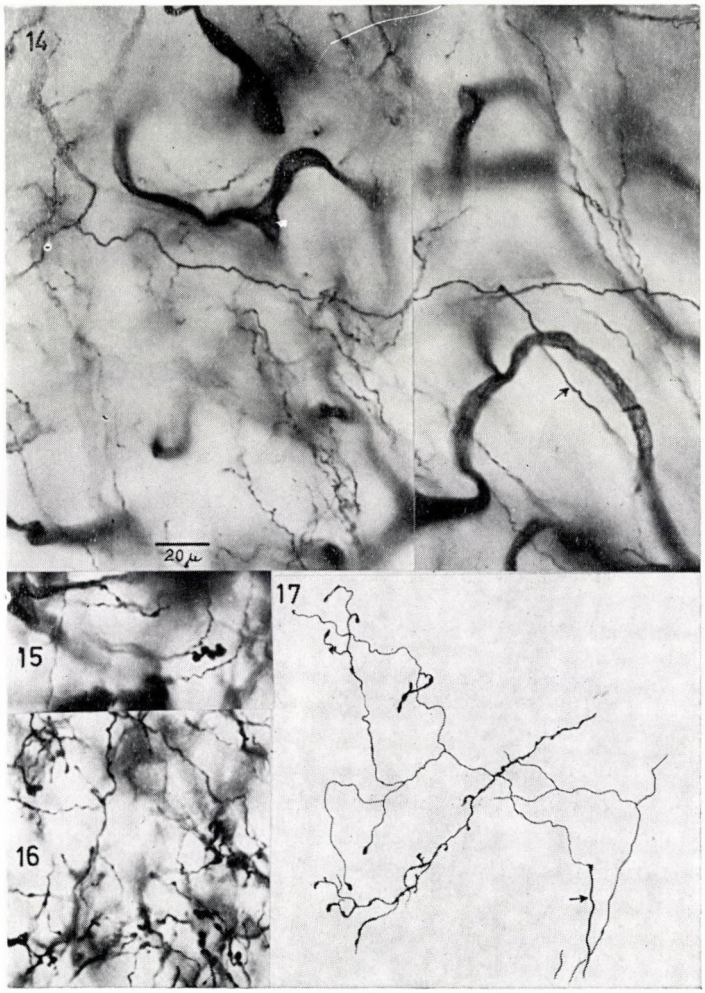
Type 4: Golgi-like cells with long axon. These cells are of medium size (20—25 μ cell body diameter), their dendritic tree resembles closely that of the Golgi II (Type 2) interneurons from which they differ by having more numerous primary dendrites and consequently a multangular cell body. The dendrites have somewhat coarser but the same kind of short spine-like side branches of drumstick shape (Figs 12, 13) which become increasingly numerous in more

distal parts of the dendrites. Their axon arises either from the cell body (Fig. 12) or from one of the principal dendrites and immediately begins to give off numerous collaterals that are always thinner than the main axon. The latter can always be traced beyond the borders of the DM nucleus. In perfusion Kopsch (adult) material it is, of course, difficult to trace axons at distances, but this type of cell could also be identified in Valverde modifications of the rapid Golgi procedure in a few days old kittens (Fig. 13). In these preparations the main branch of the axon could be traced in several instances in caudo-ventral direction, where it was seen to join the periphery of the fasciculus retroflexus. The numerous thinner collaterals of the main axon appear to arborize within the DM nucleus itself. While working on the VPL, we have not been aware of this kind of cell (TÖMBÖL 1967) so that some, mainly of the larger interneurons that have not been identified on the basis of their axons as really local i.e. Golgi II type cells, may in fact belong to this hitherto not sufficiently recognized cell group.

2. Termination of thalamo-petal fibre systems in the MD nucleus

As mentioned in the chapter on Material and methods the classical rapid Golgi procedures are excellent for tracing fibre systems and their preterminal arborizations, while not quite satisfactory for establishing the real mode of termination of the axons and their synaptic contacts. Whether this is due mainly to the fact that the synaptic structures are not sufficiently developed or whether they do not stain well in very young animals is not quite clear. The perfusion Kopsch procedure, conversely, is excellent for staining the terminal axon ramifications and synaptic contacts in the adult or near adult thalamus, but shows little of the axons and their preterminal branchings. This account is based mainly on perfusion Kopsch preparations, but also series of young kittens prepared according to the Valverde modification of the rapid Golgi procedure and Nauta preparations of adult degeneration material have been examined for additional information.

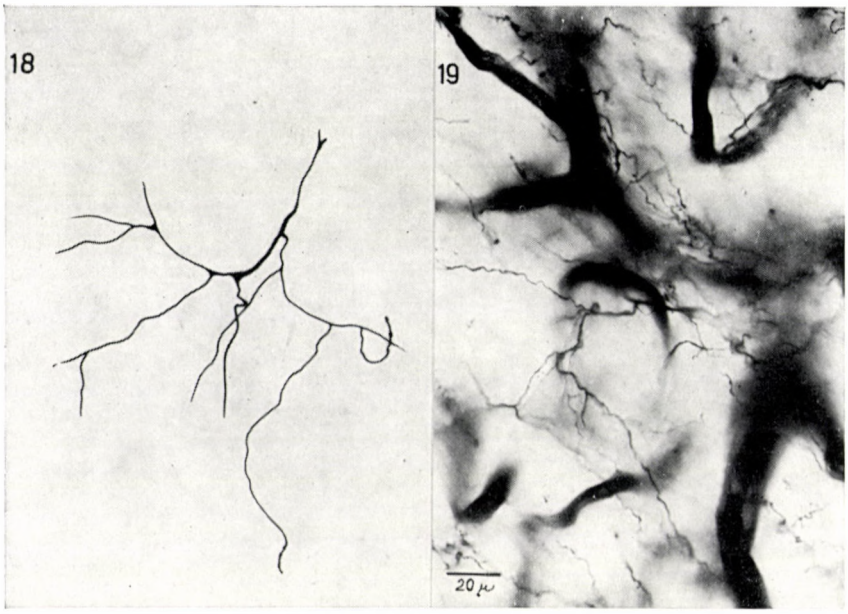
Four different types of afferent fibres can be distinguished both according to direction of entry into the DM nucleus and according to their character and pattern of arborization: *(a)* Relatively coarse ascending fibres entering the nucleus from the ventro-medial direction; *(b)* axons of medium size running in medio-lateral direction; *(c)* corticothalamic fibres; *(d)* thick fibres entering from the ventro-lateral direction. Neither afferent nor efferent fibres of this nucleus are forming solid bundles while entering or leaving the nucleus and there are no major bundles which would simply traverse this as in the case of many other thalamic nuclei. The fasciculus retroflexus marks the border of the nucleus but apart from being joined by few of DM-efferents — axons of Type 4 neurons — and some afferents, it has no specific relation to the nucleus.



Figs 14 to 17. Arborization of (*a* type) axons arriving from the ventral direction. Parental fibre is indicated by arrow in Fig. 14. Drawing in Fig. 17 shows complete arborization of the fibres indicated in Fig. 14

(a) The relatively coarse fibres entering from the ventro-medial direction can be seen to arrive at the level of the nucleus from the rostral, straight ascending and from the caudal directions. The caudal fibres usually join the fasciculus retroflexus. As sites of origin various mediobasal regions of the brain have to be considered such as the posterior hypothalamic area and supramammillary nuclei (Le Gros-Clark, 1932); lateral septal nuclei, anterior part of the medial forebrain bundle and ventral tegmental regions (Guillery 1959); and also, the amygdaloid complex (Nauta and Valenstein 1958). — Reaching different depths of the DM during their ascent, the coarse afferents begin

to give off thin preterminal branches establishing an irregular plexus throughout the nucleus (Figs 14—17). The same afferent may send branches to medial as well as lateral regions of the thalamus. The terminal arborizations of these fibres are shown in Figs 15 and 16. The endings themselves are of two kinds; some of the thin terminal axons form sinusoid or leaf-like terminal expansions



Figs 18 and 19. Medium size (*b* type) fibre entering the nucleus from the lateral side

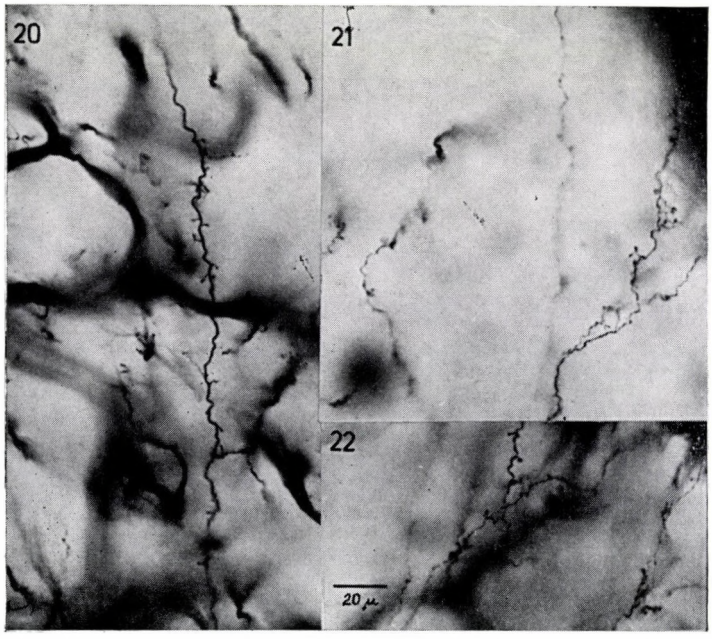
(Fig. 15), while others break up into several fine branches each of which terminates in one or sometimes a row of successive knob-shaped thickenings (Fig. 16).

(b) Axons of medium size, entering the nucleus from the lateral side, have smooth contours and irregular expansions into a wide field of the nucleus. The terminal branches of the fibres are lost from sight after having given rise to brush-like arborizations into straight very fine fibres (Figs 18, 19). No suggestion can be offered concerning the possible source of these fibres except the vague possibility that they arrive from the pallidum (Le Gros-Clark 1932) or the amygdaloid complex (Nauta and Valenstein 1958).

(c) Cortico-thalamic fibres have been identified only tentatively on the basis that entirely similar fibres in the ventrobasal complex have been shown by the degeneration method, to be of cortical origin. The preterminal parts of these fibres are most characteristic, having a straight or undulating course and very typical spine-like side branches which terminate in tiny knobs (Figs 20, 21, 22). As these fibres can be observed over considerable lengths in

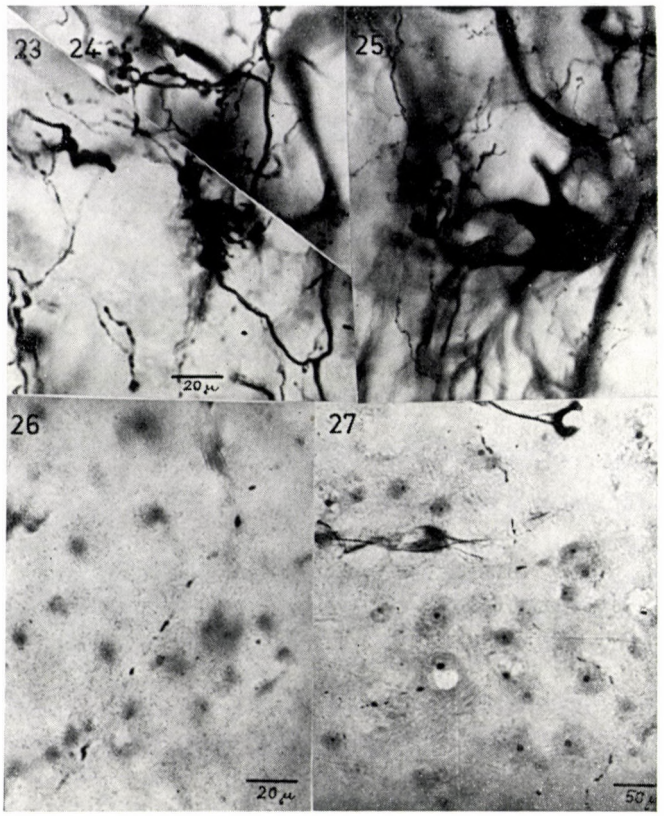
great abundance in frontal sections of the brain, one might assume that they are the branches of larger corticothalamic fibres expanding within frontally oriented disks of the thalamus. This kind of radiate preterminal expansion of corticothalamic fibres has been shown by SCHEIBEL and SCHEIBEL (1966a, b) and the preterminal axons with their spine-like terminals demonstrated in Figs 20, 21, 23 might well be parts of this diskoid branching of descending cortical fibres.

(d) Coarse fibres entering the DM nucleus from the ventro-lateral side have an arborization pattern that resembles that of specific afferents in the ventrobasal complex (Figs 23, 24, 25). The large fibres have smooth contours



Figs 20 to 22. Straight preterminal axons giving off short terminal side branches (*c* type). There was some evidence in the VPL that such fibres are of cortical origin

up to their terminal expansions. The terminal expansions are large multiple thickenings, sometimes grape-like or otherwise resembling the so-called rosettes of the mossy fibres in the cerebellum (Figs 23, 24). Their likeness to the lemniscal afferents of the VPL as well as the direction from which they appear to come has raised the possibility that they might originate from the medial lemniscus. Indeed there can be found rows of degeneration fragments in the DM after lesions in the dorsal column nuclei of the medulla oblongata (Figs 26, 27). The fragments are not too abundant and also not very large as one would expect from the large size of the preterminal fibres. Thus the question of the origin of these fibres cannot be settled unequivocally.



Figs 23 to 25. Coarse fibres (*d* type) entering the DM nucleus from the ventro-lateral side. Their terminal arborizations resemble that of the specific afferents in the VPL—VPM. 2—3-month-old cat, perfusion Kopsch procedure

Figs 26 and 27. Nauta fragments in DM of adult cat 5 days after lesion in dorsal column nuclei

3. *Local interneuronal connections*

(a) Connections established by the axons of Golgi II type neurons. As already mentioned, the axons of Golgi type interneurons (Type 2) arborize in the neighbourhood of the cell body. Arborization begins sometimes not farther than 4—5 μ from the origin of the axon. Often the first branches of the axon are very delicate collaterals and the main branching of the axon begins a few microns distally. The axonal branching is profuse, although not so dense than is usual in the cerebral cortex. Most of the arborizations are well inside the territory reached by the dendrites of the same cell. This often gives the impression as if the synapses would be established with the dendrites of the cell of origin, as has been displayed in many illustration of the cerebral cortex and designers of nervous network models have built in such connections in their logical networks. It is, however, almost impossible to tell on the basis of the

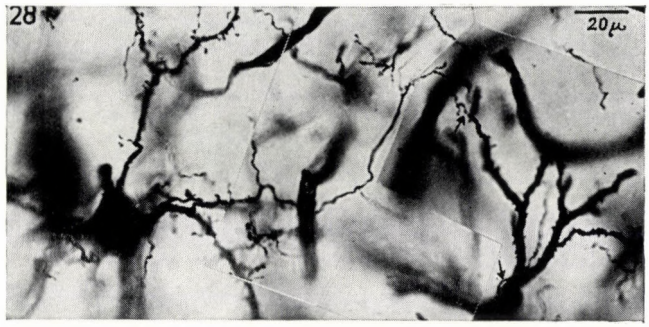
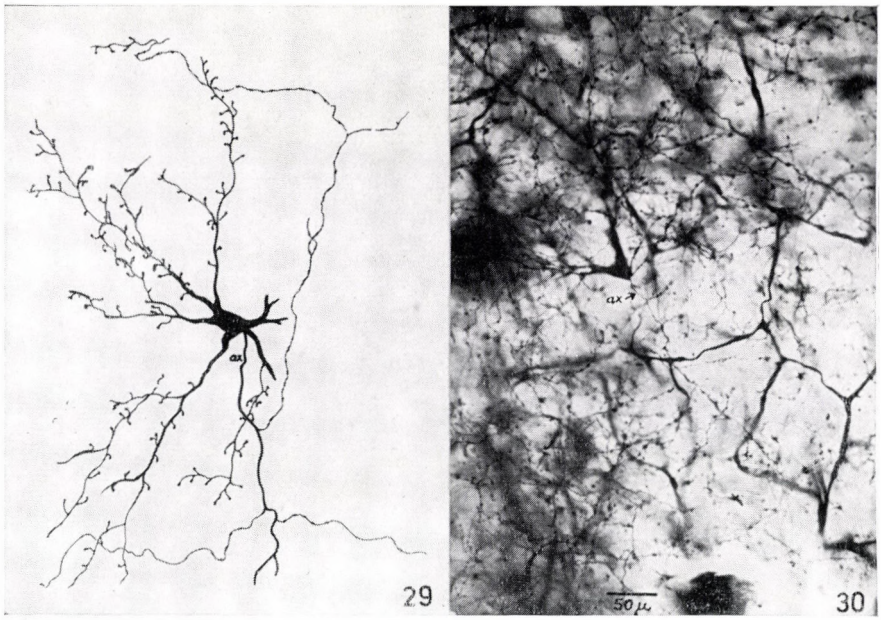


Fig. 28. Relations between Golgi II type interneuron (left) and thalamo-cortical relay cell (right). Axon ramifications of the Golgi cell are establishing contacts with dendrite of relay neuron (upper arrow). They are also in close relation to the cell body (lower arrow)



Figs 29 and 30. Drawing and microphotograph of Type 4 neuron. The drawing shows ramification pattern of initial axon branches. Main branch of axon leaves the DM. Valverde modification of the rapid Golgi procedure, 3-day-old kitten

Golgi picture whether any contact observed is in reality a synapse. In the cerebellar cortex, where the situation is uniquely favourable for the identification of synapses belonging to various neurons both on the light and the electron microscopic level, there is no indication of synapses established with own dendrites. One should, therefore, treat this observation with scepticism until conclusive evidence has been offered. Synaptic, or at least very close, contacts can be observed between Golgi axon branches and dendrites (Fig. 28) of Type 1

projective neurons. Whether occasional intimate relations to the cell bodies (Fig. 28) are true synapses is difficult to decide, particularly on account of the scarcity of axo-somatic synapses in the EM picture of large cells. The abundant grape-like terminal expansions of Golgi II type axons observed recently in the lateral geniculate body (SZENTÁGOTHAI et al. 1966) have not been found in the MD. This might indicate that synapses in this nucleus are generally not of glomerular character. As has been shown in the LGB (SZENTÁGOTHAI et al. 1966) the Golgi axon endings are mainly participating in the complex glomeru-

Fig. 31. Axon terminal ramification and distal part of dendrite engaged in forming a synaptic glomerulus. Such synaptic glomeruli are rather scarce in the DM

lar synapses. Final conclusions on synaptic relations should, however, be reserved for the forthcoming EM analysis of this nucleus.

(b) Connections established by the collaterals of Type 4 cell axons. Collaterals are given in abundance by the main axon branch in the close neighbourhood of the cell of origin (Figs 29, 30). The further branching of these initial collaterals does not significantly differ from that of the Type 2 axons. Their contacts are also similar mainly to the dendrites of Type 1 or Type 3 cells. From the beaded character of the terminal collateral branches and occasional close contacts with dendrites one gets the impression that synapses are established either with dendrites by means of small solitary terminal knobs or with dendritic spines in "crossing-over" contacts.

(c) Connections of initial axon collaterals of thalamocortical projection (Type 1) cells. Observations of such initial collaterals in adult or near adult material are scarce, so that it is difficult to get information concerning their further course and their terminal arborizations. They seem to arborize soon and to give rise to delicate terminal branches entering the fine neuropil of the nucleus (Fig. 7).

Discussion

The present observations are considered a preparatory step to further studies using both degeneration and EM methods, first separately and then in combination. However, to be able to combine degeneration and electron microscopy effectively, one has to have a clear knowledge (1) of the architecture of the nucleus under study, and (2) of its main fibre connections. These studies satisfy as yet only condition (1), while in consideration of the scarcity of data particularly on afferent fibre connections, systematic studies are needed for fulfilling condition (2).

Recent studies on the architecture of various thalamic nuclei (LEONTOVICH and ZHUKOVA 1963; SCHEIBEL and SCHEIBEL 1966a, b, c; TÖMBÖL 1967) have convincingly shown how little confidence can be placed into pure cytoarchitectonics, apart from some basic (and quite important) quantitative information on numbers and densities of various kinds of cells. Unfortunately, for a reliable differentiation of cells in the Nissl picture — if at all possible — a previous thorough Golgi analysis of the region on adult material is an essential condition. It is thus to be expected that future Nissl studies conducted in knowledge of the more recent Golgi information will be rewarding. Although remarkable attempts have been made by the classical cytoarchitectonic schools to elaborate the Nissl technique further by making plastic reconstructions of cells and their main dendrites, this cumbersome procedure can hardly substitute Golgi studies. GIHR (1964) has distinguished 11 cell varieties with the procedure. However, it becomes obvious from Golgi studies that rough form, size and initial dendrite patterns are of limited value for the subdivision of neurons in various groups if compared with the decisive criteria: the whole dendritic arborization, spines or spine-like structures and character, and ramifications of the axon. Due to the high selectivity of the Golgi procedure it is of course always possible that one or more important kinds of cell have not yet been observed, or that cells classified in a single group will later be subdivided into two or more subgroups on the basis of hitherto unknown criteria. But granted this possibility of later corrections, we may still be confident that some of the major neuron types and some of their relations have been understood correctly.

As it appears from the present study, in gross Golgi architecture the DM nucleus does not differ significantly from that of the specific sensory relay nuclei VPL, VPM or from that of the Va, VL. The same cell types can be observed although probably their relative numbers are different. Also, the neuropil formed by several kinds of terminal axon ramifications, apart from being more delicate, with more fine preterminal fibres and small terminals, is not principally different from that of the sensory relay nuclei. Thalamic mossy fibres which are so characteristic of the anterior (CAJAL 1911) and particularly

of the posterior lateral group of nuclei PL and pulvinar (MAJOROSSY et al. 1965) are practically lacking. The large sinusoid terminal expansions or rosettes of the mossy fibres are found mainly in nuclei possessing complex glomerular synapses. As few such glomerular structures can be seen in the Golgi picture, like the one shown in Fig. 31, it may be assumed that glomerular synapses will not be found in large abundance in this nucleus. This is, however, not certain and final conclusions on minute synaptic structures should be drawn only from EM studies.

From the similarity of the Golgi architecture one can nevertheless extrapolate as much that the vast majority of synapses might be rather small axodendritic boutons. Axosomatic synaptic contacts are rare in the VPL (TÖMBÖL 1967), particularly on the larger cells. On small cells which may be identified as Golgi II type cells in the VL, boutons contacting the cell body surface are more frequent, although one has to look for them thoroughly in order to find a few. The initial parts of the principal dendrites are also practically devoid of synaptic contacts. From the region of the first branching of the dendrites down to the finest branches the whole surface of the dendrite is covered by synaptic contacts. This is probably the case also in the DM, as judged from the short blunt dendritic protrusions (Figs 5, 6), which are known to be specific postsynaptic sites of thalamic neurons. They are most abundant in the regions of the dendritic tufts. On the smaller secondary and tertiary dendrites both of Type 1 and Type 3 cells (Figs 6, 7, 11) there are many thorn-shaped "spines", which according to all EM evidence in the VPL, VPM, VL are incompletely stained boutons (TÖMBÖL 1967; ECCLES et al. 1967), so that it is certain that there are terminal knobs in the DM. As mentioned in the description of Type 2 and Type 4 cells, the long drumstick-shaped side branches may be real spines. True axon-spine synapses can be observed — although not in abundance — in most specific thalamic nuclei. It is probably due to the relatively long and often curved shafts of the spines that it is difficult to trace them back to dendrites. As the number of Type 2 and Type 4 neurons is quite considerable in this nucleus, a thorough EM analysis might furnish important information on the minute synaptic relations of these cells.

So far as one can judge from the Golgi architecture, processing of information might occur according to similar elementary mechanisms as in the VPL, VPM, VA and VL. The delicacy of the neuropil, the large number and diffuse arrangement of small terminal knobs of mostly axodendritic localization, indicate that considerable convergence from the various scarcely known afferent pathways may be required for bringing the TCR neurons to action. The abundant connections of Golgi II type neurons with TCR neurons suggest that inhibitory shaping of the activity patterns of TCR neurons might be an important feature of transmission. The existence of initial collaterals of TCR neurons and their probable connections with Golgi II

type interneurons indicate the presence of a recurrent inhibitory pathway introducing a phasing of discharges by recurrent inhibition, as has been shown by ANDERSEN et al. (1964a, b) and ECCLES et al. (1964) in the sensory relay nuclei. This has probably a similar anatomical basis (TÖMBÖL 1967). — Although afferents with large terminations confined to circumscript regions of the neuropil appear to be rare, the dendritic tree of the TCR neurons with its tufted arborization pattern and the density of dendrites of the same cell in a shell surrounding the nucleus of 40—80 μ radius leads to the inference that the TCR neurons might be stimulated effectively by the axon ramifications having terminals in this shell. In spite of the delicacy of the neuropil this might ensure specific patterns of transmission of small groups of TCR neurons having overlapping "shells" in the above sense. These possibilites have recently been discussed from the geometric point of view for the sensory relay nuclei by SZENTÁGOTHAI (1967). His considerations can be applied with minor adjustments to the DM. Such speculations on the possible functional grouping of neurons in this nucleus should, however, be reserved for the future when more information on the EM architecture and on fibre connections will be availabe.

REFERENCES

1. ANDERSEN, P., BROOKS, C., ECCLES, J. C., SEARS, T. A.: (1964e) The Ventrobasal Nucleus of the Thalamus: Potential Fields, Synaptic Transmission and Postsynaptic Components. J. Physiol. (Lond.) **174,** 348—369. — 2. ANDERSEN, P., ECCLES, J. C., SEARS, T. A.: (1964b)The Ventro-basal Complex of the Thalamus: Types of Cells, Their Responses and T heir Functional Organization. J. Physiol. (Lond.) **174,** 370—399. — 3. CAJAL, S. R.: (1911) Histologie du système nerveux de l'homme et des vertébrés. Vol. 2, Maloine, Paris. — 4. ECCLES, J. C., ITO, M., SZENTÁGOTHAI, J.: (1967) The Cerebellum as a Neuronal Machine. Springer, Berlin–Heidelberg–New York. — 5. ECCLES, J. C., LLINAS, R., SASAKI, K.: (1964) Excitation of Cerebellar Purkinje Cells by the Climbing Fibres. Nature (Lond.) **203,** 245—246. — 6. FOX, C. A., HILLMAN, D. E., SIEGESMUND, K. A., DUTTA, C. R.: (1967) The Primate Cerebellar Cortex: A Golgi and Electron Microscope Study. In: Progress in Brain Research, vol. 25, Eds. C. A. Fox and R. S. Snider, Elsevier, Amsterdam—London—New York. — 7. GIHR, M.: (1964) Die Zellformen des Nucleus medialis dorsalis thalami des Menschen. In: Progress in Brain Research, Vol. 5, eds. W. Bargmann and J. P. Schadé, Elsevier, Amsterdam, 74—86. — 8. GUILLERY, R. W.: (1959) Afferent Fibres of the Dorsomedial Thalamic Nucleus in the Cat. J. Anat. (Lond.) **93,** 403—419. — 9. KUHLENBECK, H.: (1954) The Human Diencephalon. Conf. neurol. (Basel). Suppl. **14,** 41—147. — 10. LE GROS-CLARK, W. E.: (1932) The Structure and Connections of the Thalamus. Brain **55,** 406—470. — 11. LEONTOVICH, T. A., ZHUKOVA, G. P.: (1963) The Specificity of the Neuronal Structure and Topography of the Reticular Formation in the Brain and Spinal Cord of Carnivora. J. comp. Neurol. **121,** 347—380. — 12. MAJOROSSY, K., RÉTHELYI, M., SZENTÁGOTHAI, J.: (1965) The Large Glomerular Synapse of the Pulvinar. J. Hirnforschung. **7,** 415—432. — 13. MCLARDY, T.: (1963) Thalamic Microneurons. Nature (Lond.) **199,** 820—821. — 14. NAUTA, W. J. H., VALENSTEIN, E.: (1958) Some Projections of the Amygdaloid Complex in the Monkey. Anat. Rec. **130,** 346. — 15. RAMON-MOLINER, E.: (1962) An Attempt at Classifying Nerve Cells on the Basis of their Dendritic Patterns. J. comp. Neurol. **119,** 211—227. — 16. SCHEIBEL, M. E., SCHEIBEL, A. B.: (1966a) Patterns of Organization in Specific and Nonspecific Thalamic Fields. In: The Thalamus, Eds. D. P. Purpura and M. D. Yahr, Columbia University Press, New York.—17. SCHEIBEL, M. A., SCHEIBEL, A. B.: (1966b) The Organization of the Nucleus Reticularis Thalami: A Golgi Study. Brain Res. **1,** 43—62.— 18. SCHEIBEL, M. E., SCHEIBEL, A. B.: (1966c) The Organization of the Ventral Anterior Nucleus of the Thalamus. A Golgi Study. Brain Res. **1,** 250—268. — 19. SHKOLNIK-YARROS, E. G.: (1955) К вопросу о строении мозгового конца зрительного анализатора церкопитеков. Пробл. физиол. оптики, Медгиз Москва II. 162.

20. SZENTÁGOTHAI, J.: (1963) The Structure of the Synapse in the Lateral Geniculate Body. Acta anat. (Basel) **55,** 166—185. — 21. SZENTÁGOTHAI, J.: (1967) Models of Specific Neuron Arrays in Thalamic Relay Nuclei. Acta morph. Acad. Sci. hung. **15,**113—124—22. SZENTÁGOTHAI, J., HÁMORI, J., TÖMBÖL, T.: (1966) Degeneration and Electron Microscope Analysis of the Synaptic Glomeruli in the Lateral Geniculate Body. Exp. Brain Res. **2,** 283—302. — 23. TÖMBÖL, T.: (1967) Short Neurons and Their Synaptic Relations in the Specific Thalamic Nuclei. Brain Res. **3,** 307—326. — 24. VALVERDE, F.: (1962) Intrinsic Organization of the Amygdaloid Complex. Trab. Inst. Cajal Invest. biol. **54,** 291—314.

ZELLULÄRE UND SYNAPTISCHE ORGANISATION DES DORSOMEDIALEN THALAMISCHEN KERNS

T. TÖMBÖL

Die Zelltypen und die synaptische Architektur des dorsomedialen thalamischen Kerns der Katze wurden in den klassischen Golgi- und Kopschschen Perfusionspräparaten untersucht. Vier Zelltypen sind zu beobachten: 1. thalamokortikale Verbindungszellen (relay cells); 2. Interneuronen Golgi II Typs; 3. fusiforme Zellen mit aspezifischer dendritischer Arborisation; 4. mittelgroße, den Zellen 2. Typs ähnliche Zellen mit einem langen und zahlreiche kurze Fortsätze enthaltenden Axon. Es lassen sich vier verschiedene Arborisationstypen der afferenten Fasern differenzieren; in bezug auf den Ursprung stehen noch keine, in jeder Hinsicht stichhaltigen Angaben zur Verfügung. Nach eingehender Erörterung der zwischen den verschiedenen Zelltypen bestehenden durch Axonkollaterale oder kurze Axonen gebildeten Verbindungen findet auch die funktionelle Rolle der neuropilen Architektur eine kurze Besprechung.

ОРГАНИЗАЦИЯ КЛЕТОК И СИНАПСОВ ДОРСОМЕДИАЛЬНЫХ ЯДЕР ТАЛАМУСА

Т. ТЁМБЁЛ

Типы клеток и архитектура синапсов в дорсомедиальных ядрах таламуса исследовались на кошках на основе классических препаратов Гольджи и перфузионных препаратов Копша. Дается описание 4 типов клеток: 1. таламо-кортикальные передаточные клетки; 2. промежуточные нейроны типа Голъджи II; 3. веретенообразные клетки, имеющие неспецифический рисунок дендритической арборизации; 4. клетки средней величины, напоминающие тип 2, однако с одним длинным аксоном и множеством коротких ветвей. Можно различать четыре различных вида арборизации афферентных волокон, однако их точное происхождение еще недостаточно выяснено. Подробно описываются взаимные связи между различными клеточными типами, осуществляемые короткими аксонами. Дается краткое обсуждение функциональной роли нейропильной архитектуры.

Dr. Teréz TÖMBÖL; Anatómiai Intézet, Budapest IX., Tűzoltó u. 58, Hungary

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 205—211, 1968*

Department of Pathology, Massachusetts General Hospital, Harvard Medical School and Department of Surgery, Tufts University, School of Medicine, Boston, Massachusetts

ENZYME HISTOCHEMICAL STUDIES ON KIDNEYS PRESERVED IN VITRO BY HYPOTHERMIA AND HYPERBARIC OXYGEN*

K. Balogh, jr.** and I. Besznyák***

(Received July, 27, 1967)

Rat kidneys were kept for 24 hours under hypothermia at ambient pressure or under hyperbaric conditions (60 lb. per sq. inch oxygen pressure). For comparison, kidneys were stored for 24 hours at room temperature (20° C). The tissues were subsequently studied for the distribution of DPNH and TPNH diaphorase, dihydrolipoic, lactic, isocitric, succinic, glucose-6-phosphate and β-hydroxybutyric dehydrogenase activities. The microscopic appearance of kidneys and the histochemical distribution pattern of a given enzyme were characteristic and consistently uniform within one experimental group. Swelling, disintegration of architecture and marked loss of enzymatic activity were seen in kidneys kept at room temperature. These changes were less prominent in kidneys stored at 4° C. The best preservation of structure and enzymatic activity was seen in kidneys kept under hypothermia combined with hyperbaric oxygen. These data are supported by the findings of previous transplantation studies with similarly stored kidneys. The present results indicate that preservation of viability of tissues is closely related to their biochemical and structural integrity.

Current interest in organ transplantation from cadavers has raised several important technical problems. One of these is the preservation of organs until transplantation. For this purpose several methods of tissue storage have been considered theoretically feasible, and promising results have been obtained by the application of hyperbaric oxygenation in combination with hypothermia. Indeed, the preservation of human cadaver kidneys has been attempted by these means [11]. Various organs of dogs have been used in similar transplantation studies by the Minnesota group [18], and by others. These investigators reported the successful preservation of canine hearts [1, 3], lungs [4], kidneys [15, 16, 17, 21, 23], and small intestine [10, 20, 22] for 24 hours or more by the combination of hypothermia and hyperbaric oxygen.

It is not fully understood by what mechanism(s) organs are preserved under these circumstances. The present study was undertaken to correlate preservation of cell integrity with enzyme activity in organs kept under hyperbaric oxygen and hypothermia. Histochemical techniques were employed to study the microscopic appearance of kidneys and the distribution pattern of certain oxidative enzyme systems.

* This work is dedicated to Professor Joseph Baló on the occasion of having completed five decades in the service of science.

** Assistant Professor of Pathology, Harvard Medical School, Boston, USA.

*** Research Fellow in Surgery, Tufts University, 1964—1965. Permanent address: Department of Cardiovascular Surgery, Budapest Medical School, Budapest.

Materials and methods

Conditions of tissue storage were similar to those in the previous study [16], in order to permit a meaningful comparison between the histochemical findings and the results of the kidney transplantation experiments. For convenience, however, rat tissues were used instead of dog kidneys. Healthy adult male rats were kept on a regular laboratory diet and water *ad libitum*. The animals were decapitated and their kidneys were removed *in toto* without stripping their capsule. The kidneys were cut in half with a sharp razor blade and the samples were divided into four groups, consisting of twelve tissue blocks each.

Group I. Controls. Kidneys were frozen on dry ice and immediately used for histochemical reactions.

Group II. Storage at 20° C. Kidneys were kept in physiological saline solution at room temperature (20° C) under ambient pressure for 24 hours.

Group III. Hypothermia. Tissues were stored in cold (4° C) physiological saline solution under ambient pressure for 24 hours.

Group IV. Combined hypothermia and hyperbaric oxygen. Kidneys were preserved similar to Group III, but under hyperbaric conditions (60 lb. per sq. inch O_2 pressure).

After 24 hours, the specimens from Groups II, III, and IV were removed from the saline solution and briefly blotted on filter paper. Afterwards, they were frozen on dry ice, together with the fresh tissue controls from Group I. All tissue blocks were mounted to be cut in a cryostat (−18° C)at 20 μ. The frozen sections were mounted on clean coverslips, thawed, and briefly dried at room temperature. Afterwards, multiple sections were utilized from each block for the histochemical demonstration of one of the following enzymes.*

DPNH diaphorase [30], TPNH diaphorase [30], dihydrolipoic dehydrogenase [2], lactic dehydrogenase [25], isocitric dehydrogenase [26], succinic dehydrogenase [24], glucose-6-phosphate dehydrogenase [26] and β-hydroxybutyric dehydrogenase [25].

Nitro-blue tetrazolium was used as the final electron acceptor in all histochemical reactions. This tetrazolium salt precipitates as a dark blue, water and fat-insoluble diformazan at the sites of enzymatic reduction [24]. The theoretical and practical aspects of the employed histochemical techniques have been discussed elsewhere [28]. All incubations were terminated by replacing the media with 10% neutral formalin. The fixed sections were mounted with Kaiser's glycerol-jelly and examined under the light microscope.

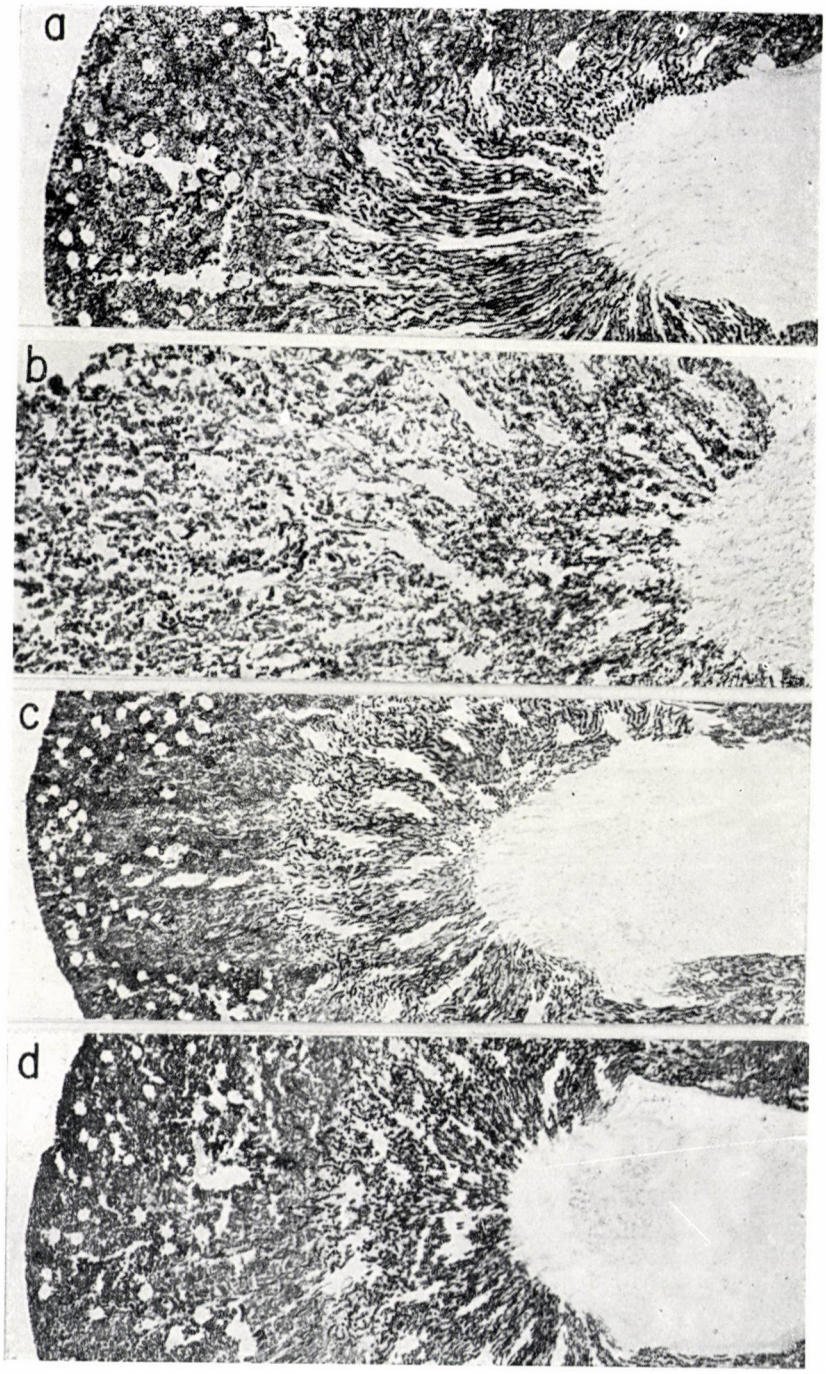
Control sections to rule out nonspecific reactions were incubated in media from which only the individual substrates were omitted. The sections failed to reveal enzymatic activity.

Results

In fresh kidneys (Group I) oxidative enzyme activities were localized in the cytoplasm of the tubular epithelium throughout the cortex and the outer zone of medulla, whereas the glomeruli and the inner zone of medulla (papilla) contained little or no demonstrable diformazan.

* *Abbreviations used.* ATP — adenosine triphosphate. DPNH — reduced diphosphopyridine nucleotide. TPNH — reduced triphosphopyridine nucleotide.

Fig. 1. Histochemical distribution pattern of succinic dehydrogenase activity in rat kidney. Sites of enzymatic activity are indicated by diformazan deposits that appear black in this picture. Enzyme activity is almost entirely limited to the tubular epithelium of the cortex and the outer zone of the medulla. Unreactive glomeruli appear as small blank dots. The inner zone of the medulla (papilla) is also negative (right edge) × 14. *(a)* Group I. Fresh kidney processed immediately after sacrificing. *(b)* Group II. Kidney stored at room temperature for 24 hours. Notice marked swelling and disintegration of cortex, with striking loss of enzyme activity. *(c)* Group III. Hypothermia (4° C) preserved considerable enzyme activity in comparison to *(b)*, although the cortex appears markedly swollen. *(d)* Group IV. Hyperbaric oxygen with hypothermia preserved more enzyme activity than hypothermia alone. The parenchyma also appears swollen. The distribution pattern of succinic dehydrogenase approximates the normal.


a
b
c
d

The microscopic appearance and histochemical distribution pattern of a particular enzyme was characteristic and consistently uniform within one experimental group. The kidneys in Group I showed the normal enzyme patterns (Fig. 1a) similar to those described in the original histochemical methodology reports. The other groups showed lesser degrees of activity than the corresponding normal values of all enzymes. In addition, all stored kidneys (Group II—IV) were oedematous. The most marked swelling and disintegration of the architecture was seen in kidneys kept at room temperature for 24 hours (Group II); this was accompanied by the lowest enzyme activity (Fig. 1b). More enzymatic activity was seen in kidneys kept at 4° C (Group III) (Fig. 1c). Among the experimental groups, the best preservation of structure and the relatively highest enzyme activity was observed in kidneys kept at 4°C under 60 lb. per sq. inch O_2 pressure (Group IV) (Fig. 1d). The visual evaluation of enzyme activities in the histochemical reactions could be summed up as follows

$$\text{Group I} > \text{Group IV} > \text{Group III} > \text{Group II}.$$

Discussion

In a previous study [16], one of us (I. B.) found that dog kidneys could be preserved in an electrolyte solution for 24 hours in a refrigerator pressure chamber maintained at 4° C and 3 to 5 ATA O_2 following which they were replanted as autographs into the pelvis of the host. Two to three weeks after replantation, contralateral nephrectomy was performed. In a series of 27 animals receiving transplants preserved in this manner, there were 12 long-term survivors. When examined six to eight months following contralateral nephrectomy the survivors were apparently healthy. At the same time hypothermia or hyperbaric oxygen alone failed to preserve viability of kidneys. Similar observations were made by the Minnesota group on a variety of canine organs [18].

It was evident from the transplantation studies that hypothermia combined with hyperbaric oxygen must preserve considerable amounts of oxidative enzyme activity in the kidneys. This was confirmed by the present results because substantially more oxidative enzyme activity was demonstrable in rat kidneys stored under hypothermia and hyperbaric oxygen than with either hypothermia or hyperbaric oxygen alone. In contrast to our results, CALNE et al. [5] reported no significant changes in the histochemical distribution pattern of acid and alkaline phosphatase, succinic dehydrogenase and DPNH diaphorase activities in dog kidneys preserved up to 17 hours in cold (4° C) Hanks' solution at ambient pressure.

Little is known about the mechanisms involved in the preservation of structure and function of tissues under the described experimental conditions.

It is well known that hypothermia lowers metabolic activity and thus decreases oxygen consumption of tissues *in vivo* as well as *in vitro*. Hyperbaric oxygen, likewise, depresses oxygen consumption [7,27], inhibits tissue respiration and oxidative phosphorylation, i.e. ATP production under aerobic conditions [14, 29]. In addition, the activity of succinic dehydrogenase [7, 31] and other enzymes, particularly of those containing —SH groups, have also been shown to be inhibited by hyperbaric oxygen [6, 8, 9, 12—14, 31—34]. Recent data indicate that hyperbaric oxygen also appears to be involved in the electron transfer chain [6]. In general, it has therefore been believed that hyperbaric oxygen sustained viability of organs *in vitro* by these mechanisms. This view was challenged by LILLEHEI et al. [19], who reported that canine hearts stored for 24 hours under hypothermia and hyperbaric oxygen, nitrogen or helium all resumed a coordinated ventricular beat after revascularization. The findings in 15 preserved hearts suggested that oxygen is apparently not essential for preservation of organs *in vitro*, since an inert gas such as nitrogen or helium also maintained viability of the hearts. The results of LILLEHEI et al. indicate that a combination of hypothermia and high gas pressure influences the rate of respiration and glycolysis of the cells in such a way as to minimize or delay irreversible cell damage and death, even in the absence of oxygen.

Obviously, a histochemical study of this nature cannot provide answers concerning the mechanisms involved in the maintenance of organ viability. However, it permits light microscopic observation of structures and simultaneous visual evaluation of histochemical enzyme reactions. The results of this study suggest that preservation of the viability of tissues closely parallels their morphological and biochemical integrity.

REFERENCES

1. ALMOND, C. H., ANIDO, H., SEABER, A., YOUNG, R., MACKENZIE, J. W.: (1965) Heart Preservation Utilizing Hyperbaric Oxygen and Hypothermia. Amer. Heart J. **69,** 425. — 2. BALOGH, K. jr.: (1964) Dihydrolipoic Dehydrogenase Activity: A Step in Formation of Acyl-Coenzyme A, Demonstrated Histochemically. J. Histochem. Cytochem. **12,** 404. — 3. BLOCH, J. H., MANAX, W. G., EYAL, Z., LILLEHEI, R. C.: (1964) Heart Preservation in vitro with Hyperbaric Oxygenation and Hypothermia. J. thorac. cardiovasc. Surg. **48,** 969. — 4. BLUMENSTOCK, D. A., LEMPERT, N., MORGADO, F.: (1965) Preservation of the Canine Lung in vitro for 24 Hours with the Use of Hypothermia and Hyperbaric Oxygen. J. thorac. cardiovasc. Surg. **50,** 769. — 5. CALNE, R. Y., PEGG, D. E., PRYSE-DAVIES, J., BROWN, F. L.: (1963) Renal Preservation by Ice-cooling. An Experimental Study Relating to Kidney Transplantation from Cadavers. Brit. med. J. 651. — 6. CHANCE, B., JAMIESON, D., COLES, H.: (1965) Energy-linked Pyridine Nucleotide Reduction: Inhibitory Effects of Hyperbaric Oxygen in vitro and in vivo. Nature (Lond.) **206,** 257. — 7. DICKENS, F.: (1945) The Toxin Effects of Oxygen on Brain Metabolism and on Tissue Enzymes. 2. Tissue Enzymes. Biochem. J. **40,** 171. — 8. DICKENS, F.: (1962) The Toxin Effects of Oxygen on Nervous Tissue. In: Neurochemistry, 2nd ed. Thomas, Springfield, Ill. P. 815. — 9. DONALD, K. W.: (1964) Oxygen Poisoning. *In:* Hyperbaric Oxygenation. Proceedings of the Second International Congress, Glasgow. Ed. I. McA. Ledingham; Livingstone, Edinburg, **1965**, P. 26. — 10. EYAL, Z., MANAX, W. G., BLOCH, J. H., LILLEHEI, R. C.: (1965) Successful in vitro Preservation of the Small Bowel, Including Maintenance of Mucosal Integrity with Chlorpromazine, Hypothermia, and Hyperbaric Oxygenation. Surgery **57,** 259. — 11. FIGUEROA, J. E., NAKAMOTO, S., STRAFFON, R. A.,

SHIBAGAKI, M., KOLFF, W. J.: (1965) Human Cadaver Kidney Transplantation: A Progress Report. Trans. Amer. Ass. Soc. artif. int. Organs. **11,** 213. — 12. HAUGAARD, N.: (1955) Effect of High Oxygen Tensions upon Enzymes. Proceedings of Underwater Physiology. Symp. nat. Acad. Sci. nat. Res. Counc. Washington, D. C., P. **8.** — 13. HAUGAARD, N.: (1964) The Toxic Action of Oxygen on Metabolism and the Role of Trace Metals. *In:* Oxygen in the Animal Organism. Ed. F. Dickens, E. Neil, Pergamon, Oxford, P. 495. — 14. HAUGAARD, N.: (1965) Poisoning of Cellular Reactions by Oxygen. Ann. N. Y. Acad. Sci. **117,** 736. — 15. LADAGA, L. G., KOO, G. C. G., MACLEAN, L. D.: (1965) Preservation of the Kidney in the Dog. Surg. Forum **16,** 337. — 16. LADAGA, L. G., NABSETH, D. C., BESZNYÁK, I., HENDRY, W. F., MCLEOD, G., DETERLING, R. A., jr.: (1966) Preservation of Canine Kidneys by Hypothermia and Hyperbaric Oxygen. Ann. Surg. **163,** 553. — 17. LEMPERT, N., BLUMENSTOCK, D. A., CARTER, R. D.: (1965) Hyperbaric Hypothermic Preservation of the Dog Kidney with and without Immediate Contralateral Nephrectomy. Surg. Forum **16,** 196. — 18. LILLEHEI, R. C., MANAX, W. G., BLOCH, J. H., EYAL, Z., HIDALGO, F., LONGERBEAM, J. K.: (1964) In vitro Preservation of Whole Organs by Hypothermia and Hyperbaric Oxygenation. Cryobiology, **1,** 181. — 19. LILLEHEI, R. C., LYONS, G. W., MANAX, W. G., DIETZMAN, R. H.: (1966) 9th International Congress on the Diseases of the Chest, Copenhagen. — 20. LYONS, G. W., LARGIADER, F., LILLEHEI, R. C.: (1965) Intestinal Absorption of Ileum Preserved by Hypothermia and Hyperbaric Oxygen. Surg. Forum **16,** 357. — 21. MAKIN, G. S., HOWARD, J. M.: (1965) Hyperbaric Oxygen and Profound Hypothermia to Preserve Canine Kidneys. Arch. Surg. **91,** 568. — 22. MANAX, W. G., BLOCH, J. H., EYAL, Z., LILLEHEI, R C.: (1964) Experimental Preservation of the Small Bowel. Amer. J. Surg. **109,** 26. — 23. MANAX, W. G., BLOCH, J. H., LONGERBEAM, J. K., LILLEHEI, R. C.: (1964) Successful 24 Hour in vitro Preservation of Canine Kidneys by the Combined Use of Hyperbaric Oxygenation and Hypothermia. Surgery, **56,** 275. — 24. NACHLAS, M. M., TSOU, K. C., DESOUZA, E., CHENG, G. S., SELIGMAN, A. M.: (1957) Cytochemical Demonstration of Succinic Dehydrogenase by the Use of a New p-Nitrophenyl Substituted Ditetrazole. J. Histochem. Cytochem. **5,** 420. — 25. NACHLAS, M. M., WALKER, D. G., SELIGMAN, A. M.: (1958) A Histochemical Method for the Demonstration of Diphosphopyridine Nucleotide Diaphorase. J. biophys. biochem. Cytol. **4,** 29. — 26. NACHLAS, M. M., WALKER, D. G., SELIGMAN, A. M.: (1958) The Histochemical Localization of Triphosphopyridine Nucleotide Diaphorase. J. biophys. biochem. Cytol. **4,** 467. — 27. NORMAN, J. N., SMITH, G., DOUGLAS, T. A.: (1966) The Effect of Oxygen at Elevated Atmospheric Pressure and Hypothermia on Tissue Metabolism. Surg. Gynec. Obstet. **122,** 778. — 28. PEARSE, A. G. E.: (1960) Histochemistry, Theoretical and Applied. 2nd ed., Little, Brown and Co., Boston. — 29. SANDERS, A. P., HALL, I. H.: (1966) Effects of Hyperbaric Oxygenation on Metabolism II. Oxidative Phosphorylation in Rat Brain, Liver and Kidney. Proc. Soc. exp. Biol. (N. Y.) **121,** 34. —30. SCARPELLI, D. G., HESS, R., PEARSE, A. G. E.: (1958) The Cytochemical Localization of Oxidative Enzymes. I. Diphosphopyridine Nucleotide Diaphorase and Triphosphopyridine Diaphorase J. biophys. biochem. Cytol. **4,** 747. — 31. STADIE, W. C., HAUGAARD, N.: (1945) Oxygen Poisoning. V. The Effect of High Oxygen Pressure Upon Enzymes: Succinic Dehydrogenase and Cytochrome Oxidase. J. biol. Chem. **161,** 153. — 32. STADIE, W. C., HAUGAARD, N.: (1945) Oxygen Poisoning. VII. The Effect of High Oxygen Pressure Upon Enzymes: Uricase, Xanthine Oxidase and d-Amino Acid Oxidase. J. biol. Chem. **161,** 181. — 33. STADIE, W. C., RIGGS, B. X., HAUGAARD, N.: (1945) Oxygen Poisoning. VI. The Effect of High Oxygen Pressure upon Enzymes: Pepsin, Catalase, Cholinesterase, and Carbonic Anhydrase. J. biol. Chem. **161,** 175. — 34. THOMAS, J. J. jr., NEPTUNE, E. M.: (1955) Chemical Mechanisms in Oxygen Toxicity. Proc. Underwater Symp. nat. Acad. Sci.-nat. Res. Counc. Washington, D. C. P. 139.

HISTOCHEMISCHE ENZYMUNTERSUCHUNGEN DES UNTER HYPOTHERMIE IN HYPERBAREM SAUERSTOFFDRUCK KONSERVIERTEN NIEREN

K. BALOGH jr. und I. BESZNYÁK

Rattennieren wurden 24 Stunden bei Hypothermie unter normalem Druck sowie in hyperbarem Sauerstoff konserviert. Zu Vergleichszwecken wurden Kontrollnieren 24 Stunden bei Zimmertemperatur (20° C) aufbewahrt. Nachfolgend wurden die Gewebe auf ihre DPNH- und TPNH-diaphorase-Gehalt, ferner auf die Dihydrolipoid-, Milchsäure-, Isozitronensäure-, Sukzinat-, Glukose-6-phosphatase- und 3-Hydroxybuttersäure-Aktivität untersucht. Das mikroskopische Bild der Niere und die histologische Verteilung des jeweiligen Enzyms waren

in den einzelnen Versuchsgruppen charakteristisch und konsequent identisch. Anschwellen, Desintegration der Struktur und ein merklicher Verlust an enzymatischer Aktivität wurde in den bei Zimmertemperatur konservierten Nieren festgestellt. Diese Veränderungen waren weniger ausgeprägt in Nieren, die bei 4° C gehalten wurden. Struktur und Enzymaktivität blieben am besten erhalten, wenn man die Nieren unter Hypothermie in hyperbarem Sauerstoff konserviert hatte. Diese Angaben fanden eine Bestätigung durch die Ergebnisse der früheren Transplantationsversuche von ähnlich konservierten Nieren. Die gegenwärtigen Ergebnisse weisen darauf hin, daß das Erhalten der Lebensfähigkeit der Gewebe eng mit ihrer biochemischen und strukturellen Integrität zusammenhängt.

ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ЭНЗИМОВ В ПОЧКАХ, ХРАНЯЕМЫХ in vitro В УСЛОВИЯХ ГИПОТЕРМИИ И ГИПЕРБАРИЧЕСКОГО ДАВЛЕНИЯ КИСЛОРОДА

К. БАЛОГ МЛ. и И. БЕСНЬЯК

Почки крыс хранялись в течение 24 часов в условиях гипотермии при нормальном давлении среды или при гипербарических условиях (давление кислорода 60 фунтов/кв. инч). В целях сравнения контрольные почки хранялись в течение 24 часов при комнатной температуре (20 °C). После этого в тканях почек были определены распределение *DPNH* и *TPNH*-диафоразы и активность дигидролипоидов, молочной кислоты, изолимонной кислоты, янтарной кислоты, глюкоз-6-фосфата и 3-гидроксимасляной кислоты. В пределах отдельных групп микроскопическая картина почек и гистохимическое распределение отдельных энзимов были характерными и последовательно однообразными. Набухание, дезинтеграция построения и заметная потеря ферментативной активности были выявлены в почках, храняемых при комнатной температуре. Эти изменения были менее выраженными в почках, храняемы при температуре в 4 °C. Структура и ферментативная активность сохранялись лучше всего в почках, консервированных при помощи гипотермии в сочетании с гипербарическим кислородным давлением. Эти данные были подтверждены результатами прежних экспериментов по пересадке почки, храняемой при подобных условиях. Результаты настоящих исследований указывают на то, что сохранность жизнеспособности тканей тесно связана с их биохимической и структурной целостностью.

Dr. KÁROLY BALOGH, jr.; Dep. of Pathology, Massachusetts General Hospital, Harvard Medical School, U.S.A.

Dr. ISTVÁN BESZNYÁK: Dep. of Cardiovascular Surgery, Univ. Med. School, Budapest, Hungary

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 213—221, 1968*

Departments of Pathology and Surgery, Albert Einstein College of Medicine — Bronx Municipal Hospital Center, New York, N. Y. 10461

HAEMORRHAGIC PANCREATITIS INDUCED BY ELASTASE*

J.J. MOLNÁR, I. J. SCHNEIDER, S. TINDEL, D. SHAPIRA and D. STATE

(Received July 29, 1967)

1. 75 mg of pancreatic elastase in 15 ml distilled water was infused into the pancreatic ducts of dogs. 2. Shortly after the infusion acute haemorrhagic pancreatitis developed in all 14 dogs. 3. Significant vascular changes were present in the elastase-induced pancreatitis, similar to those described by RICH and DUFF in 1936. 4. According to literary data and our own material, vascular changes were less prominent in the trypsin-infused pancreases than in the elastase-infused ones. 5. It is suggested that the commercial trypsin used in 1936 by RICH and DUFF was not free of elastase and the vascular changes described by these authors were due to the elastase admixture rather than to the trypsin itself. 6. For the experimental production of haemorrhagic pancreatitis in dogs, trypsin-free crystalline elastase is strongly recommended.

The recognition of enzymes and their role in health and disease has long preceded their names and chemical purification. Pancreatic juice was said to be used by the noted John HUNTER [1] for the debridement of infected wounds. The theory, that pancreatic juice is the disease-producing factor in acute haemorrhagic pancreatitis, was proposed almost a century ago by KLEBS [2]. OPIE, the father of the common channel theory [3, 4], claimed that trypsin is responsible for the peculiar necrosis of the pancreas in acute haemorrhagic pancreatitis. RICH and DUFF [5] proved that the haemorrhage in that condition results from a type of necrosis of the vessel walls in the pancreas, which can easily be reproduced by pancreatic juice as well as by crystalline trypsin. In later paper [6] the same authors emphasized the similarity between acute necrotizing vasculitis induced by trypsin, and the small vessel changes in human hyaline arteriosclerosis.

The study of the necrotizing effect of trypsin on the vessel walls was reopened by the discovery and crystallization of another proteolytic enzyme from the pancreas. BALÓ and BANGA [7] isolated an enzyme from a watery solution of fat-free pancreas, and named it elastase on the basis of its powerful action on the removal of elastic fibres from the walls of arteries. The possible role of elastase in human arteriosclerosis has been duly investigated [8], and extensive biochemical studies clarified its mode of action on elastic fibres [9], but no experiments have been reported on the role of elastase in acute haemorrhagic

* This work is dedicated to Professor Joseph Baló on the occasion of having completed five decades in the service of science.

pancreatitis prior to our presentations [10, 11]. In this paper we summarize our unpublished results and establish a definite role of elastase in experimental haemorrhagic pancreatitis.

Materials and methods

Twenty fasting mongrel dogs of both sexes, weighing 15 to 22 kg were used in the experiments. After intravenous sodium pentobarbital anaesthesia a midline abdominal incision was made to expose the body of the pancreas. The accessory pancreatic duct (which is the major pancreatic duct in dogs) was identified and cannulated with the largest polyethylene catheter possible (Fig. 1). The catheter was advanced 2 cm into the intrapancreatic portion of the duct,

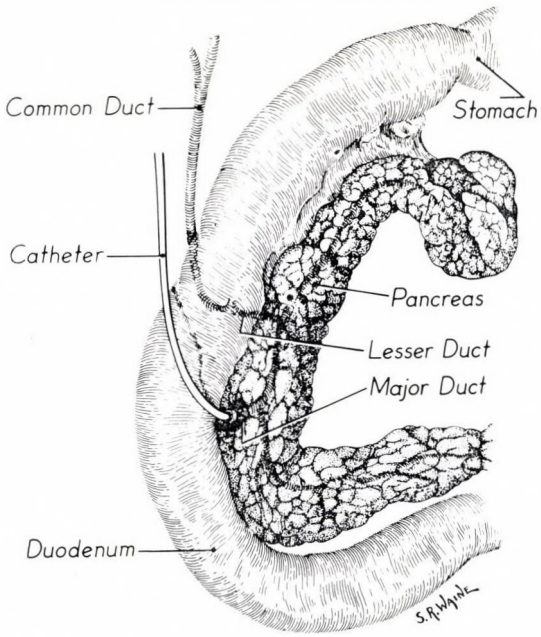


Fig. 1. Anatomy of duodenum and pancreas in dog with catheter inserted into major pancreatic duct

and secured with a silk ligature. Reflux of clear pancreatic juice occurred in every instance, indicating that the catheter was properly inserted. Under sterile conditions, a solution of 75 mg pancreatic elastase dissolved in 15 ml distilled water, as prepared by the method of GRANT and ROBBINS [12], was allowed to drip *via* the catheter into the pancreatic duct. Assays of this preparation yielded 160 elastolytic units per mg. In six control animals a solution of 15 ml of distilled water was used. The elastase solution was slowly elevated above the animal until the hydrostatic pressure was sufficient to initiate flow into the pancreatic duct. The required pressure ranged from 40—60 cm water, and the duration of flow varied from 15 to 30 minutes. At the conclusion of the infusion the catheter was removed, and both sides of the pancreatic duct were ligated. All animals were sacrificed five hours after the completion of the infusion, and the whole pancreas from each was removed for gross and microscopic study. All tissues were immediately fixed in ten per cent buffered formalin and representative sections were taken from each pancreas. The tissues were embedded in paraffin and subsequent sections stained with H&E and van Gieson elastic preparations.

Results

The 14 elastase infused dogs developed striking gross changes. Approximately one hour following the completion of the infusion, progressive oedema, congestion and confluent haemorrhages appeared on the surface of the pancreas, resembling the early changes in human haemorrhagic pancreatitis. The haemorrhages were prominent adjacent to the infusion site and extended throughout the body and the tail of the pancreas. In multiple cross sections these changes involved most of the parenchyma along the interstitial septa. In three animals gross foci of peripancreatic fat necrosis were present. The six control animals showed minimal oedema and hyperaemia of the pancreas.

Microscopic examination showed wide, swollen interstitial septa infiltrated by leukocytes, dilated veins and irregular areas of wide spread haemorrhages destroying the acini (Figs 2, 3). Vascular changes were prominent and varied according to the size of the artery. In the larger arteries the internal elastic membrane was uncoiled and appeared frayed (Fig. 4). In the medium sized muscular arteries fibrinoid-like necrosis of the wall with loss of elastica and muscularis was seen occasionally (Fig. 5). Hyaline degeneration or complete dissolution of the vascular structures was frequent, often producing an unstained outline of the arteriole (Fig. 6). Fresh thrombi were found in the medium sized veins. The pancreatic duct and its branches were usually intact, their mucosa surfaces uninvolved, but inflammatory cells were occasionally seen in their lumen. In addition to the three animals which showed gross foci of fat necrosis, four more displayed microscopic evidence of fresh peripancreatic fat necrosis (Fig. 7). All control animals were studied histologically and showed some extravasation of blood near the incisional margin, with mild oedema and vascular dilatation, but the classical vascular and inflammatory changes were absent in the remaining tissue.

Discussion

The role of proteolytic enzymes in the pathogenesis of acute haemorrhagic pancreatitis has been investigated by several authors [13, 14, 15, 16, 17, 18, 19, 20]. It is generally agreed that intrapancreatic activation of the inactive zymogens to proteolytic enzymes will produce the characteristic autodigestion of the pancreas. The histological reaction to this autodigestion would then be the classical morphological appearance of acute haemorrhagic pancreatitis. Of the three well known proteolytic enzymes of the pancreas (trypsinogen, chymotrypsinogen, procarboxypeptidase), only trypsinogen was usually selected for these studies, because its conversion to trypsin is necessary for the liberation of the other two enzymes.

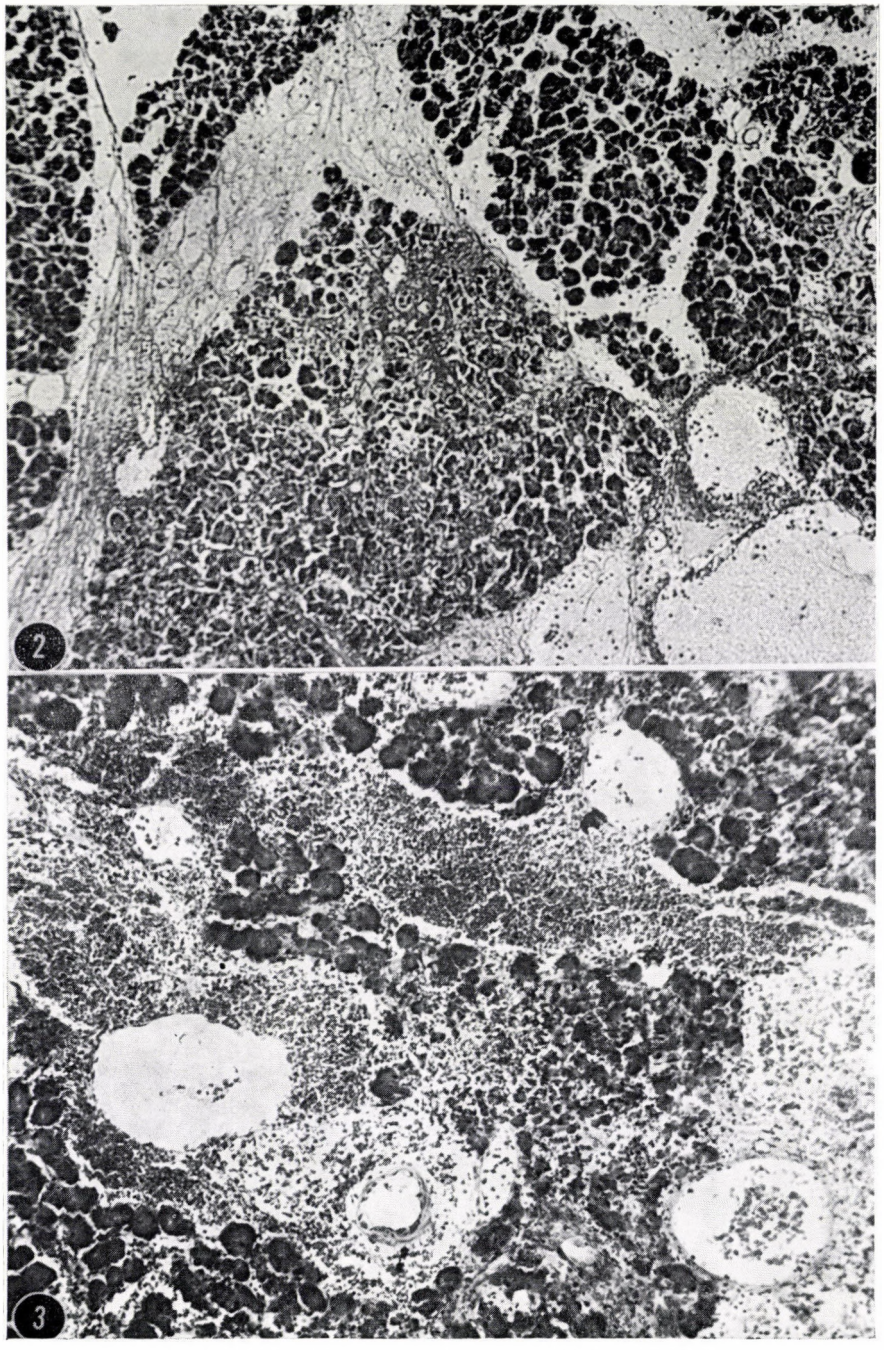


Fig. 2. Oedematous interstitial septa dividing the pancreatic lobules. Dilated veins and scattered migrating inflammatory cells. H&E ×60

Fig. 3. Widespread haemorrhagic areas extending into the lobules, destroying the acini. H&E × 60

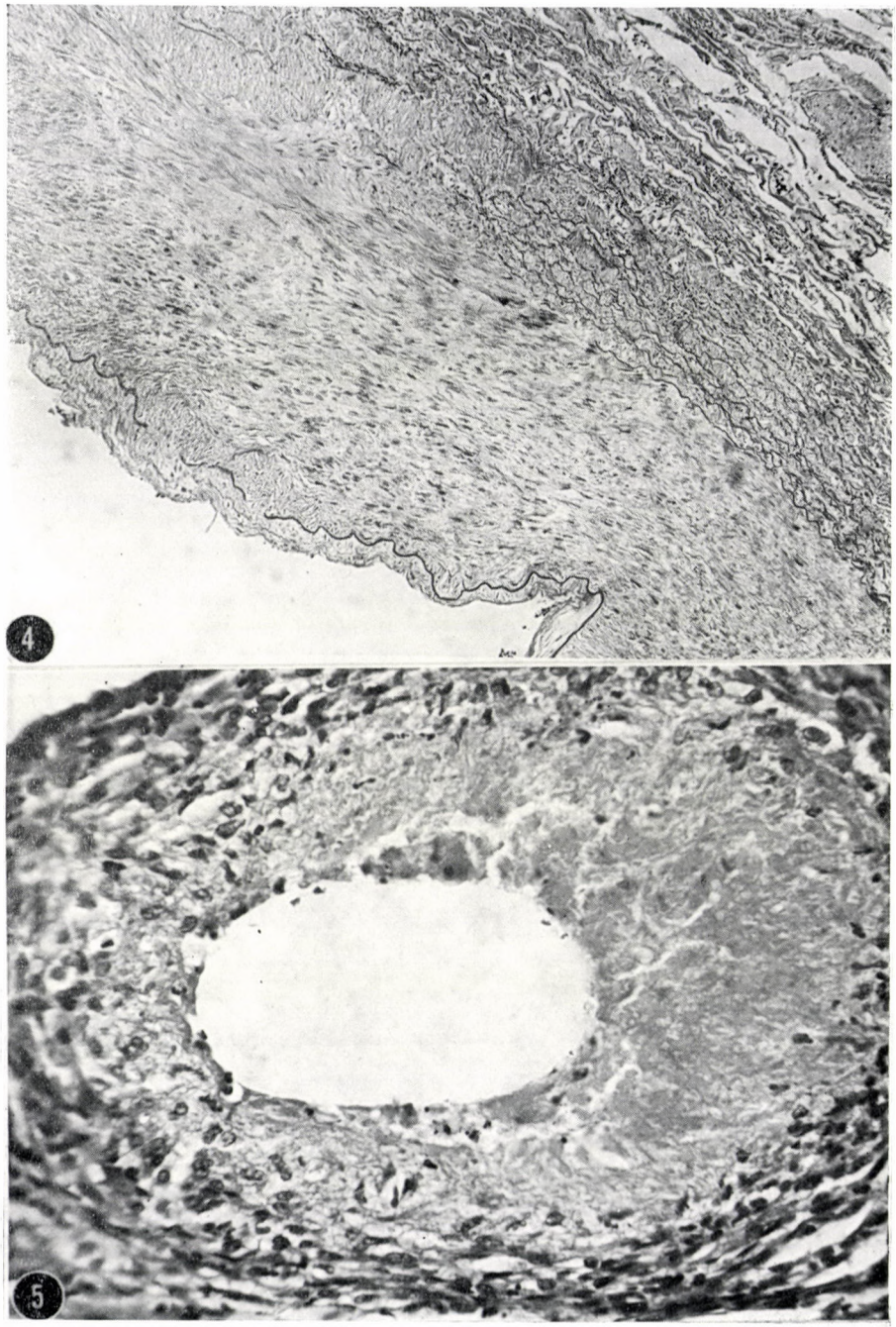


Fig. 4. Portion of a large artery. The internal elastic membrane is frayed. H&E × 200
Fig. 5. Fibrinoid necrosis in a medium size artery. H&E × 200

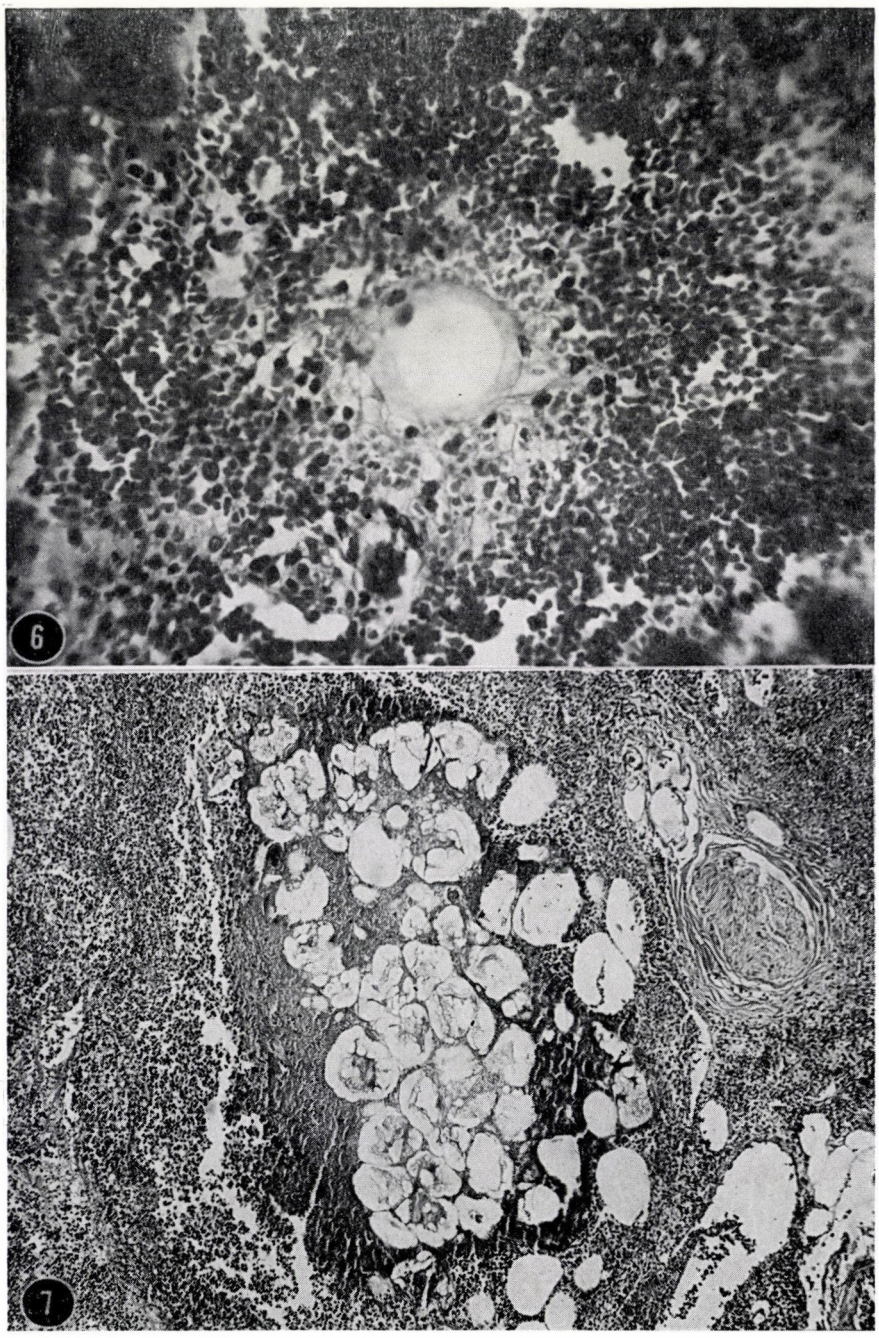


Fig. 6. Shadow of a small arteriole in the centre of haemorrhage. H&E ×360
Fig. 7. A focus of fat necrosis surrounded by acute inflammatory exudate in the peripancreatic fat

The pathology of pancreatitis produced by autodigestive trypsin was studied by RICH and DUFF [5]. They emphasized first the presence of vascular changes, and suggested that the haemorrhage was the result of the peculiar vascular necrosis produced by the action of escaped pancreatic juice. It was shown furthermore that not only pancreatic secretion, but purified crystalline trypsin at the site of injection anywhere in the body, will give rise to similar vascular necrosis and haemorrhages found in cases of haemorrhagic pancreatitis. The question of how specific these vascular changes are, as described by RICH and DUFF, certainly becomes an interesting point, since our experiments with elastase showed similar if not identical vascular changes. In recent studies, BECK et al. [21] compared the morphological appearance of bile-induced and trypsin-induced experimental pancreatitis in dogs, and concluded that no difference was seen histologically in the two groups one hour after the experiment had been started. They found no free trypsin in the bile-induced cases, but definite proteolytic activity in the trypsin-induced group, only in the first 15—20 minutes, and noted a remarkable absence of any conspicuous vascular necrosis. They found vascular necrosis only in the centre of extensive parenchymal necrosis. The vascular changes seemed to show a parallel course with the duration of the experimental damage. They therefore concluded that the vasculitis in bile- or trypsin-induced experimental haemorrhagic pancreatitis, was probably secondary to the parenchymal damage. The frequently cited RICH and DUFF experiment involved three cases of experimentally produced haemorrhagic pancreatitis. In one case they used bile, and in the other two cases they used commercial trypsin. Since crude trypsin can be used as a source for purified elastase [22], it is more than probable that the commercial pancreatic trypsin (Fairchild Bros. and Foster, New York City) as utilized by RICH and DUFF in their 1936 experiments, contained other proteolytic enzymes and most probably a generous amount of elastase. The peculiar vascular changes, therefore, could be attributed to the elastase-contaminated commercial trypsin, rather than to trypsin alone. As BECK et al. [21] pointed out, the vascular changes in trypsin-induced acute pancreatitis are rather mild and secondary, and in our experience [23] they were almost non-existent at the same period when elastase-induced cases already showed advanced vascular necrosis. In a recent study WANKE et al. [24] used a battery of enzymes to produce pancreatitis in rats. Among others, they injected elastase into the pancreatic duct of two rats, but failed to produce the changes we have induced in dogs.

The pathogenesis of acute haemorrhagic pancreatitis is still obscure. When a morphological lesion, so unique in appearance and so classical in manifestations and in some of the biochemical alterations, can be produced experimentally by at least fifty different techniques, it is probable that none of them is the right one to apply for human cases. With the introduction of more sensi-

tive biochemical tests and by improved laboratory facilities, we hope that in the future some of the serum levels of enzymes and their inhibitors will be measured, and we will obtain a better understanding of the natural course of human haemorrhagic pancreatitis. Until that time, however, we strongly recommend that a simple technique for the experimental model of haemorrhagic pancreatitis be used by the infusion of elastase at physiological pressure into the pancreatic ducts of dogs.

REFERENCES

1. HUNTER, J.: cit. REISER, H. G., PATTON, R., ROETTIG, L. C.: (1951) The Tryptic Debridement of Necrotic Tissue. Arch. Surg. **63,** 568. — 2. KLEBS, T. A. E.: (1868) Handbuch der pathologischen Anatomie. Hirschwald, Berlin. — 3. OPIE, E. L.: (1901) The Etiology of Acute Hemorrhagic Pancreatitis. Bull. Johns Hopkins Hosp. **12,** 182. — 4. OPIE, E. L., MEAKINS J. C.: Data concerning the Etiology and Pathology of Hemorrhagic Necrosis of Pancreas. J. exp. Med. **11,** 561. — 5. RICH, A. R., DUFF, G. L.: (1936) Experimental and Pathological Studies on the Pathogenesis of Acute Pancreatitis. Bull. Johns Hopkins Hosp. **58,** 213. — 6. RICH, R. A. DUFF, G. L.: (1937) The Production of Hyaline Arteriosclerosis and Arteriolonecrosis by Means of Proteolytic Enzymes. Bull. Johns Hopkins Hosp. **61,** 63. — 7. BALÓ, J., BANGA, I.: (1954) Die Zerstörung der elastischen Fasern der Gefässwand. Schweiz. Z. Path. Bakt. **12,** 350. — 8. LANSING, A. I.: (1954) Ageing of Elastic Tissue and the Systemic Effects of Elastase. Ciba Found. Coll. on Ageing. Churchill, London. P. 88.— 9. BALÓ, J., BANGA, I.: (1961) Über die Ergebnisse der Elastase und Kollagen-muko-proteinase-Forschung. Z. Vitamin-, Hormon- u. Fermentforsch. **12,** 59.— 10. MOLNAR, J. J., SCHNEIDER, I. J., TINDEL, S., GUEFT, B.: (1962) Pathologic Studies on Pancreatitis and Vasculitis Induced by Trypsin-free Elastase. 59th Meeting of Amer. Ass. Path. Bact. Montreal. — 11. SCHNEIDER, I. J., TINDEL, S., SHAPIRA, D., MOLNAR, J. J., STATE, D.: (1962) Elastase-Induced Hemorrhagic Pancreatitis. 4th Ann. Meeting of Gastroent. Res. Forum. New York. — 12. GRANT, N. H., ROBBINS, K. C.: (1957) Studies on Porcine Elastase and Proelastase. Arch. Biochem. **66,** 396.—13. BECK, I. T.: (1963) On the Role of Proteolytic Enzymes in the Pathogenesis of Acute Pancreatitis, Ph. D. Thesis, McGill Univ., Montreal, Pp. 190. — 14. DREILING, D.: (1959) Experimental Pancreatitis: a Critical Review. J. Mt. Sinai Hosp. **25,** 128. — 15. KEITH, L. M., JR., BARNES, J. E., DENKEWALTER, F. R.: (1958) Experimental Study of Interstitial Injection of Trypsin and Trypsinogen into the Pancreas. Arch. Surg. **77,** 416. — 16. POWERS, S. R., JR., BROWN, H. H., STEIN, A. A.: (1955) The Pathogenesis of Acute and Chronic Pancreatitis. Ann. Surg. **142,** 690. — 17. HANSSON, K., LUNDH, G., STENRAM, U.: (1961) Morphological and Secretory Studies in Experimentally Induced Pancreatitis in Rats. Acta chir. scand. **121,** 274.—18. EINSENHARDT, R. H., HARZELL, G. W., JR., KUBISTA, T., HIMEL, C.: (1961) The Etiology of Acute Hemorrhagic Pancreatitis in Relation to the Mechanism of Activation of Pancreatic Proteases. Fed. Proc. **20,** 252. — 19. DOERR, W.: (1964) Pathogenese der akuten und chronischen Pankreatitis. Verh. dtsch. Ges. inn. Med. **70,** 718. — 20. NAGEL, W., ROBEL, K. P., WILLING, F.: (1965) Über die Aktivierung proteolytischer Proenzyme des Pankreas. Klin. Wschr. **43,** 173. — 21. BECK, I. T., KAHN, D. S., SOLYMAR, J., MCKENNA, R. D., ZYLBERSZAC, B.: (1964) The Role of Pancreatic Enzymes in the Pathogenesis of Acute Pancreatitis. III. Gastroenterology **46,** 531. — 22. LEWIS, U. J., WILLIAMS, D. E., BRINK, N. G.: (1956) Pancreatic Elastase: Purification, Properties and Function. J. biol. Chem. **222,** 705. — 23. MOLNAR, J. J., TINDEL, S., SCHNEIDER, I. J.: (1966) Unpublished Data. — 24. WANKE, M., NAGEL, W., WILLING, F.: (1966) Formen der experimentellen Pankreatitis patho-anatomisch gesehen. Frankf. Z. Path. **75,** 207.

DURCH ELASTASE HERBEIGEFÜHRTE HÄMORRHAGISCHE PANKREATITIS

J. J. MOLNAR, I. J. SCHNEIDER, S. TINDEL, D. SHAPIRA und D. STATE

1. In die Ausführungsgänge der Bauchspeicheldrüse von Hunden wurden 75 mg Pankreaselastase in 15 ml destilliertem Wasser infundiert.

2. Bei allen 14 Hunden entwickelte sich bald nach der Infusion eine akute hämorrhagische Pankreatitis.

3. Bei der durch Elastase herbeigeführten Pankreatitis waren signifikante Gefäßveränderungen zu beobachten, die den von RICH und DUFF im Jahre 1936 beschriebenen ähnlich waren.

4. Die Gefäßveränderungen in der Bauchspeicheldrüse nach Trypsininfusion waren weniger ausgeprägt als nach Elastaseinfusion.

5. Es wird angenommen, daß das 1936 von RICH und DUFF benutzte handelsübliche Trypsin nicht frei von Elastase war, und daß die durch diese Autoren beschriebenen Gefäßveränderungen eher durch die Elastasenbeimischung als durch das Trypsin selbst herbeigeführt waren.

6. Es wird empfohlen, für die experimentelle Erzeugung einer hämorrhagischen Pankreatitis an Hunden Trypsin-freie kristalline Elastase zu verwenden.

ГЕМОРРАГИЧЕСКИЙ ПАНКРЕАТИТ, ВЫЗВАННЫЙ ЭЛАСТАЗОЙ

Й. Й. МОЛЬНАР, И. Й. ШНЕЙДЕР, С. ТИНДЕЛ, Д. ШАПИРА и Д. СТЕЙТ

1. В протоки поджелудочной железы собак авторы проводили вливание 75 мг панкреатической эластазы, разбавленных в 15 мл дистиллированной воды.

2. Скоро после вливания развился острый геморрагический панкреатит у всех 14 собак.

3. При панкреатите, вызванном эластазой, наблюдались достоверные изменения сосудов, подобные изменениям, описанным Рич и Дафф в 1936 году.

4. Согласно литературным данным и собственому материалу авторов изменения сосудов поджелудочной железы были менее выраженными после вливания трипсина, чем после введения эластазы.

5. Предполагается, что примененный Рич и Даффом в 1936 году имеющийся в продаже трипсин не был свободным от эластазы, и что описанные этими авторами изменения сосудов были вызваны скорее примесью эластазы, чем самим трипсином.

6. Для экспериментального создания геморрагического панкреатита у собак рекомендуется применять кристаллическую эластазу, не содержащую примесь трипсина.

John J. MOLNÁR
Iral J. SCHNEIDER
Seymour TINDEL
Daniel SHAPIRA
David STATE

Dep. of Path. and Surgery, Albert Einstein College of Medicine — Bronx Municipal Hospital Center, New York, N. Y. 10461

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 223—236, 1968*

Division of Anatomical Pathology and Pathology Institute, Dalhousie University, Halifax, Nova Scotia, Canada

THE POSTNATAL TRANSFORMATION OF THE PINEAL GLAND*

N. A. KERÉNYI and K. SARKAR

(Received July 29, 1967)

Postnatal transformation of the pineal gland has been shown on human material. The transformation begins at birth and is complete at 2—3 weeks of age, producing a characteristic mosaic arrangement. The mosaic pattern becomes less prominent at about six months and disappears completely at 9—12 months. An analogy has been noted between the postnatal transformation of the pineal gland and the postnatal involution of the foetal zone of the adrenal cortex. Although the two processes are different in morphogenesis they show a striking parallelity in regard to their progress, being related to the age of the infant as well as to certain pathological conditions such as congenital cyanotic heart disease. The authors propose to use this phenomenon as an experimental model to facilitate a better understanding of the postnatal transformation of the pineal gland.

Philosophers and scientists have been trying for two thousand years to give the pineal gland a metaphysical or a physiological role. Considering the smallness of the organ, it is surprising how much space it has occupied in both speculative and scientific literature. Histological as well as physiological conceptions in regard to this organ have frequently changed. During a span of more than twenty centuries, the theories about the epiphysis reflect the leading ideas of medical science, often under the influence of philosophical conceptions. First, it was stated that the epiphysis functions as an organ of meditation enabling man to remember his past life. According to HEROPHILOS of Alexandria [1], the pineal gland acts as a sphincter controlling the stream of thoughts. GALENO in 1562 [2] described the epiphysis and he regarded this organ as a gland with internal secretion. DESCARTES [3] saw in the pineal the noblest of all organs, the seat of the soul. MAGENDIE [4] suggested that the epiphysis is an organ for reflex regulation of the cerebrospinal fluid and of intracranial pressure. The pineal was also considered as a rudimentary organ, supposedly of sensory nature and related to the third eye of Lacertidae. In 1898, Otto HEUBNER [5] published a case report of a young boy who had shown precocious puberty and was found to have a pineal tumour. At the beginning of this century, MARBURG [6] was the first to suggest that the mammalian epiphysis would have an endocrine function related to the development of sexual organs. According to MARBURG, the epiphysis inhibits sexual develop-

* This work is dedicated to Professor Joseph BALÓ on the occasion of having completed five decades in the service of science.

ment. At the time of puberty, atrophy of the pineal gland begins and sexual maturation takes place. This theory was somewhat supported by the fact that tumours destroying the epiphysis were often associated with precocious puberty. There were observations to the contrary, however, and countless experiments either administering pineal extracts or, at the other extreme, performing pinealectomy, failed to yield conclusive evidence one way or another [4]. Standard endocrine tests designed to determine the possible glandular functions of the pineal were also inconclusive.

A review of the literature on pineal tumours by KITAY [7] showed that most of the tumours associated with precocious puberty were not really pineal in origin but were either tumours of supporting tissues or teratomas. On the other hand, tumours associated with delayed puberty were, in most cases, true pineal tumours. Both BARGMANN [8] and HORÁNYI [9] have demonstrated that the epiphysis does not necessarily atrophy after puberty; it has also been stated that the appearance of calcification does not alter the function of the pineal gland [10].

In 1958, LERNER et al. [11] achieved a major breakthrough. An earlier observation by MCCORD and ALLEN [12] that bovine pineal extract would blanch the skin of the tadpole, was re-examined by LERNER's group, and as a result they isolated melatonin; the structure of this compound is N-acetyl-5-methoxytryptamine. Melatonin produces a contraction of the pigment granules in melanocytes. In 1960, AXELROD and WEISSBACH [13] discovered the enzyme hydroxyindole-O-methyltransferase and showed this to be responsible for catalyzing the transformation of N-acetyl serotonin to melatonin. In mammals, only the pineal gland can synthesize melatonin and hydroxyindole-O-methyltransferase is found to be present only in the pineal gland. Melatonin is an antagonist of the melanocyte-stimulating hormone (MSH) [14].

The pineal suddenly became a centre of interest. The number of publications dealing with different functions of the epiphysis multiplied with remarkable speed. It was demonstrated that the minimum rate of rat pineal blood flow per gram exceeds all endocrine organs with the exception of the neurohypophysis [15]. Experiments have indicated that the pineal gland synthesizes serotonin during the day and melatonin at night [16]. It was demonstrated that the pineal gland probably secrets a substance that inhibits some aspects of gonadal function [17]. In the past few years, the epiphysis has become known to be a light-dependent biologic clock that, amongst other functions, exercises control over sexual maturation in mammals [18]. The epiphysis was identified as a fourth neuroendocrine transducer, over and above the previously recognized three neurosecretory systems (hypothalamus — posterior pituitary, hypothalamus — anterior pituitary, adrenal medulla) [19].

This fast progress in discovering the functions of the pineal gland was in no way matched by any significant amount of new information as far as the

morphology of the pineal gland is concerned. With the exception of a recent study by GYORKEY et al. [20], practically no attempt has been made to correlate the biochemical findings with the morphology of the gland.

Examination of the human epiphysis is one of the most neglected subjects in pathology. Even in otherwise well completed autopsies, the pineal is often not examined. Since the detailed study of RIO-HORTEGA in 1932 [4] few data have been published [1, 9, 21] and none of these took notice of an extremely important morphological feature; namely, the postnatal transformation of the pineal gland. The excellent work of GLOBUS and SIBERT [22] touches upon the phenomenon, and Dorothy RUSSELL [23] also mentions it in her textbook on tumours. The most detailed textbooks or recent complete reviews of the morphology of the epiphysis fail even to mention this important morphological feature [1, 24, 25].

This striking paucity of information and the continuously growing recognition of functions of this important organ have prompted our investigations. Their main purpose was to throw fresh light on the morphological appearance of the pineal gland of the newborn and the infant, with particular emphasis on the postnatal transformation of the gland.

Materials and methods

Five hundred unselected pineal glands from all age groups, including 100 from neonates and infants under one year of age, were examined. All material was fixed in buffered formaldehyde. Each gland, whenever feasible, was divided, either vertically or horizontally; one half was embedded in paraffin and the other half was used for the preparation of frozen sections. This, of course, often could not be carried out in the newborn and foetal epiphyses, where a decision had to be made either for embedding *in toto*, or using the whole gland for frozen section. In a number of cases, ultra-thin sections were also examined. A few of the foetal and postnatal glands were cut and studied serially. The following staining methods were used: Haematoxylin-eosin, phosphotungstic acid-haematoxylin, PAS, Prussian blue reaction, Masson's trichrome, Reticulin, van Gieson, Verhoeff's elastin, von Kossa, Cajal's stain, Hortega's and Bielschowsky's silver impregnation method.

Results

The shape of the neonatal pineal gland in the first few weeks of life is rather irregular, and its relative size is much smaller than that of other endocrine organs at the same age. Its dimensions are usually less than 0.3 cm except the length which may be 0.3—0.6 cm. The pineal gland in neonates is situated deep in a hollow, bounded by the corpora quadrigemina. No habenulae can be demonstrated and the gland is somewhat similar to a sessile skin tag with poor demarcation towards the base. In the first six months of life the shape of the pineal gland gradually becomes ovoid. However, the adult gross appearance with the habenulae can hardly ever be demonstrated before one and a half years of age.

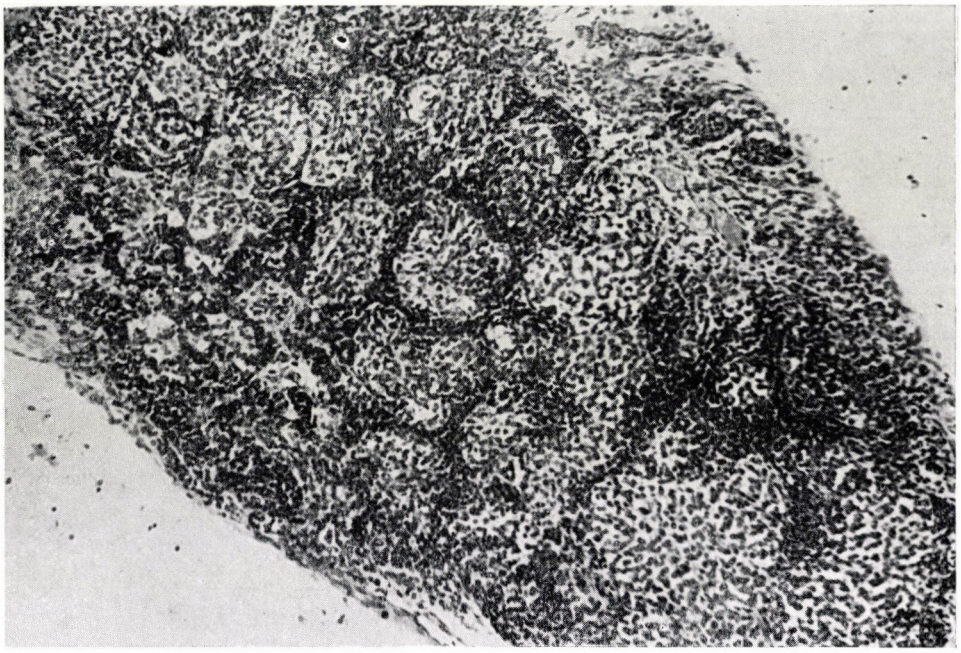


Fig. 1. Section of pineal body of infant at three weeks illustrating the so-called mosaic appearance. Haematoxylin-eosin × 125

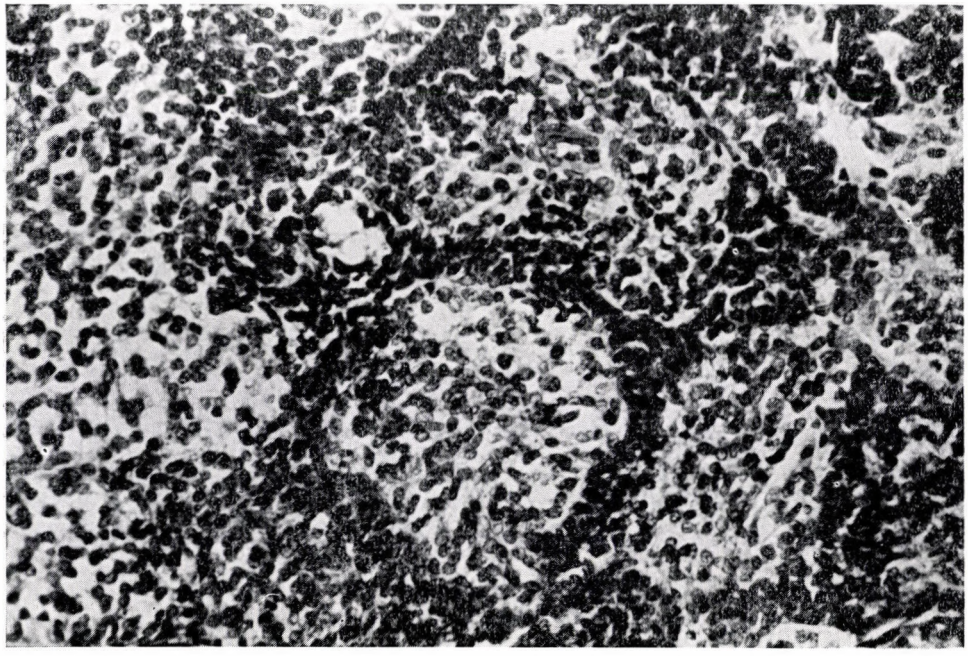


Fig. 2. Higher magnification of the section of the pineal body shown in Fig. 1 to illustrate the structure of "mosaic appearance". Haematoxylin-eosin × 315

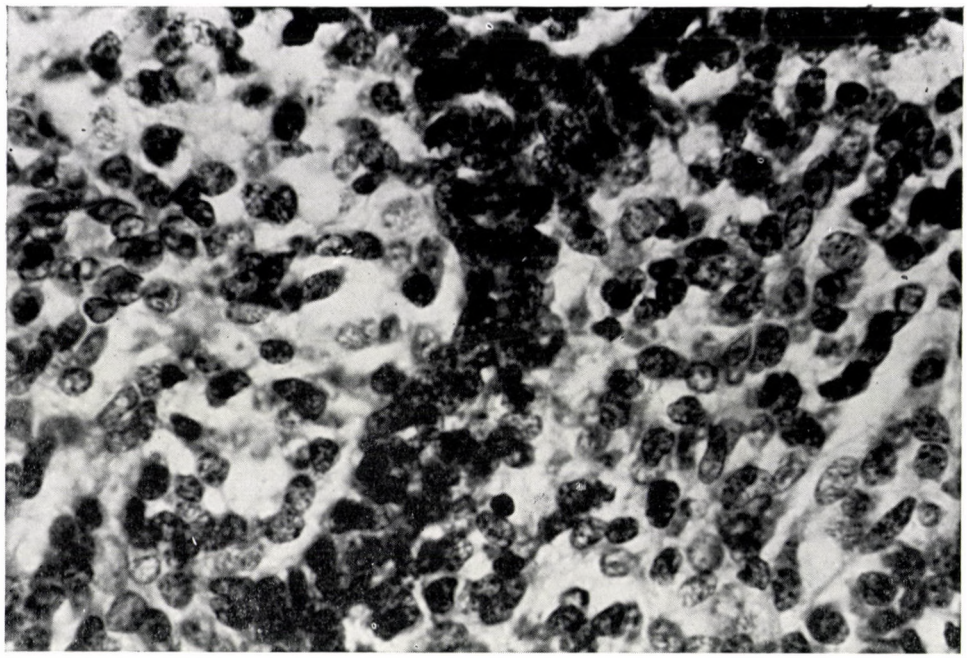


Fig. 3. Higher magnification of the section of pineal body shown in Figs 1—2, to demonstrate cellular details. Haematoxylin-eosin × 800

Fig. 4. Section of pineal body of infant six months of age to show the somewhat less marked but still preserved mosaic pattern. Small cyst-like structure filled with thin PAS positive material is seen at the left margin. Haematoxylin-eosin × 125

The architectural changes observed by microscopical examination are even more striking. The first few days of postnatal life are marked by profound alterations in the histology of the pineal gland. A characteristic pattern of clear areas surrounded by narrow zones or bands of deeply staining cellular elements slowly emerges. Although this pattern is not present at the time of birth it invariably develops by the end of the third postnatal week. The development of this so-called mosaic appearance is independent of the maturity

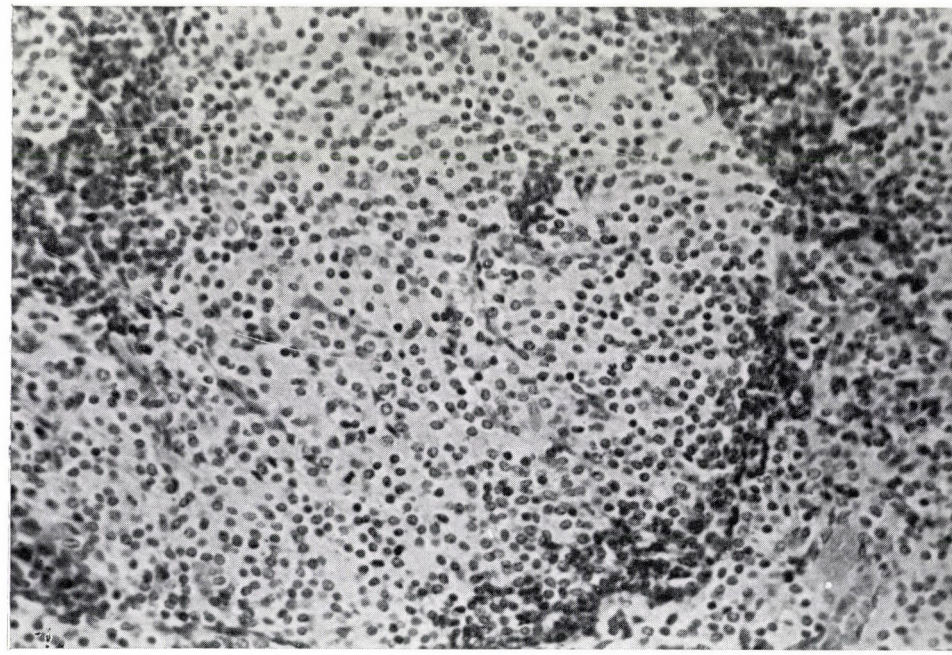


Fig. 5. Higher magnification of pineal body shown in Fig. 4, to demonstrate the still preserved lines of the relatively smaller, strongly chromophilic cells separating the groups of central paler and larger cells. Haematoxylin-eosin × 315

of the newborn and it develops in surviving premature infants as well as in fully mature neonates. The mosaic appearance is due to the aggregation of rather large and pale cells into round and polygonal masses, which are separated from one another by anastomosing narrow bands of densely packed smaller cells with chromatin-rich nuclei. The majority of the nuclei of the pale cells is irregularly ovoid in shape, and the cells are loosely distributed. On the other hand the peripheral bands of smaller cells are closely packed with chromatin-rich nuclei and with very little cytoplasm (Figs 1 to 3). These smaller cells are somewhat similar in size and in appearance to lymphocytes. There are, however, transitional forms between the large pale cells in the centre and the small chromatin-rich cells at the periphery. The mosaic appearance is fully

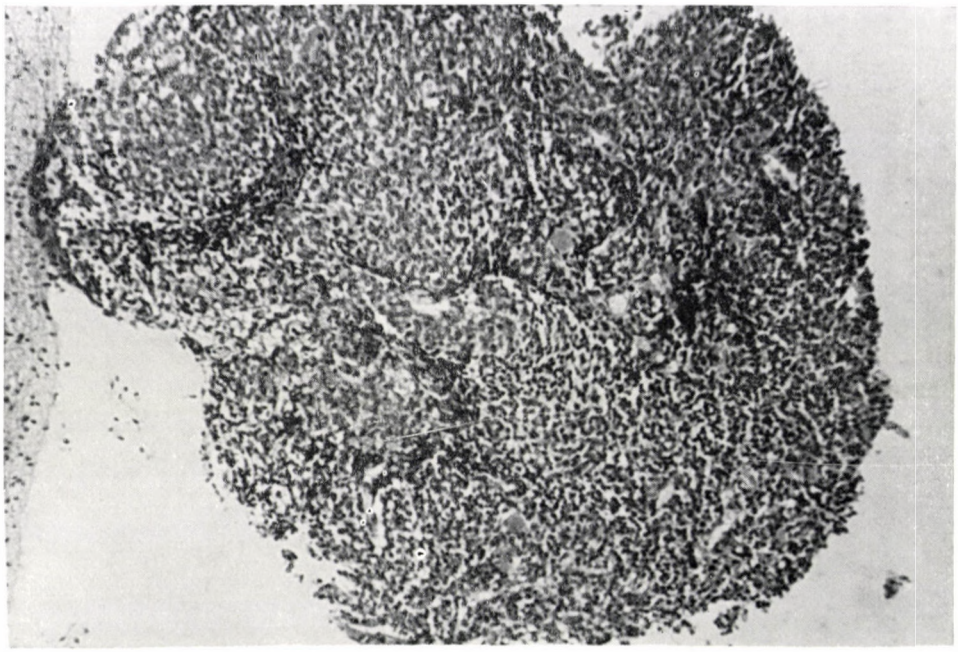


Fig. 6. Section of pineal body of an infant aged nine months illustrating the almost complete loss of the mosaic arrangement through a marked decrease in the number of the small dark cells. Haematoxylin-eosin × 125

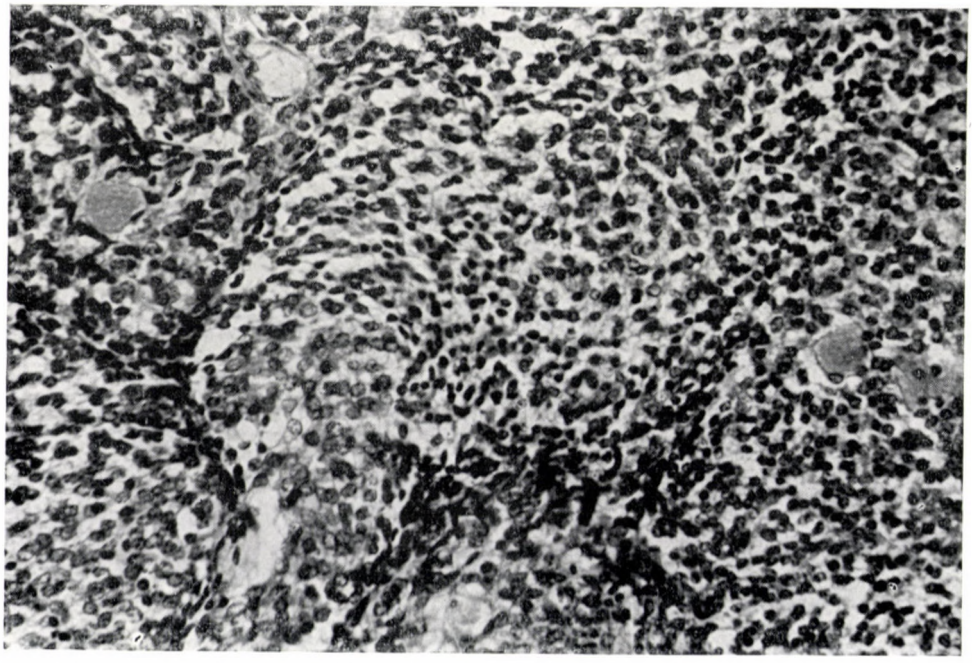


Fig. 7. Higher magnification of the section of pineal body shown in Fig. 6 to demonstrate the break-up of the mosaic arrangement. Haematoxylin-eosin × 315

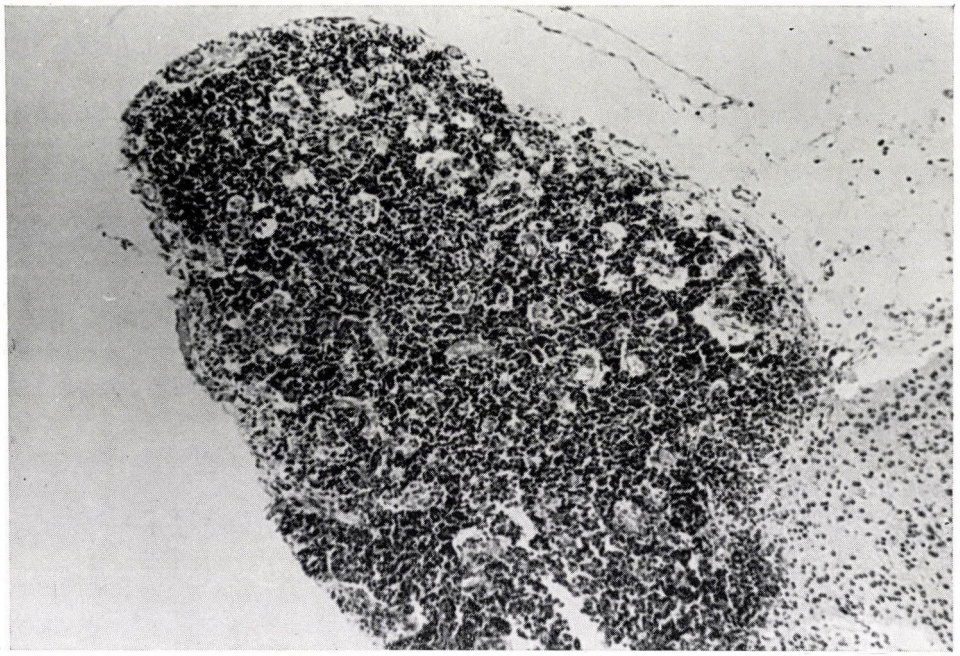


Fig. 8. Section of pineal body of a newborn at 24 hours showing the absence of the mosaic arrangement. Haematoxylin-eosin × 125

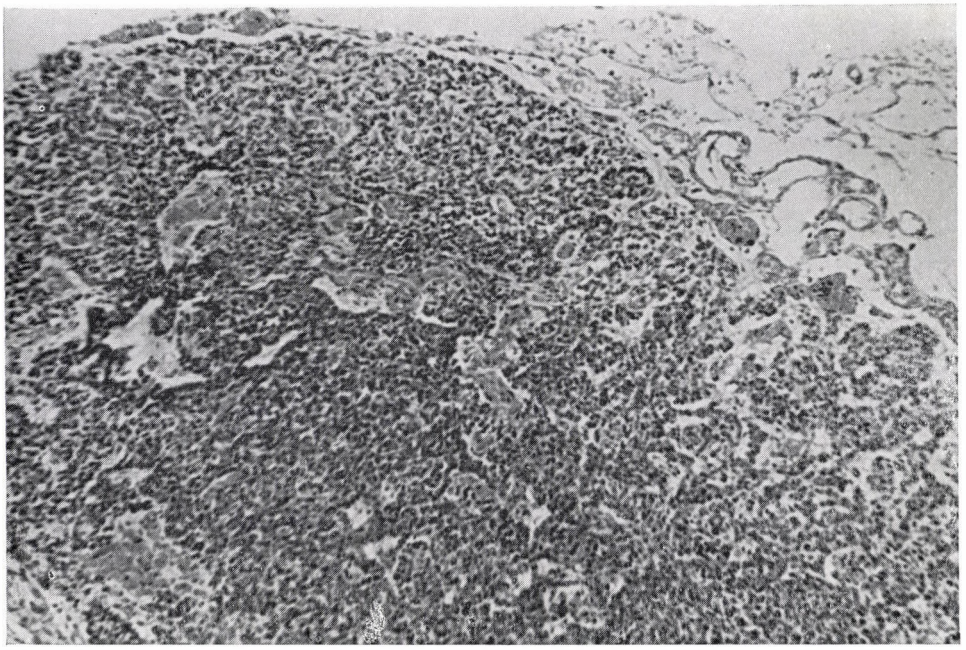


Fig. 9. Section of pineal body of an infant with cyanotic congenital heart disease, aged four weeks. The mosaic arrangement is absent. Haematoxylin-eosin × 125

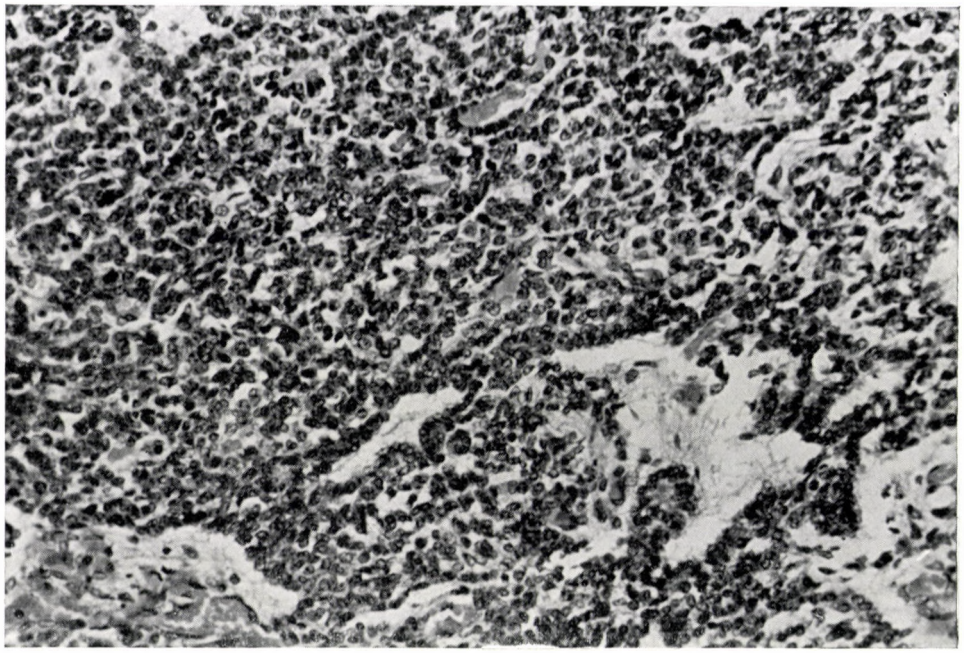


Fig. 10. Higher magnification of the section of pineal body shown in Fig. 9 to demonstrate irregular gland-like spaces. Haematoxylin-eosin × 315

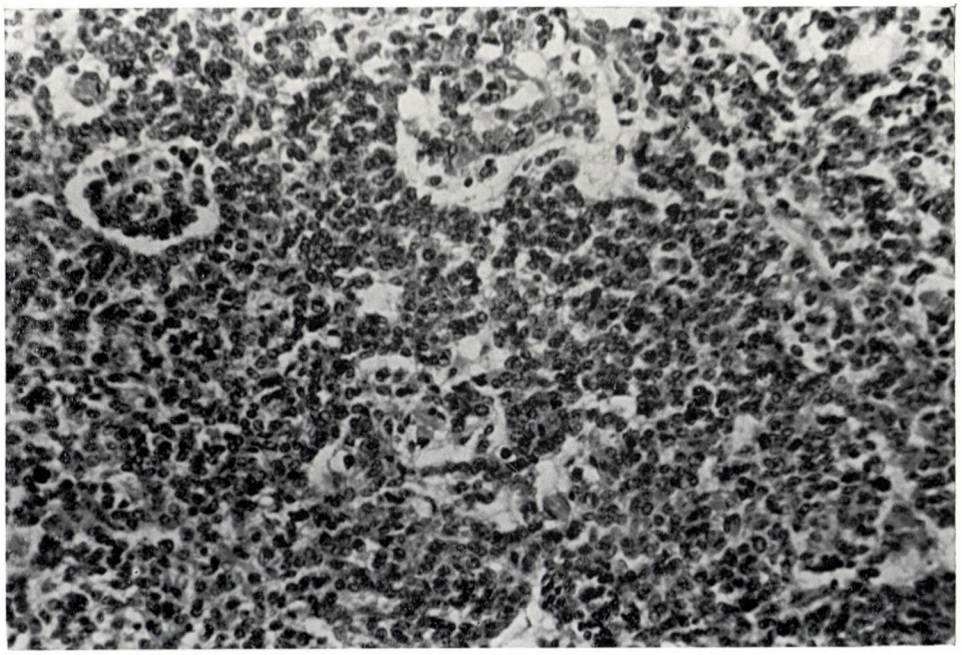


Fig. 11. Higher magnification of the section of pineal body shown in Fig. 9, to demonstrate a glomerulus-like structure. Haematoxylin-eosin × 315

developed by three weeks of age and it is very well preserved until approximately 6 months of age, at which time the pattern is gradually becoming less well-defined (Figs 4—5). By 9 months of age there is an almost complete loss of the mosaic arrangement. The number of the small dark cells is markedly decreased. The aggregates of the larger and paler cells in a few areas are still partially fringed by semilunar bands but in other areas the difference between the two cell groups has completely disappeared. The small cells seem to assume

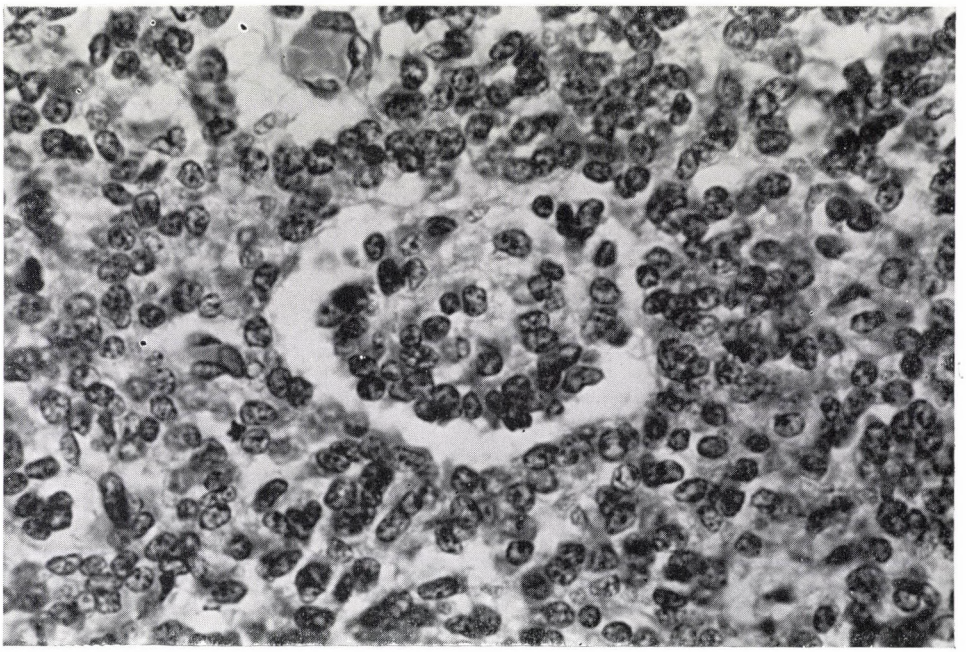


Fig 12. Higher magnification of the section shown in Fig. 11, to illustrate details of the "glomeruloid structure". Haematoxylin-eosin × 800

a new character, becoming elongated and more and more similar to fibroblasts. The pale, large cell aggregates on the other hand are becoming smaller and more chromophilic (Figs 6—7). In contrast to adult pineals where both capillaries and small arterioles are present, only capillaries are seen in the neonatal pineal gland. They are easily observed within the central pale cell conglomerates, but their presence in the darker zone is doubtful. As the parenchymal cells mature they develop thin argyrophil cytoplasmic processes. In the perikaryon clusters of tiny rods and dots resembling the blepharoblasts of ependymal cells are demonstrable by phosphotungstic-acid haematoxylin staining. A moderate number of fibrillary astrocytes are also present. At about 18 months of age the characteristic adult type of architecture is becoming more and more apparent. At 2½ to 6½ years of age the general topography of the mature pineal

gland is completely developed. Isolated alveolar nests of cells are separated by connective tissue septa. Corpora acervuli, cyst formation and other morphological signs of "degeneration" begin to appear as early as 3 years of age. The presence of septa, which give the gland a pseudoalveolar or lobulated architecture, varies from one pineal to another and also shows variations within the same gland. In most cases the septa are thicker in the distal (posterior) area than in the basal (anterior) part; the lobulation is also more distinct in the cortical (peripheral) zone than in the medullary (central) zone. In three pineal glands from blind subjects the interlobular septa were very thin or absent. Although the small number examined does not allow any firm conclusion, this observation seems worth recording.

As mentioned previously, the mosaic arrangement is absent at the time of birth (Fig. 8) and invariably develops by three weeks of age in normal or premature infants. This development is markedly delayed in infants with congenital cyanotic heart disease. The mosaic arrangement in these cases may still be absent as late as 4 weeks after birth. The pineal gland of the newborn with congenital heart disease shows irregular gland-like spaces or "glomeruloid" structures (Figs 10 to 12).

Discussion

The postnatal transformation of the pineal gland is a well-defined morphological process which according to our observations takes place on a predetermined schedule. The development of the so-called mosaic arrangement starts at dramatic speed immediately after birth. The transformation is not dependent on maturity because it starts at once in both premature and fullterm infants. The characteristic mosaic pattern will be demonstrable in most cases in the first post-natal week and the mosaic arrangement will be fully developed by three weeks of age. The appearance shows little change from the first to sixth months. The epiphyses at the above period are characterized by usually round conglomerates of larger, loosely arranged, less chromophilic cells, separated by continuous anastomosing bands of smaller, densely packed cells, with chromatin-rich nuclei and a narrow rim of cytoplasm. Although these small cells resemble lymphocytes and appear to be quite distinct from the central groups of cells, they probably represent immature forms of the larger cells and transitions can be demonstrated between the two forms.

After six months of age the continuity of the small anastomosing bands of cells is beginning to break up. They gradually become larger, elongated, lighter staining and somewhat similar to the central cells. By the end of 9 months, only remnants of the mosaic structure are left.

It is extremely interesting to note the analogy between the above described postnatal transformation of the pineal gland and the postnatal involution

of the foetal adrenal cortex. This is not an analogy in morphogenesis but an analogy regarding the sequence of events that takes place immediately after birth. The involution of the foetal adrenal cortex starts at birth, irrespective of the maturity of the neonate. The involution is more or less complete within three weeks. Remnants of the foetal cortex, however, can be demonstrated up to 9—12 months. LANMAN [26] has reported that in infants with cyanotic congenital heart disease, there is a delayed involution of the foetal adrenal cortex. As demonstrated above, a similar delay of postnatal transformation of the pineal gland occurs in infants with cyanotic congenital heart disease.

There are further evidences that both adrenal cortex and medulla may be related to the pineal gland. In rats reared in darkness a progressive activation of the epiphysis and of the glomerular zone of the adrenal cortex has been demonstrated. There is a remarkable similarity in many ways between the pineal organ and the adrenal medulla as regards structure and function; both are of neural origin and have a characteristic cell type that receives autonomic innervation; both receive a high fraction of the cardiac output and contain pharmacologically active amines as well as an almost unique methylating enzyme producing epinephrine in the adrenal and melatonin in the pineal [1].

The postnatal transformation of endocrine glands is poorly understood. The central nervous system seems to play an important role in the development of the foetal zone of the adrenal cortex. The foetal zone is absent in anencephalic monsters and it is also absent in cases of "anencephalic type" of hypoplasia of the adrenal [27]. Efforts at a better understanding of this phenomenon were frustrated by the difficulties of finding suitable experimental animals. Amongst mammals only the primates and larger felidae are reported to have a foetal adrenal cortex similar to that in man [26]. The postnatal transformation of the pineal gland occurs also in rodents and it can be studied in commonly used experimental animals. Considering the close relationship between light stimulation and the function and morphology of the pineal, the authors intend to conduct experimental studies, trying to influence the postnatal transformation of the pineal gland by keeping the experimental animals in constant darkness using a variety of methods (enucleation, suturing the eyes, dark room without surgical interference). The characteristic mosaic arrangement of the postnatal transformation of the pineal gland lends itself to an easy evaluation using light microscopy and simple staining procedures.

REFERENCES

1. KAPPERS, J. A., SCHADE, J. P.: (1965) Progress in Brain Research. VI. 10. Structure and Function of the Epiphysis Cerebri. Elsevier, Amsterdam. — 2. GALENO: (1562) De usu partium corporis humani. Venezia. — 3. DESCARTES, P. (1649) Traite des passions de l'âme. — 4. RIO-HORTEGA, P. DEL.: (1932) In: Cytology and Cellular Pathology of the Nervous System. Ed. W. Penfield. (Section XIV). Hoeber, New York. — 5. HEUBNER O.: (1898) Tumor der Glandula pinealis. Dtsch. med. Wschr. **24,** 214. — 6. MARBURG, O.: (1908) Zur Kenntnis der normalen und pathologischen Histologie der Zirbeldrüse: Die Adipositas cerebralis. Arb. neurol. Inst. Univ. Wien **17,** 217. — 7. KITAY, J. T.: (1954) Pineal Lesions and Precocious Puberty. A Review. J. Clin. Endocr. **14,** 622. — 8. BARGMANN, W.: (1943) Die Epiphysis cerebri. In: Mollendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen. Springer, Berlin. — 9. HORÁNYI, B.: (1966) A corpus pineale szerkezete és működési jelentősége. Orv. Hetil. **107,** 1585. — 10. DEMARTINO, C., TONIETTI, G., ACCINNI, L.: (1964) Electron Microscopic Study of Pubertal and Adult Rats' Pineal Body. — 11. LERNER, A. B., CASE, J. D., TAKAHASHI, Y., LEE, T. H., MORI, W.: (1958) Isolation of Melatonin, Pineal Gland Factor that Lightens Melanocytes. J. Amer. chem. Soc. **80,** 2587. — 12. MCCORD, C. P., ALLEN, F. P.: (1917) Evidences Associating Pineal Gland Function with Alteration in Pigmentation. J. exp. Zool. **23,** 207. — 13. AXELROD, J., WEISSBACH, H.: (1960) Enzymatic O-methylation of N-acetylserotonin to Melatonin. Science **131,** 1312. — 14. NOVALES, R. R., NOVALES, B. J.: (1965) Analysis of Antagonism between Pineal Melatonin and other Agents which Act on Amphibian Melanophore. Progr. Brain Res. **10,** 507. — 15. GOLDMAN, H., WARTMAN, R. J.: (1964) Flow of Blood to Pineal Body of Rat. Nature (Lond.), **203,** 87. — 16. RELKIN, R.: (1966) The Pineal Gland. New Engl. J. Med. **274,** 944. — 17. WURTMAN, R. J., AXELROD, J., CHU, E. W.: (1963) Melatonin, Pineal Substance: Effect on Rat Ovary. Science **141,** 227. — 18. QUAY, W. B.: (1964) Circadian and Estrous Rhythms in Pineal Melatonin and 5-hydroxyindole-3-acetic acid. Proc. Soc. exp. Biol (N. Y.) **115,** 710. — 19. WURTMAN, J. W., AXELROD, J.: (1965) The Pineal Gland. Sci. Amer. **213,** 50. — 20. GYORKEY, F., MIN, K-W., OTANI, T., FARREL, G., GYORKEY, P.: (1967) The Human Pineal Body: Histochemical, Biochemical and Ultrastructural Observations. Sixty-fourth Annual Meeting of the American Association of Pathologists and Bacteriologists. P. 14. — 21. KITAY, J. T., ALTSCHULE, M. D.: (1954) The Pineal Gland. Harvard Univ. Press, Boston, Mass. — 22. GLOBUS, J. H., SIBERT, S.: (1931) Pinealomas. Arch. Neurol. Psychiat. **25,** 937. — 23. RUSSELL, D. S., RUBINSTEIN, L. J.: (1963) Pathology of Tumours of the Central Nervous System. Arnold, London. — 24. LIU, N.: (1966) Some Aspects of Endocrinology in the Fetus and the Newborn. Pediat. Clin. N. Amer. **13,** 1047. — 25. DANOWSKY, T. S.: (1962) Clinical Endocrinology Vol. I. Williams and Wilkins, Baltimore. — 26. LANMAN, J. T.: (1953) The Fetal Zone of the Adrenal Gland. Medicine **32,** 389. — 27. KERENYI, N.: (1961) Congenital Adrenal Hypoplasia. Arch. Pathol. **71,** 336.

POSTNATALE TRANSFORMATION DER ZIRBELDRÜSE

N. A. KERÉNYI und K. SARKAR

Die postnatale Transformation der Zirbeldrüse wurde an menschlichen Material untersucht. Sie beginnt bei der Geburt und ist im Alter von 2—3 Wochen vollendet, wobei eine charakteristische Mosaikstruktur entsteht. Das Mosaikmuster ist im Alter von etwa 6 Monaten weniger merkbar, und im Alter von 9—12 Monaten verschwindet es vollkommen. Zwischen der postnatalen Transformation der Zirbeldrüse und der postnatalen Involution der fötalen Zone der Nebennierenrinde wurde eine Analogie festgestellt. Obwohl die beiden Prozesse morphogenetisch verschieden sind, zeigen sie eine auffallende Parallelität ihrer Progression mit dem Alter des Kindes sowie auch auf gewissen pathologischen Verhältnissen wie z.B. der Zyanose bei angeborenen Herzfehlern.

Die Erscheinung wird als experimentelles Modell zum besseren Verständnis der postnatalen Transformation der Zirbeldrüse empfehlen.

ПОСЛЕРОДОВОЕ ПРЕОБРАЗОВАНИЕ ШИШКОВИДНОЙ ЖЕЛЕЗЫ

Н. А. КЕРЕНЬИ и К. ШАРКАР

Авторы выявили на человеческом материале послеродовое преобразование шишковидной железы, начинающееся при рождении и кончающееся в 2—3-недельном возрасте, причем получается характерное мозаичное строение. В около 6-месячном возрасте мозаичность менее заметна и к 9—12-месячному возрасту она совершенно исчезает. Между послеродовым преобразованием шишковидной железы и послеродовой инволюцией фетальной зоны коры надпочечников была выявлена аналогия. Хотя эти процессы в отношении морфогенеза различны, они показывают поразительный параллелизм в отношении прогрессирования, как с точки зрения возраста детей, так и отдельных патологических условий, напр., врожденных пороков сердца с ранним цианозом.

Авторы предлагают использовать этот феномен в качестве экспериментальной модели для выяснения послеродового преобразования шишковидной железы.

N. A. Kerényi; Division of Anat. Path. Dalhousie Univ., Halifax, Nova Scotia, Canada

K. Sarkar; Resident Physician, Pathology Inst., Dalhousie Univ., Halifax, Nova Scotia, Canada

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 237—242, 1968*

Department of Dermatology (Director: L. Szodoray), University Medical School, Debrecen

SULPHATASE ACTIVITY IN HUMAN EPIDERMIS AND VARIOUS TUMOURS

Klára Vezekényi

(Received May 15, 1967)

In normal skin the live layers up to the stratum granulosum show uniform sulphatase activity while that of the stratum corneum is weak. The activity in eccrine perspiratory glands and follicles is high, in sebaceous glands very high. In capillary endothel weak, in inflammatory tissue elements intensive activity was observed. Sulphatase activity in benign epithelial tumours and basalioma was weak, in malignant epithelial tumours, intensive. Connective tissue tumours behaved differently. Tumours of neural origin and pigment tumours displayed high activity. Pigmented tumour cells and melanophages were sulphatase negative.

The localization and role of enzymes acting on various sulphate esters in plant, animal and human tissues have been studied by numerous authors. As to sulphatase activity in human epidermis and related tissues, however, only the paper of Hewitt et al. [5] was available. Among the enzymes belonging to this group, phenyl and arylsulphatases are detectable by histochemical methods. Roy [9] and Dogson et al. [3] differentiated three types of sulphatase. The occurrence of these enzymes varies in the different animal species. It is supposed that enzyme types A and B are lysosomal, while type C is microsomal, in origin. According to Roy [11] the three types cannot be differentiated histochemically, as they all act on the same substrate. Woohsmann and Brosowski [14] claimed that the use of various inhibitors makes it possible to distinguish the three types.

Huggins and Smith [6] have shown that the concentration of arylsulphatases is 6 to 8 times higher in rat tumours than in normal tissues. The purpose of the present experiments was to study sulphatase activity in normal skin and in tumours originating from different dermal elements.

Materials and methods

A total of 97 specimens removed in local lidocaine anaesthesia was examined: 6 normal skin specimens, 5 specimens from inflammatory conditions (scleroderma, lichen ruber planus, vasculitis nodularis) and 86 specimens from different tumours. The diagnoses are shown in Table I.

Estimation of sulphatase activity was performed by the azo-dye method of Rutenberg et al. [12]. As formalin fixation inhibits sulphatase activity in human tissues, 15 to 20 μ thick frozen sections were prepared. As human tissues do not split benzoyl-naphthyl sulphate [4], potassium 6-bromo-2-naphthyl sulphate (Mann Research Laboratories, New York) was used

at pH 6.1. Diffusion was prevented by supplementing the incubation medium with sodium chloride. The optimal incubation time was 2 hours at 37° C. Coupling of the azo dye was performed at 4° C in 25 ml 0.05 *M* phosphate buffer pH 7.6 containing 25 mg Diazo Blue B [7].

High sulphatase activity was indicated by a blue colour reaction. Weak reactions were characterized by a red colour. Sometimes the reaction product dissolved in lipids with red colour.

Control specimens incubated without substrate gave no colour reactions.

Table I

Distribution of specimens

Benign and malignant epithelial tumours		*Melanotic tumours*	
Condyloma acuminatum	1	Naevus pigmentosus	8
Papilloma	2	Melanoma	5
Naevus verrucosus	1		
Naevus sebaceous	1	*Connective tissue and other tumours*	
Verruca seborrhoica	9		
Keratoacanthoma	6	Fibroma	3
Keratoma seniel	2	Fibrosarcoma	1
Basalioma	15	Histiocytoma	3
Carcinoma spinocellulare	16	Neuroma	1
Carcinoma mixtum	1	Neurofibroma	1
Carcinoma medullare	1	Haemangioma	2
Metatypic carcinoma	2	Granuloma fungoides	1
Bowen type carcinoma	1		
Adenocarcinoma	1		
Hidradenoma	1		
Carcinoma mammae	1		

Results

Normal skin and inflammatory conditions. The stratum corneum showed no or a weak activity. In hyperkeratosis in the so called intermediate zone was the strongest reaction observed. In other (from the basal to the granular) layers a similar blue colour reaction appeared in the cytoplasm (Fig. 1). Within the cytoplasm activity was not uniform: two active zones were situated around the nucleus and beside the cell membrane. Under high power the intracellular bridges seemed to react positively. The basal layer reacted weakly or not at all if melanin was present in larger amounts. In acanthosis no significant changes were observed.

Among esodermal elements activity was highest in the sebaceous glands, especially in peripheral undifferentiated cells but differentiated cells, too, were

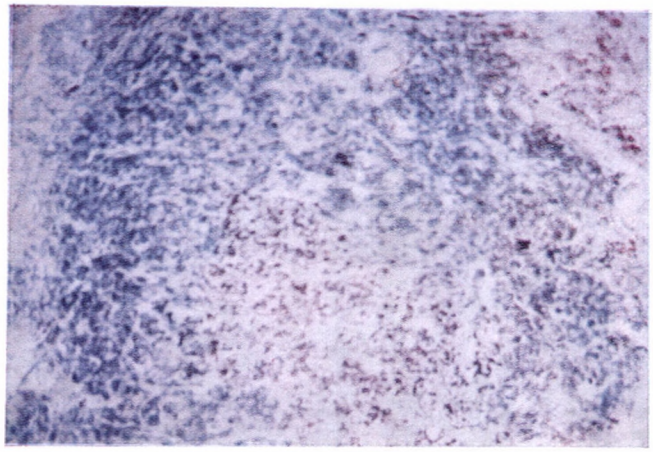


Fig. 9. Melanoma metastasis in lymph node. High sulphatase activity in the tumour cells

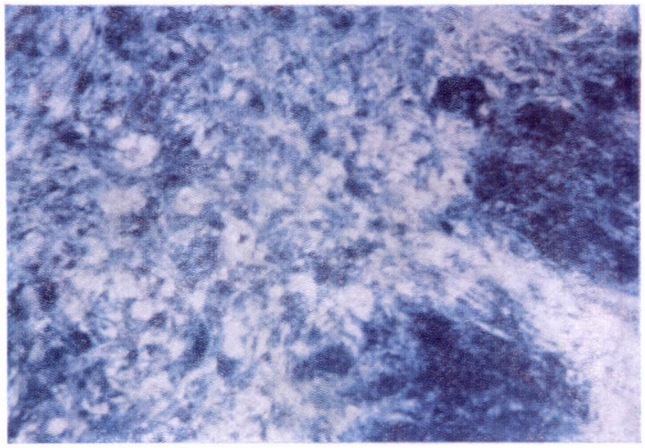


Fig. 10. Strong reaction in cellular elements of histiocytoma

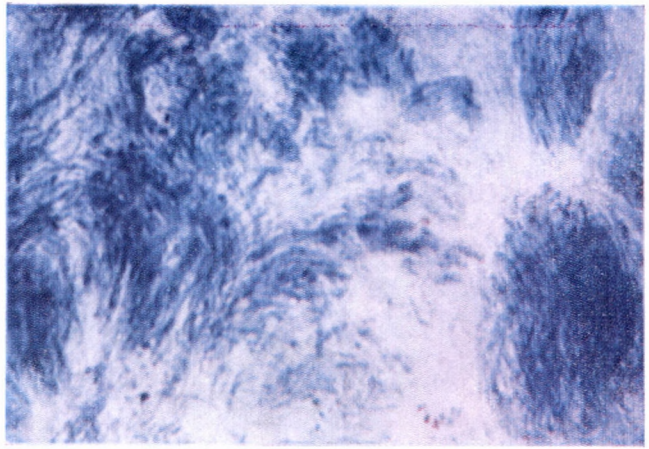


Fig. 11. High sulphatase activity in proliferating neuroma nerve fibres

definitely positive. The red colour in the centre of the lobes was probably caused by dissolved reaction products. Sometimes the structure of the gland was indistinguishable owing to the intensive colour reaction (Fig. 2).

The outer root sheath of follicles showed higher activity than the epithelium. The hair shaft was mostly stained red.

Sulphatase activity in sweat glands (Fig. 3) was somewhat higher than in the epithelium. Secretory cells of the alveoli contained coarser, the cells lining the ducts finer, blue granules. A faint diffuse staining was observed in unstriated muscles and in the endothelium of capillaries. Cellular elements in inflammatory infiltrations showed high activity. Connective tissue fibres and cells never contained sulphatase under normal conditions.

Benign and malignant epithelial tumours. In condyloma acuminatum sulphatase activity was somewhat higher than in normal epithelium. Papillomas and naevus verrucosus behaved as the normal epithelium. Sebaceous gland lobes in naevus sebaceus displayed intensive activity.

In verruca seborrhoeica the basal cell-type epithelial proliferation showed stronger activity than the normal epithelium; the intensity of the reaction was, however, not uniform (Fig. 4). In pigmented areas the reaction was weak; it was stronger around keratinization centres. Keratinized parts were bright red in colour. In keratoacanthoma the epithelial lobes reacted similarly as or somewhat stronger than the normal epidermis, the outer parts stained intensively, in others the cytoplasm contained coarse granules. Keratinous pearls stained red (Fig. 5). Senile keratoma behaved similarly to normal epithelium.

There was no difference in sulphatase activity between solid, adenoid, cystic, superficial and keratotic forms of basalioma. The reaction was sometimes stronger in the outer row of cells (Fig. 6). Highly pigmented parts in pigmented basalioma gave weak or negative reactions.

In spinocellular carcinoma, sulphatase activity was stronger than in normal epithelium, especially in the outer cell rows of tumour nests and in less differentiated areas. The reaction sometimes revealed coarse granules. Keratinous pearls stained red. In a spinalioma developing from the wall of an epithelial cyst the keratinized parts contained cells that maintained their intensive sulphatase activity.

In metatypic carcinoma sulphatase activity was intensive (Fig. 7) and quite especially so in medullary carcinoma. In mixed carcinoma the activity corresponded to the prickle or basal cell character of various parts. In sections prepared from a Bowen type carcinoma high sulphatase activity was revealed. Extremely strong reactions were observed in some larger cells (clumping cells).

In a case of hidradenoma activity was moderate. Tumour cell nests of a rectal adenocarcinoma spreading to the skin showed increased activity and the metachromatic substance produced by the tumour also gave a blue colour reaction (false positivity?).

In a skin metastasis of breast cancer, the tumour cell nests situated among the fibrous bundles of the corium were strongly positive (Fig. 8).

Melanotic tumours. Nevus cells in compound and dermal types of nevus pigmentosus showed high activity. Pigmented nevus cells gave weak or negative reactions. In the cytoplasm of some cells the reaction was gnanular. Melanophage cells were negative.

In melanoma and its metastases (Fig. 9) the tumour cells, with the exception of pigmented ones, exhibited strong activity, sometimes of granular appearance. Eventual segregated cells in the epithelium over the tumour, were well distinguishable from the epithelial cells by their higher activity.

Tumours of connective tissue and other origin. In cell-rich fibromas the reaction was weak and diffuse. A similar diffuse, but stronger reaction was observed in dermatofibrosarcoma protuberans.

The cellular elements in histiocytoma usually displayed an intensive activity (Fig. 10). In a neuroma the tumour tissue composed of fibrous bundles were highly active (Fig. 11).

In neurofibroma the reaction was strong and diffuse. The endothelial lining of cavernous haemangiomas showed weak activity. In the infiltrative stage of granuloma fungoides the cellular elements gave a coarse granular reaction.

Discussion

There are only assumptions as to the physiological role of arylsulphatases. In certain processes the arylsulphates may act as sulphate donors. The problem is rendered more difficult by the fact that of various arylsulphates detected in urine only tyrosine sulphate has been demonstrated in normal tissues [3].

The finding that arylsulphatases are able to sulphatate a number of steroids, especially dehydroepiandrosterone; less intensively androsterone, testosterone, oestrone, pregnanolone, etc. [10] indicates their role in steroid metabolism. For this reason they are often referred to as steroid sulphatases.

A further interesting observation is that the sulphatase activity is higher in the tissues of male than of female animals [2, 14]. According to Huggins, and Smith [6] the highest activity can be observed in liver, spleen and kidney. The degree of sulphatase activity in various organs varies with the animal species.

It has been supposed that arylsulphatase plays a part in sulphatation of polysaccharides [5] although it is not identical with sulphotransferase [16].

The present studies have shown that sulphatase activity can be detected in all cellular elements of the skin. Nuclei of cells and connective tissue are negative. As to normal skin, our results were almost identical with those of Hewitt et al. [5]. The live layers of the epidermis from the basal layer to the stratum granulosum showed uniform positivity. In the stratum corneum activ-

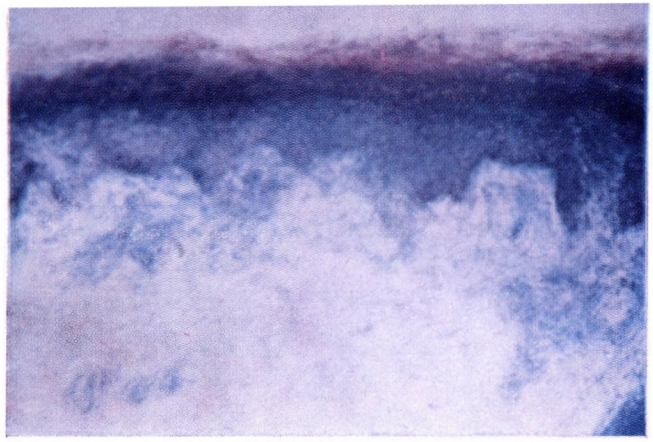


Fig. 1. Uniform distribution of sulphatase activity in normal epidermis from stratum basale to stratum granulosum

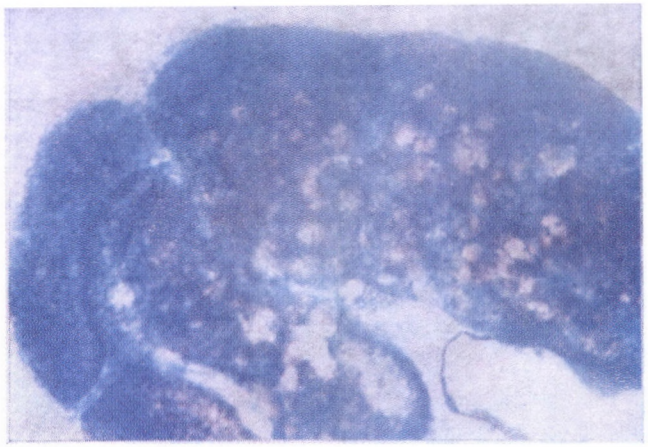


Fig. 2. High sulphatase activity in sebaceous gland

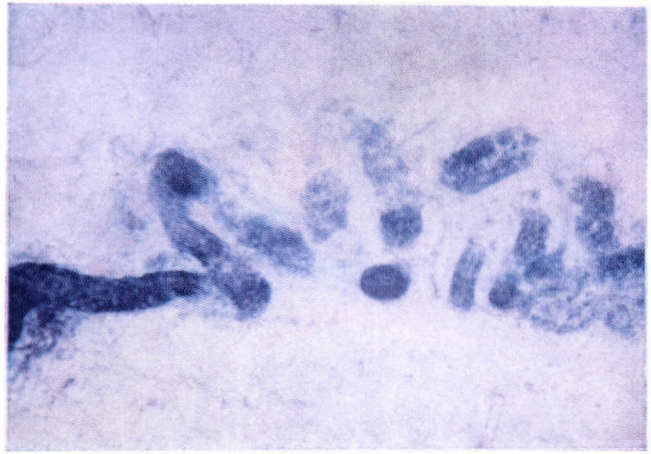


Fig. 3. Sulphatase activity in the alveoli of an eccrine sweat gland

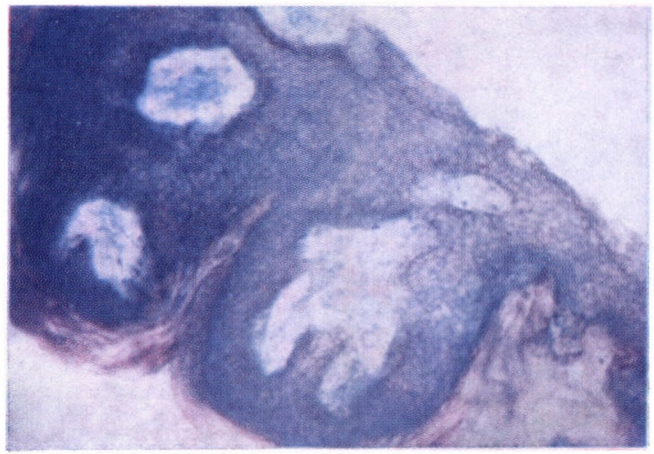


Fig. 4. Uneven reaction in verruca seborrhoeica. The reaction is somewhat stronger than in normal epidermis

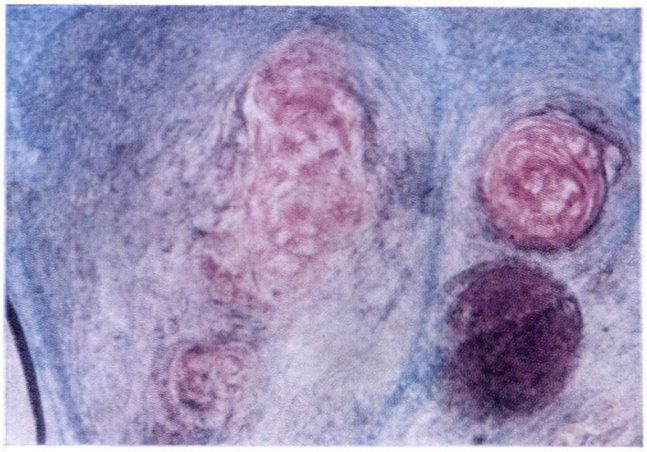


Fig. 5. Sulphatase activity in keratoacanthoma; it is similar as or, in the outer row of cells, slightly more intensive than, in normal epidermis. Keratinous pearls stain red

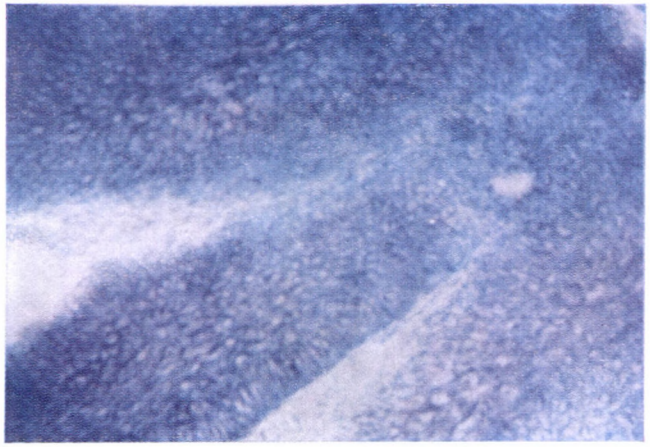


Fig. 6. Sulphatase activity in basalioma

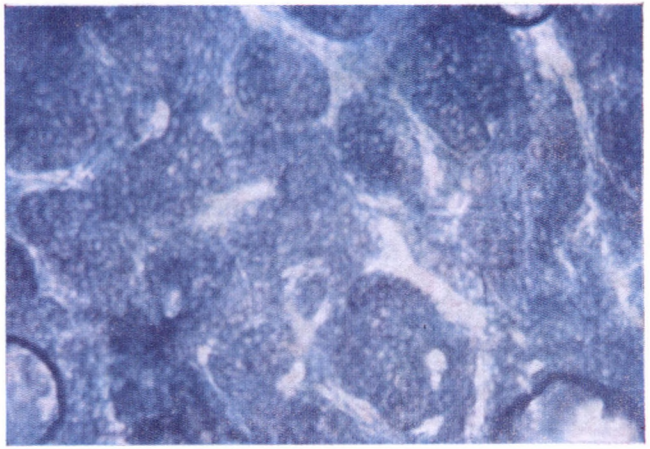


Fig. 7. High sulphatase activity in metatypic carcinoma

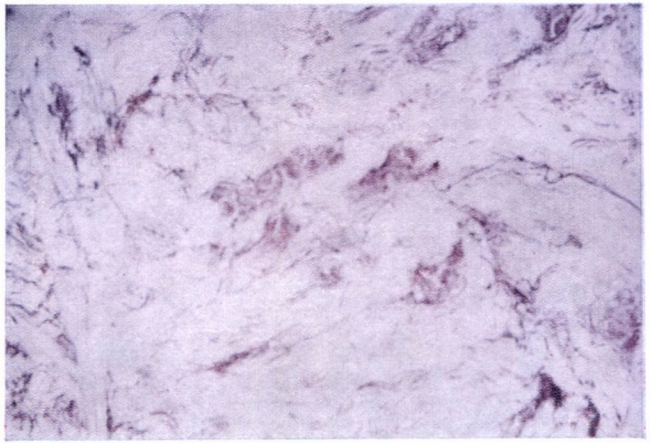


Fig. 8. Sulphatase activity in the tumour cell groups of a skin metastasis of breast cancer

ity was weak. This finding differs from data concerning the epidermal distribution of other hydrolytic enzymes which usually accumulate in the so-called intermediate zone [13].

In the alveoli of sweat glands, ducts and the outer root sheath of follicles activity was marked, and most marked in sebaceous glands.

No sex difference in activity has been revealed but this might have been due to difficulties in quantitative evaluation of the histochemical reactions. In agreement with the finding of AUSTIN and BISCHERL [1], leukocytes in inflammatory infiltrations showed strong sulphatase positivity.

In benign epithelial tumours the activity was similar to, or somewhat higher than, in the normal epidermis.

Malignant epithelial tumours were usually characterized by increased activity. Basaliomas were exceptions in this respect; in these tumours however, other enzymes also fail to exhibit increased activity [13].

Melanotic tumours exhibited high sulphatase activity. In melanoma the reaction was stronger than in pigmented naevi. Pigmented tumour cells either in the above tumours or in seborrhoeic verruca and pigmented basalioma, similarly to melanophage cells, were mostly negative.

The behaviour of connective tissue tumours was not consistent. Fibrosarcoma showed weaker activity than benign histiocytoma. Tumours of neural origin (neuroma and neurofibroma) were highly active. As shown in rats by WOOHSMANN and BROSOWSKI [14] the medullary sheath of nerve fibres contains sulphatase.

REFERENCES

1. AUSTIN, J. H., BISCHERL, M.: (1961) Blood, **17,** 216. — 2. DOGSON, K. S., SPENCER B., THOMAS, J.: (1953) Biochem. **53,** 452. — 3. DOGSON, K. S., ROSE, F. A., TUDBALL, N.: (1959) Biochem. J. **71,** 10. — 4. GÖMÖRI, G.: (1952) Microscopic Histochemistry. Univ. Chicago Press. Chicago. — 5. HEWITT, J., WEGMANN, R., GUIGON, M.: (1961) Ann. Histochem. **6,** 131. — 6. HUGGINS, C., SMITH, D. R.: (1947) J. biol. Chem. **170,** 391. — 7. KISZELY, GY., PÓSALAKY, Z.: (1964) Mikrotechnische und histochemische Untersuchungsmethoden. Akademischer Verlag. Budapest, p. 361. — 8. PEARSE, S. G., EVERSON,: (1960) Histochemistry, Theoretical and Applied. Churchill, London. — 9. ROY, A. B.: (1953) Biochem. J. **53,** 12. — 10. ROY, A. B.: (1956) Biochem. J. **63,** 294. — 11. ROY, A. B.: (1962) J. Histochem. Cytochem. **10,** 106. — 12. RUTENBERG, A. M., COHEN, R. B., SELIGMAN, A. M.: (1952) Science, **116,** 538. — 13. SZODORAY, L., VEZEKÉNYI, K.: (1966) Acta morph. Acad. Sci. Hung. **14,** 79. — 14. WOOHSMANN, H., BROSOWSKI, K. H.: (1964) Acta histochem. (Jena) **18,** 169. — 15. WOOHSMANN, H., HARTRODT, W.: (1964) Histochemie, **4,** 336. — 16. WORTMAN, B.: (1961) J. biol. Chem. **236,** 974.

SULFATASEAKTIVITÄT DER MENSCHLICHEN EPIDERMIS UND VERSCHIEDENEN TUMOREN

KLÁRA VEZEKÉNYI

Die Sulfataseaktivität der normalen und entzündlichen Haut sowie verschiedener Tumoren wurde untersucht.

In der normalen Haut zeigen die lebenden Schichten der Haut bis zum Stratum granulosum dieselbe Aktivität, die Hornhautreaktion ist jedoch schwach. Die Reaktion der ekkrinen

Schweißdrüsen und der Follikel ist lebhaft, am intensivsten reagieren die Talgdrüsen. Die Reaktion des Kapillar-Endothels ist schwach, die der entzündlichen Elemente dagegen stark.

Gutartige Epithelneubildungen und Basaliom verfügen über eine schwache Aktivität, bösartige Epitheltumoren dagegen über eine gesteigerte. Die Aktivität der Bindegewebegeschwülste ist verschieden, die der Nerven- und Pigmenttumoren indessen lebhaft. Pigmentierte Tumorzellen und Melanophagen sind in dieser Beziehung als negativ zu bezeichnen.

АКТИВНОСТЬ СУЛЬФАТАЗЫ В ЭПИДЕРМИСЕ И В РАЗЛИЧНЫХ ОПУХОЛЯХ ЧЕЛОВЕКА

К. ВЕЗЕКЕНИ

Автором была исследована активность сульфатазы в невредимой и воспалительной коже и в опухоля.

В невредимой коже живые слои эпителия показывают до зернистого слоя одинаковую активность, реакция рогового слоя слабая. Экринные потовые железы и фолликулы дают интенсивную реакцию, активнее всего оказались сальные железы. Эндотелий капиллярных сосудов дает слабую, воспалительные элементы клеток — сильную реакцию.

Доброкачественные опухоли эпителия и базалиомы показывают слабую, злокачественные опухоли эпителия — повышенную активность. Поведение соединительнотканных опухолей неодинаковое. Активность нейрогенных опухолей и меланом очень интенсивна. Пигментированные опухолевые клетки и меланофаги дают отрицательную реакцию.

Dr. Klára Vezekényi; Debrecen 12, Hungary

Acta Morphologica Acad. Sci. Hung. **16** *(2), pp. 243—250, 1968*

Institute of Pathology (Director: Prof. J. Ormos), University Medical School, Szeged

TWO RARE FORMS OF TUMOUR IN THE HYPOTHALAMO-HYPOPHYSIAL SYSTEM

INFUNDIBULAR CHORISTOMA AND GLIOBLASTOMA INFILTRATING THE PITUITARY*

D. Bara and P. Lantos

(Received July 5, 1967)

Two cases have been reported. The first was that of a 62-year-old female with an infundibular choristoma which did not affect the hypothalamus and the short portal vessels, thus did not lead to necrosis of the anterior lobe, and allowed the passage of part of the neurosecretory fibres of the supraopticohypophysial tract. The second case was that of a female aged 40 with Central Recklinghausen's disease due to a hypothalamic spongioblastoma. This had caused amenorrhoea and diabetes insipidus then transformed into a glioblastoma and invaded the major part of the hypophysis.

Two cases of tumour are reported below. They were of interest on account of their rare occurrence and their relation to pituitary function.

Choristoma of the hypophysial stalk. Mrs. Z. S., a patient aged 62, suffering from carcinoma of the sigmoid, was subjected 5 days before her death to resection of the colon and adhaesiolysis in Szeged Hospital No. 1. Examination of the removed specimen (Dr. I. Török) revealed an exulcerated adenocarcinoma. The direct cause of death was general peritonitis. Other essential findings established post mortem were postoperative atelectasis of the lower pulmonary lobes; degeneration of the parenchymal organs; subendocardial haemorrhage; acut splenic hyperplasia; and cachexia.

As a secondary finding, necropsy revealed behind the chiasm at the initial part of the stalk a greyish-white node, the size of a cherry stone, from which the narrow stalk advanced towards the neurohypophysis (Fig. 1). The hypothalamus was not compressed by the tumour. The node consisted of large cells oval or rounded and for the most part indistinctly demarcated, with a finely granulated, faintly eosinophilic structure and small nuclei.

Small groups of such cells are sometimes observable in the neural lobe and infundibulum. Their origin is unknown. Some authors regard them as neurogenous elements, others as degenerated basophilic cells (onkocytes). From these cells small tumours may arise; they were termed choristoma by Sternberg (1921) and Priesel (1922). Other terms suggested by later authors are adenoma deriving from anterior lobe cells (Löffler, 1930); granular-cell pituicytoma (Feyrter, 1949); tumourette tumour (Lüthy and Klingler, 1951,

* Based on a paper read at the Congress of the Hungarian Society of Pathologists, Szeged, 1966.

SHANKLIN, 1953); granular-cell myoblastoma (HARLAND, 1953). Including minor tumour-like cell aggregations of microscopic size the incidence of choristoma amounts to 17% according to SHANKLIN (1953) and 6.4% according to LUSE and KERNOHAN (1955).

Apart from some episodes of vertigo which occurred three or four months before the patient's death and could well be attributed to other causes, the tumour suspending the hypophysio-hypothalamic connection did not cause

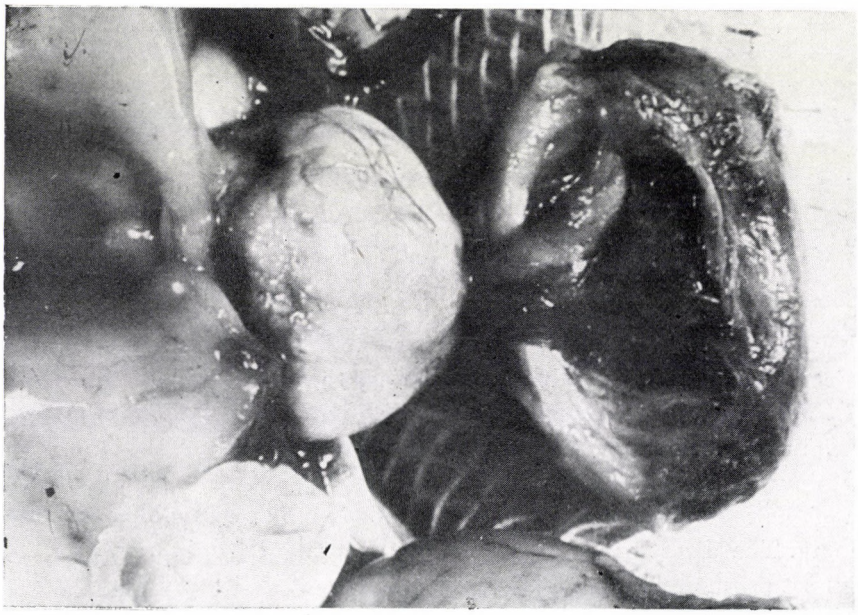


Fig. 1. Choristoma of the hypophysial stalk

any symptoms. The thyroid weighed 21.4 g and displayed medium dilated acini mostly lined with cuboid cells, eosinophilic colloid in the lumen, and an increase of interstitial connective tissue. The ovaries weighed 4.2 g. They contained corpora albicantes and hyaline-walled vessels. The adrenals weighed 9.3 g, with discernible cortical layers, slight nodular hyperplasia and areas of depleted lipoid. In the adenohypophysis the ratio of distribution on the basis of 2000 cells was 63.3% chromophobic, 26.1% eosinophilic, 10.6% basophilic. PAS preparations presented 12.1% PAS positive and 87.9% PAS negative cells. Thus the number of acidophilic cells was near the lower limit of normal. The practically negative findings explain themselves through the observations of DANDY (1940), XUEREB et al. (1954) and RUSSEL (1956) that high stalk injuries leave the main substance of the anterior-lobe parenchyma intact since blood supply is ensured by the short portal vessels in the lower part of the stalk.

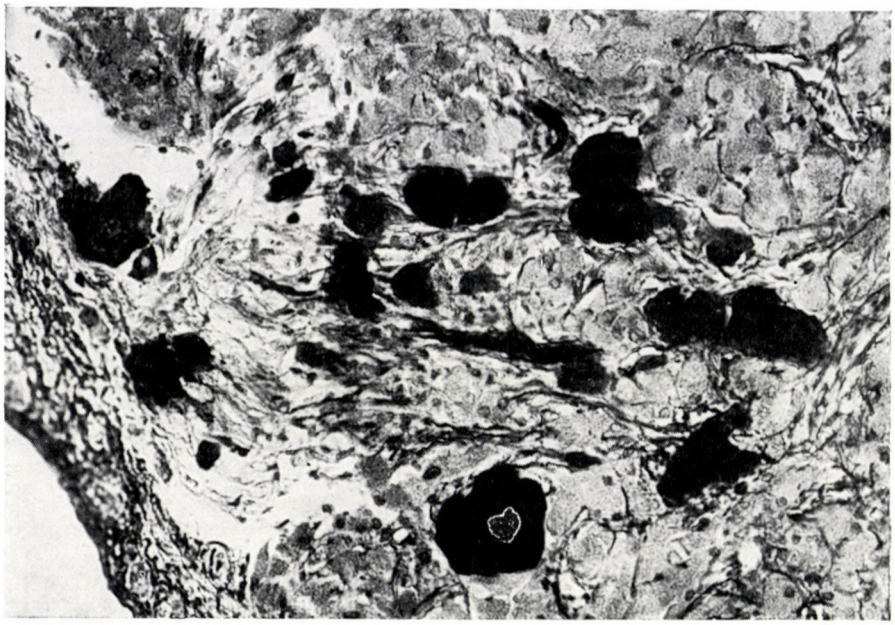


Fig. 2. Some swollen fibres of the supraopticohypophysial tract in the peripheral portion of the choristoma. 4% neutral formol fixation, aldehyde-fuchsin, × 224

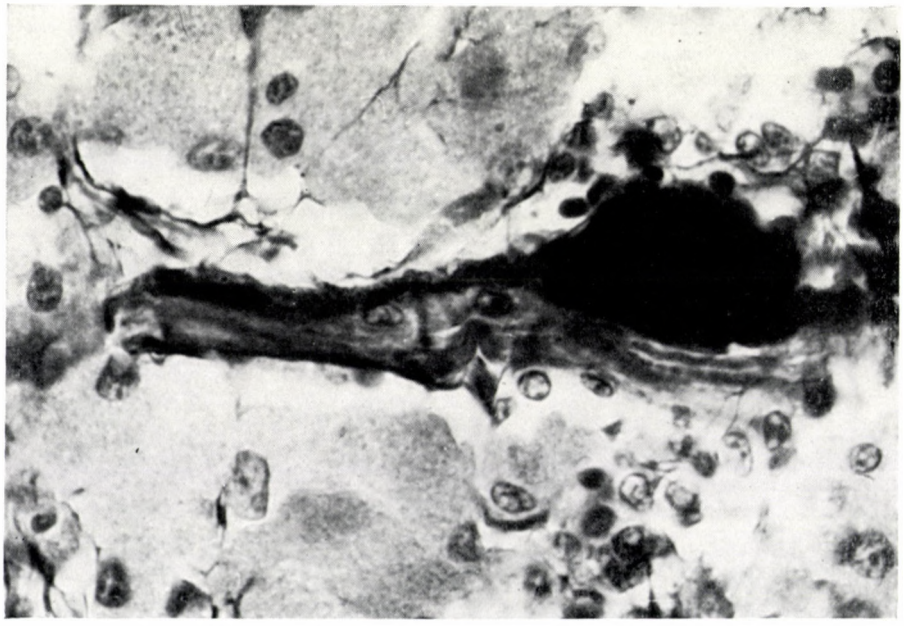


Fig. 3. Large Herring bodies. Aldehyde-fuchsin, × 896

There was neither polyuria nor polydipsia. Pre-operative serum electrolyte values were: Na 132, K 4.9, Cl 102 meq/l. The haematocrit value was normal. Aldehyde-fuchsin staining, however, indicated a considerable swelling of certain fibres of the supraopticohypophysial tract, with large Herring bodies both in the choristoma and the infundibulum (Figs 2, 3). According to the general view, this finding probably points to a stasis of neurosecretion or in other words that the ADH cannot reach the neurohypophysis. Similar observations were made in humans by MÜLLER (1955), SLOPER and ADAMS (1956) as well as KORPÁSSY (1960). On the other hand, the neurohypophysis showed no atrophy and contained a normal quantity of secretion. Since a number of thin neurosecretory fibres were revealed amidst the choristoma cells, we assume one part of the supraopticohypophysial tract fibres made their way across the tumour into the neurohypophysis and retained their secretion-transporting function. Post-mortal phenomena have unfortunately rendered the magnocellular nuclei of the anterior hypothalamus unamenable to a closer cytologic study.

Glioblastoma infiltrating the hypophysis. Sch. I., a 40-year-old female patient had had amenorrhoea six months, polydipsia and polyuria (appr. 5 litres daily) four months before death. Examination at the Municipal Hospital of Makó revealed a low specific gravity of the urine which, together with the result of water deprivation and chlorothiazide tests in the absence of renal disease, ruled out other types of polyuria and confirmed the diagnosis of diabetes insipidus. Excessive emaciation and neurologic symptoms were also observed. With the suspicion of a diencephalic tumour she was admitted to the Department of Neurology of the University Medical School, Szeged 12 days before her death. By that time the former symptoms of polydipsia and polyuria were not striking, whereas several subcutaneous nodes and café au lait spots appeared in the hyperpigmented skin. These symptoms together with the result of skin biopsy (Dr. A. TÓSZEGI), allowed to establish the diagnosis of neurofibromatosis. The patient's neural symptoms grew worse but no neurosurgical intervention could be risked on account of the patient's weak general condition.

Post-mortem examination revealed a tumour which had destructed the whole of the hypothalamus and invaded the surrounding mesencephalon, with haemorrhages (Fig. 4) and some parts presenting the picture of spongioblastoma (Fig. 5). Elsewhere the tumour was more abounding in anaplastic cells; there were regressive changes, pseudorosettes, a pseudopalisading of cells and capillary proliferation (Fig.6), all this apparently pointing to a fusiform glioblastoma. The brain and the hypophysis were removed separately. No normal stalk tissue was seen. Adhering to the surface of the hypophysis (weighing 1.2 g) as well as to the stalk orifice was a piece of grey tissue; this proved to be a tumour which had spread from the environment through the capsule and destroyed the

Fig. 4. Tumour in the hypothalamus and environments, with local haemorrhages

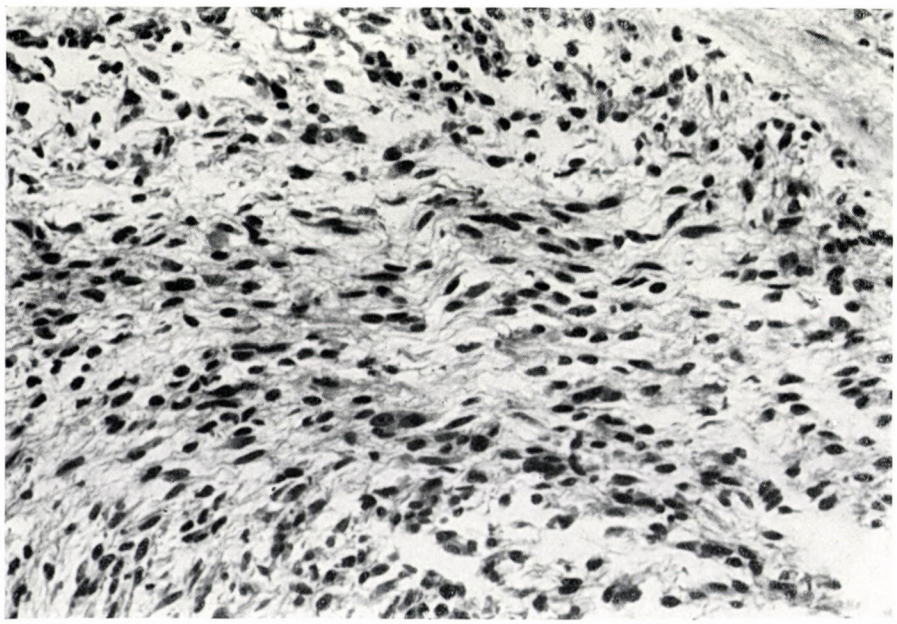


Fig. 5. Structure of spongioblastoma shown in Fig. 4; 4% formol, haematoxylin-eosin, × 224

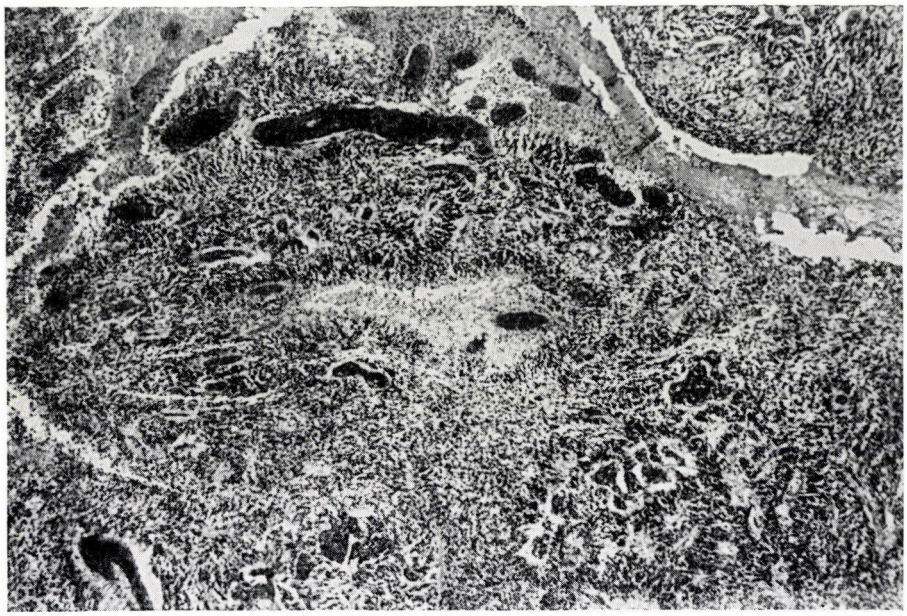


Fig. 6. Changes pointing to glioblastoma: cell abundance, necrosis, pseudopalisading of cells, and capillary proliferation, in the tumour shown in Fig. 4. Haematoxylin-eosin, × 35

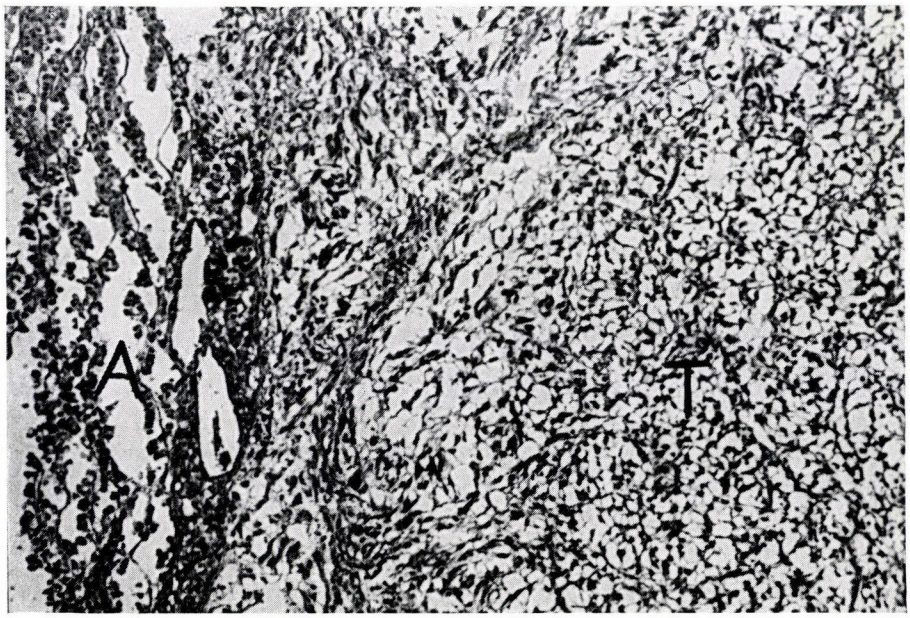


Fig. 7. Loose tumour tissue in the vicinity of a narrow strip of adenohypophysial parenchyma. 4% formol, PAS orange, × 113. A: adenohypophysis, T: tumour

greater part of the parenchyma. The peripheral portion of the tumour presented the picture of glioblastoma. There was a remaining 1 to 1.5 mm wide strip of adenohypophysis tissue; progressive oedema in its vicinity did not allow a reliable evaluation of the spongious tumour (Fig. 7). There was also a small area of fibrous, cell-rich neurohypophysis-like tissue, void of secretion. Ripening follicles or yellow bodies were seen neither in the left ovary (8 g) nor in the right one (40 g) but in the latter there was a walnut sized haemorrhagic cavity with no lining. Dilated acini abounding in colloid with a flat lining of epithelium were present in the thyroid (60 g). The adrenals (15 g) presented distinct cortical layers. A large amount of lipoid was identifiable with Oil-Red O in the fasciculate zone. Some portions of the zona glomerulosa appeared thicker than usual. Among the post-mortem findings, bilateral hypostatic pneumonia and a high degree of cachexia deserved attention.

Patients suffering from neurofibromatosis are known to present a variety of endocrine symptoms for which the responsibility is attributed to a gene lesion developmental disorder respectively, as the essential causative factor (Szondi et al. 1925), to increased STH release (Hallervorden, 1952), to circulatory disorders due to endarteritis and periarteritis, or to a state of excitement or injury of the peripheral vegetative nerves (Reubi, cit. Orthner, 1955). In a morphologically distinct group are listed those cases (including the one just dicussed) which are known as central Recklinghausen's disease (Orthner, 1955) and are due mostly to a so-called chiasmatic (optic or hypothalamic) spongioblastoma.

In our case there were some special features such as the transformation of the hypothalamic spongioblastoma into fusiform glioblastoma; the infiltration of the hypophysis by the tumour; and the improvement of the diabetes insipidus owing to a gradual destruction of the adenohypophysis.

We are indebted to Professor Dr. Huszák and Head Physician Dr. D. Szabó, for the records of the patients.

REFERENCES

1. Dandy, W. E.: (1940) Section of the Human Hypophysial Stalk. Its Relation to Diabetes Insipidus and Hypophysial Functions. J. Amer. med. Ass. **114,** 312—314. — 2. Feyrter, F.: (1949) Über die granulären neurogenen Gewächse. Beitr. path. Anat. **110,** 181—208. — 3. Hallervorden, J.: (1952) Bemerkungen zur zentralen Neurofibromatose und tuberosen Sklerose. Dtsch. Z. Nervenheilk. **169,** 308—321. — 4. Harland, W. A.: (1953) Granular-cell Myoblastoma of the Hypophyseal Stalk. Cancer (Philad.) **6,** 1134—1138. — 5. Korpássy, B.: (1960) Neurosekretion und hypothalamische Kontrolle der adrenokortikotrophen Funktion der Adenohypophyse. In: Symposia Biologica Hungarica. I. Akadémiai Kiadó, Budapest, Pp. 13—24. — 6. Löffler, E.: (1930) Über ortsfremde Zellen und Geschwülste im Hinterlappen und im Stiel der Hypophyse. Virchows Arch. path. Anat. **274,** 326—349. — 7. Luse, S. A., Kernohan, J. W.: (1955) Granular-cell Tumors of the Stalk and Posterior Lobe of the Pituitary Gland. Cancer (Philad.) **8,** 616—622. — 8. Lüthy, F., Klingler, M.: (1951) Der Tumorettentumor des Hypophysenhinterlappens. Schweiz. Z. allg. Path. **14,** 721—729. — 9. Müller, W.: (1955) Neurosekretstauung im Tractus supraoptico-hypophyseus

des Menschen durch einen raumbeengeden Prozeß. Z. Zellforsch. **42,** 439—442. — 10. ORTHNER H.:(1955) Pathologische Anatomie und Physiologie der hypophysär-hypothalamischen Krankheiten. In: Handbuch der speziellen pathologischen Anatomie und Histologie. ed : Springer, Berlin, Vol. 1, part 5. Pp. 543—939. — 11. PRIESEL, A.: (1922) Über Gewebsmißbildungen in der Neurohypophyse und am Infundibulum des Menschen. Virchows Arch. path. Anat. **238,** 423—440. — 12. RUSSEL, D. S.: (1956) Effects of Dividing the Pituitary Stalk in Man. Lancet **1,** 466—468. — 13. SHANKLIN, W. M.: (1953) The Origin, Histology and Senescence of Tumorettes in the Human Neurohypophysis. Acta anat. (Basel) **18,** 1—20. — 14. SLOPER, J. C., ADAMS, C. W. M.: (1956) The Hypothalamic Elaboration of Posterior Pituitary Principle in Man. Evidence Derived from Hypophysectomy. J. Path. Bact. **72,** 587—602. — 15. STERNBERG, C.: (1921) Ein Choristom der Neurohypophyse bei ausgebreiteten Oedemen. Zbl. allg. Path. path. Anat. **31,** 585—591. — 16. SZONDI, L., KENEDY, D., MISKOLCZY, D.: (1925) Die Beziehungen des Morbus Recklinghausen zum endokrinen System. Beiträge zur Klinik und Histologie der Neurofibromatose. Arch. Derm. Syph. (Berl.) **148,** 519—533. — 17. XUEREB, G. P., PRICHARD, M. M. L., DANIEL, P. M.: (1954) The Arterial Supply and Venous Drainage of the Human Hypophysis Cerebri. Quart. J. exp. Physiol. **39,** 190—217. — 18. XUEREB, G. P., PRICHARD, M. M. L., DANIEL, P. M.: (1954) The Hypophysial Portal System of Vessels in Man. Quart. J. exp. Physiol. **39,** 219—230.

SELTENE NEUBILDUNGEN IM HYPOTHALAMUS-HYPOPHYSE-SYSTEM: CHORISTOM DES HYPOPHYSENSTIELS, FERNER »ZENTRALER RECKLINGHAUSEN FALL« MIT TUMORÖSER INFILTRATION DER HYPOPHYSE

D. BARA und P. LANTOS

Zwei seltene Sektionsbefunde werden erörtert: Im ersten Fall (62jährige Frau) handelte es sich um ein hochliegendes, kirschgroßes Hypophysenstiel-Choristom, das den Hypothalamus nicht komprimierte, keine Adenohypophysennekrose verursachte, da die sog. kurzen portalen Gefäße intakt blieben. Ein Teil der neurosekretorischen Fasern des Tr. supraopticohypophyseus zeigte einen normalen Verlauf. Stellenweise bildeten sich wegen der keulenförmigen Anschwellung einiger dieser Fasern dagegen riesige Herringsche Körper. Im zweiten Fall führte ein mit Hypothalamus-Spongioblastom einhergehender »zentraler Recklinghausen« bei einer 40-jährigen Frau zu Diabetes insipidus. Der Tumor umwandelte sich in ein Glioblastom und infiltrierte den größeren Teil der Hypophyse.

РЕДКИЕ ОПУХОЛИ В ГИПОТАЛАМО-ГИПОФИЗАРНОЙ СИСТЕМЕ: ХОРИСТОМА НОЖКИ ГИПОФИЗА И «ЦЕНТРАЛЬНЫЙ РЕКЛИНГГАУЗЕН» С ОПУХОЛЕВОЙ ИНФИЛЬТРАЦИЕЙ ГИПОФИЗА

Д. БАРА и П. ЛАНТОШ

Сообщаются два редких наблюдения на вскрытии: высоко локализированная хористома ножки гипофиза величиной с черешневой косточки у 62-летней женщины. Опухоль не сжимала окружающего гипоталамуса, оставила так наз. краткие воротные сосуды незатронутыми, и, следовательно, не вызвала некроза передней доли и пропустила часть нейросекреторных волокон супраоптикогипофизарного тракта. Эти волокна местами кольбовидно набухали и образовались гигантские тельца Герринга. Второй случай, это «центральный Реклинггаузен» со спонгиобластомой гипоталамуса, приводивший у 40-летней женщины к синдрому несахарного мочеизнурения. Опухоль преобразовалась в глиобластому и проникла в большую часть гипофиза.

Dr. Dénes BARA
Dr. Péter LANTOS } Kórbonctani Intézet, Szeged, Hungary

RECENSIONES

D'Arcy Thompson: On Growth and Form

Abridged Edition. Ed. J. T. Bonner, Cambridge Univ. Press 1966. — 346 Pp., 181 Illustrations, 2 Plates.

Thompson's book on growth and form appeared first in 1917 and was subsequently published in amplified form in 1942. The present volume is a reprint of the 1961 edition.

There are two features to show that the book was originally written in 1917. First, it contains numerous data which reflect biological concepts as they were 50 years ago, concepts which have since either been overshadowed or sunk into oblivion by new knowledge and new notions, although they are still worthy of note. Second, some of the interpretations, if viewed from the angle of present knowledge, are obsolete as certain problems have since been solved by molecular biology and genetics.

The work under review is interesting and instructive nevertheless because it analyses functions governing growth and form from the angle of physics and mathematics, and treats them not statically but dynamically. The problems dealt with in the work direct attention to some aspects of evolution which have recently been more or less neglected.

The book contains 10 chapters. The following are of especial interest.

Chapter II discusses dimensional problems. It is through mathematical relationships that absolute and relative size, surface, body weight and extension are projected onto the body's heat and energy balance, on nutrition, on kinetic phenomena (jump, flight) and on the function of sense organs, etc. It is also in a physical sense that the question involves problems regarding the upper and lower limits of the size of organisms.

Chapter III, dealing with cell forms, is highly interesting, although it treats the subject somewhat mechanistically. The author recognized the significance of surface tension and membrane tension, the analogy of splashes and drops, he analysed the surfaces of revolution, and raised the problems of asymmetry and anisotropy at a time when scientific analyses were still lacking in these respects. The author attempts to substantiate his arguments by biological examples, e. g. on protozoa, but it is now clear that he is actually presenting models.

Chapter IV on tissue forms, cell aggregates, the evolution of organic forms and space-filling, is likewise interesting and instructive. The wide range of this chapter includes Darwin's pertinent theories as also Leduc's diffusion models and the branching of blood vessels.

Dealing with the connections between mechanical stress and form, Chapter VIII approaches the problem of mechanical efficiency. The question of primariness in the matter of structural-functional correlations is, in the main, treated somewhat mechanistically. In the concluding parts of the book the attention is directed to the whole of the organism, and the author refers to the evolutionary aspect of phylogenesis. All this shows his awareness of the fact that the formative development and change of the various parts of the organism are no isolated phenomena.

The logical connection between the foregoing and Chapter IX, the most interesting one of the book, is, thus, quite evident. Chapter IX namely deals with the theory of transformation and the phenomena of relative growth (allometry). The problem is approached by mathematical formulas and the Cartesian system of co-ordinates. Starting from a deformation of the rectangular co-ordinates, the author turns to the systems of radial co-ordinates, and analyses the formative evolution and transformations of living systems mathematically, and refers to the significance of the three-dimensional co-ordinate systems. The manner in which the examples are derived from a deformation of rectangular co-ordinates is really a classical one; the serial examples illustrate the laws of allometric growth and facilitate their analysis.

The preface written by J. T. Bonner, the editor of the book, as also his footnotes containing references to recent papers are lending an up-to-date spirit to D'Arcy Thompson's work.

Although somewhat obsolete, the book makes very interesting reading and shows well that however rapidly science is advancing, the study of forgotten works is always instructive and useful.

GY. KISZELY

Antti U. Arstila: Electron Microscopic Studies on the Structure and Histochemistry of the Pineal Gland of the Rat

Supplement to "Neuroendocrinology" Vol. 2. (S. Karger, Basel—New York, 1967), 101 Pp., 72 Figs.

This little monograph presents an excellent summary of present knowledge on the pineal body. The author's electron microscopic and histochemical studies have clarified numerous controversial problems arisen in context of this endocrine gland.

The text, on a total 67 pages, includes seven chapters. Chapter 1 is a short introduction, summarizing the main structural and functional aspects of the pineal body. Chapter 2 describes the author's electron microscopic findings; this is perhaps the most valuable part of his work. Correlating his own results with well-selected references, he concludes that the pineal body contains three different cell types, i.e. dark and light main cells and interstitial cells. An unequivocal and persuasive differentiation of the three cell types is presented, based on their submicroscopic features. Inconsistently with the majority of authors, Arstila states that the interstitial cells are special parenchymal cells, definitely differing from the glial elements of the central nervous system. Chapter 3, dealing with histochemistry, discusses the hydrolytic enzymes of the pineal body. The main cells of this gland exhibit acid phosphatase, aryl-sulphatase and thioacetic acid esterase activities, but do not give positive alkali phosphatase and cholinesterase reactions. In Chapter 4 is described the ultrastructure of pineal nerve fibres. A short experimental part appended to this chapter discusses the submicroscopic changes taking place in the nerve fibres under the influences of various experimental interventions such as bilateral cervical sympathectomy, treatment with reserpine or oxypertine. In Chapter 5 are presented the conclusions drawn by the author. Chapter 6 contains a concise description of the contents, whereas Chapter 7 a compilation of carefully selected references, including more than 150 papers.

The demonstration material (Chapter 8) includes 72 figures arranged in 18 plates and occupies 37 pages. Except for 2 schematic drawings, the whole material consists of original electron micrographs and light and electron microscopic pictures of histochemical reactions.

B. MESS

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója Műszaki szerkesztő: Farkas Sándor

A kézirat nyomdába érkezett 1968. V. 22. — Terjedelem: 9,25 (A/5) ív, 91 ábra (14 színes), 3 melléklet

68.65687 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

INDEX

Morphologia Normalis et Experimentalis

Pathologia

Oncologia

NEUROBIOLOGY OF INVERTEBRATES

Proceedings of a Symposium held at the Biological Research Institute of the Hungarian Academy of Sciences (Tihany) September 4—7, 1967

Ed. by J. Salánki

In English · Approx. 480 pages · Cloth

The thirty-four papers presented in this volume and written by specialists in the field of invertebrate neuromorphology, neurophysiology and zoology give an account of recent results in searching the nervous system of annelids, molluscs, crustaceans and insects.

Studies of the first part present mainly submicroscopical and histochemical investigations from the viewpoint of functional morphology of structures involved in neural regulation. Papers of the second part, devoted to elementary processes and to chemical specificity at cellular level, are partly of biophysical, partly of pharmacological interest. The third part contains papers discussing selected problems of active substances and enzymes in the nervous system and heart, and also transport of materials along nerves. The fourth part includes studies on neural integration, especially on problems of learning and control of behaviour.

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
Budapest

The Acta Morphologica publish papers on experimental medical subjects in English, German, French and Russian.

The Acta Morphologica appear in parts of varying size, making up volumes.

Manuscripts should be addressed to:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Correspondence with the editors and publishers should be sent to the same address.

The rate of subscription to the Acta Morphologica is 165 forints a volume. Orders may be placed with "Kultúra" Foreign Trade Company for Books and Newspapers (Budapest I., Fő utca 32. Account No. 43-790-057-181) or with representatives abroad.

Les Acta Morphologica paraissent en français, allemand, anglais et russe et publient des travaux du domaine des sciences médicales expérimentales.

Les Acta Morphologica sont publiés sous forme de fascicules qui seront réunis en volumes.

On est prié d'envoyer les manuscrits destinés à l'adresse suivante:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Toute correspondence doit être envoyée à cette même adresse.

Le prix de l'abonnement est de 165 forints par volume.

On peut s'abonner à l'Entreprise du Commerce Extérieur de Livres et Journaux «Kultúra» (Budapest I., Fő utca 32. Compte-courant No. 43-790-057-181) ou à l'étranger chez tous les représentants ou dépositaires.

«Acta Morphologica» публикуют трактаты из области экспериментальных медицинских наук на русском, немецком, английском и французском языках.

«Acta Morphologica» выходят отдельными выпусками разного объема. Несколько выпусков составляют один том.

Предназначенные для публикации авторские рукописи следует направлять по адресу:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

По этому же адресу направлять всякую корреспонденцию для редакции и администрации.

Подписная цена «Acta Morphologica» — 165 форинтов за том. Заказы принимает предприятие по внешней торговле книг и газет «Kultúra» (Budapest I., Fő utca 32. Текущий счет № 43-790-057-181) или его заграничные представительства и уполномоченные.

40,— Ft

Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

ALBANIA

Ndermarja Shtetnore e Botimeve
Tirana

AUSTRALIA

A. Keesing
Box 4886, GPO
Sydney

AUSTRIA

Globus Buchvertrieb
Salzgries 16
Wien I

BELGIUM

Office International de Librairie
30, Avenue Marnix
Bruxelles 5
Du Monde Entier
5, Place St. Jean
Bruxelles

BULGARIA

Raznoiznos
1, Tzar Assen
Sofia

CANADA

Pannonia Books
2, Spadina Road
Toronto 4, Ont.

CHINA

Waiwen Shudian
Peking
P. O. B. 88

CZECHOSLOVAKIA

Artia
Ve Směčkách 30
Praha 2
Poštovná Novinová Služba
Dovoz tisku
Vinohradská 46
Praha 2
Mad'arská Kultura
Václavské nám. 2
Praha I
Poštová Novinová Služba
Dovoz tlače
Leningradská 14
Bratislava

DENMARK

Ejnar Munksgaard
Nörregade 6
Copenhagen

FINLAND

Akateeminen Kirjkauppa
Keskuskatu 2
Helsinki

FRANCE

Office International de Documentation et Librairie
48, rue Gay Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC

Deutscher Buch-Export und Import
Leninstraße 16
Leipzig 701
Zeitungsvertriebsamt
Clara Zetkin Straße 62
Berlin N. W.

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
Postfach 46
7 Stuttgart S.

GREAT BRITAIN

Collet's Holdings Ltd.
Dennington Estate
London Rd.
Wellingborough. Northants.
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
Oxford

HOLLAND

Swetz and Zeitlinger
Keizersgracht 471—487
Amsterdam C
Martinus Nijhof
Lange Voorhout 9
The Hague

INDIA

Current Technical Literature
Co Private Ltd.
India House OPP
GPO Post Box 1374
Bombay I

ITALY

Santo Vanasia
Via M. Macchi 71
Milano
Libreria Commissionaria Sansoni
Via La Marmora 45
Firenze

JAPAN

Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
Tokyo
Maruzen and Co. Ltd.
P. O. Box 605
Tokyo-Central
Far Eastern Booksellers
Kanda P. O. Box 72
Tokyo

KOREA

Chulpanmul
Phenjan

NORWAY

Johan Grundt Tanum
Karl Johansgatan 43
Oslo

POLAND

RUCH
ul. Wronia 23
Warszawa

ROUMANIA

Cartimex
Str. Aristide Briand 14—18
Bucureşti

SOVIET UNION

Mezhdunarodnaya Kniga
Moscow G—200

SWEDEN

Almquist and Wiksell
Gamla Brogatan 26
Stockholm

USA

Stechert Hafner Inc.
31, East 10th Street
New York, N. Y. 10003
Walter J. Johnson
111, Fifth Avenue
New York, N. Y. 10003

VIETNAM

Xunhasaba
19, Tran Quoc Toan
Hanoi

YUGOSLAVIA

Forum
Vojvode Mišića broj 1
Novi Sad
Jugoslovenska Knjiga
Terazije 27
Beograd

26. IX. 1968

Index: 26.017

Acta Morphologica

Academiae Scientiarum Hungaricae

TOMUS XVI * FASCICULUS 3

Akadémiai Kiadó Budapest

1968

ACTA MORPH. HUNG.

ACTA MORPHOLOGICA

A MAGYAR TUDOMÁNYOS AKADÉMIA ORVOSTUDOMÁNYI KÖZLEMÉNYEI

SZERKESZTŐSÉG ÉS KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY U. 21.

Technikai szerkesztő:
Dr. Somogyi Endre

Az Acta Morphologica német, angol, francia és orosz nyelven közöl értekezéseket a kísérletes orvostudomány tárgyköréből.

Az Acta Morphologica változó terjedelmű füzetekben jelenik meg. Több füzet alkot egy kötetet.

A közlésre szánt kéziratok a következő címre küldendők:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Ugyanerre a címre küldendő minden szerkesztőségi és kiadóhivatali levelezés.

Az Acta Morphologica előfizetési ára kötetenként belföldre 120, külföldre 165 Ft. Megrendelhető a belföld számára az Akadémiai Kiadónál (Budapest V., Alkotmány utca 21. Bankszámla 05-915-111-46), a külföld számára pedig a „Kultúra" Könyv- és Hírlap Külkereskedelmi Vállalatnál (Budapest I., Fő utca 32. Bankszámla: 43-790-057-181) vagy annak külföldi képviseleteinél és bizományosainál.

Die Acta Morphologica veröffentlichen Abhandlungen aus dem Bereiche der experimental-medizinischen Wissenschaften in deutscher, englischer, französischer und russischer Sprache.

Die Acta Morphologica erscheinen in Heften wechselnden Umfages. Mehrere Hefte bilden einen Band.

Die zur Veröffentlichung bestimmten Manuskripte sind an die folgende Adresse zu senden:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

An die gleiche Anschrift ist auch jede für die Schriftleitung und den Verlag bestimmte Korrespondenz zu richten.

Abonnementspreis pro Band: 165 Forint. Bestellbar bei dem Buch- und Zeitungs-Außenhandels-Unternehmen »Kultúra« (Budapest I., Fő utca 32. Bankkonto Nr. 43-790-057-181) oder bei seinen Auslandsvertretungen und Kommissionären.

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 253—263, 1968*

Research Institute of the Roumanian Academy of Sciences (Head: Prof. M. GÜNDISCH);
Department of Anatomy (Head: Prof. T. MAROS) of the Institute of Medicine
and Pharmacology, Tîrgu-Mureş

INVOLVEMENT OF THE OPTIC SYSTEM IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE DOG

T. MAROS and L. LÁZÁR

(Received August 4, 1966)

Changes occurring in the optic system of dogs in the course of experimental allergic encephalomyelitis have been studied under the light microscope. The optic apparatus was found to be involved as frequently as in cases of human demyelinating encephalomyelitis and multiple sclerosis. Also, a symptomatic morphological change appeared early in this area.

The fact that the optic apparatus is affected early and frequently might be due to the close connection between neuroglia and connective tissue in the retrobulbar portion of the optic nerve. An important role in the development of demyelination and sclerotic foci is attributed to the reactive and astrocytic interfascicular oligodendroglial elements. The conspicuous frequency of perivascular processes in the occipital pole seems to be connected with increased functional vasomotor activity.

Frequent morphological changes in the optic system are characteristic of experimental allergic encephalomyelitis (EAE). Such changes sometimes give rise to conspicuous manifestations such as irreversible or temporary blindness, while sometimes the changes cannot be observed by routine methods because no objective symptoms are present.

Since damages of the optic system are frequent in multiple sclerosis as also in other human demyelination diseases [15], it seemed justified to study it in the EAE of dogs. The study was designed in order to investigate the factors which determine the nature of the morphological changes and to establish why they occur so frequently in certain parts of the optic system.

Material and method

Twenty mongrel dogs of both sexes and different ages were used. The model experiment by which EAE was induced as well as the observed neurological symptoms and morphological changes have been described earlier [18, 19, 21]. Eyeball, optic nerve, optic chiasma, optic tract and occipital pole of the animals were removed, fixed in a 1 : 10 solution of formol and bromformol, and stained with haematoxylin–eosin, Masson's method, myelin stain and silver impregnation (for the demonstration of neuronal and glia elements).

Results

Clinical symptoms. In 3 animals bilateral complete blindness accompanied by the absence of pupillary reflexes was observed, while disorders of vision manifesting themselves indirectly were registered in 6 animals. They fell wed with the eyes non-existent objects moving in the air, or snapped at non-existing flies, a phenomenon indicative of scotoma; some of them missed the offered food, presumably owing to diplopia. All these animals displayed ophthalmo-leucoencephalitic lesions that were frequently combined with acute myelitis. Changes in the optic apparatus were observed in nearly all animals irrespective of whether they displayed manifest signs of disturbed vision.

Circulatory disturbances were observed in the optic system of 10 animals. Localization and frequency of the gross changes are listed in Table I.

Table I

Nature of alterations	Affected areas			
	Optic nerve	Optic chiasma	Optic tract	Occipital pole
Hyperaemia	8			8
Minor haemorrhage	10	1	1	7
Haematoma	2			
Foci of haemorrhage and malacia				2

Changes in the optic nerve affected the retrobulbar space in the first line. In one of the cases extravasated blood surrounded the eyeball and coursed between the sheaths of the optic nerve as far as the optic chiasma. In another case the entire white substance around the calcarine fissure turned into an extensive soft focus.

Microscopic picture. The morphological changes characteristic of EAE [18, 19], could all be found in the optic apparatus.

Mixed perivascular foci appearing at the different stages of myelin sheath destruction were registered in the following parts of the optic apparatus.

(i) Retrobulbarly, 1 cm behind the lamina cribrosa (11 dogs)

(ii) In the optic chiasm (5 dogs)

(iii) In the optic tract (9 dogs)

(iv) In the optic radiation (16 dogs). In these, demyelination was the predominant phenomenon in 10 cases, vascular and perivascular phenomena in 5 cases, cerebral oedema and a swelling of the myelin sheath in one animal.

Conspicuous signs of demyelination were observed in the optic nerve, optic chiasma and optic tract in 12 animals.

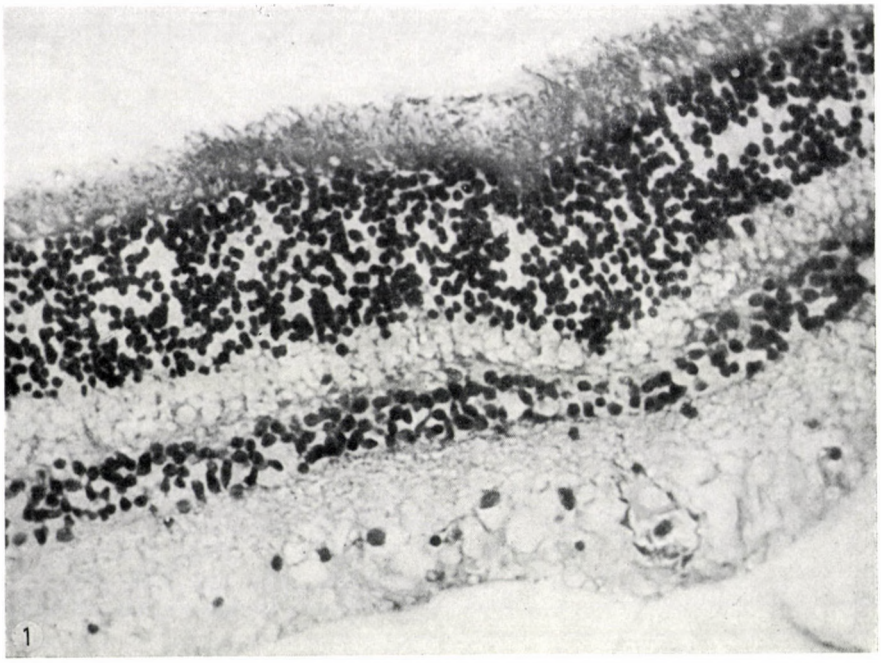


Fig. 1. Vesicles of different sizes in the ganglionic layer. Mallory's stain. Eyepiece, 6 ×, Lens, 24/0, 42

Nature and frequency of changes

(a) *Retina.* This organ occasionally showed microscopic haemorrhages and frequently perivascular lymphocytic-plasmacytic infiltrations, precisely corresponding to those described by Fog [6] in multiple sclerosis.

Retinal phlebitis and periphlebitis were also observed, likewise phenomena known from multiple sclerosis [9]. Nevertheless, most ganglion cells were of a normal aspect, and it was the appearance of serous microcysts which pointed to the circulatory disturbance (Fig. 1).

Reactive forms of the macroglia without signs of essential reorganization of the other retinal elements were frequent in the chronic phase. Thickening of the adventitia, a constant concomitant sign, made the blood vessels visible to the naked eye in the form of stripes.

(b) *Optic nerve.* Connective tissue proliferation a practically constant occurrence, was followed by glial hyperplasia and then by gliosis or even focal sclerosis. Gliosis and cicatrization were most pronounced in the retrobulbar portion of the optic nerve, and especially in the immediate vicinity of the lamina cribrosa (Fig. 2). Proceeding towards the chiasma, the cicatricial aspect of the widening connective tissue septa became gradually less pronounced and the number of interfascicular glial elements (mostly oligodendroglia) grew gradually lower (Fig. 3).

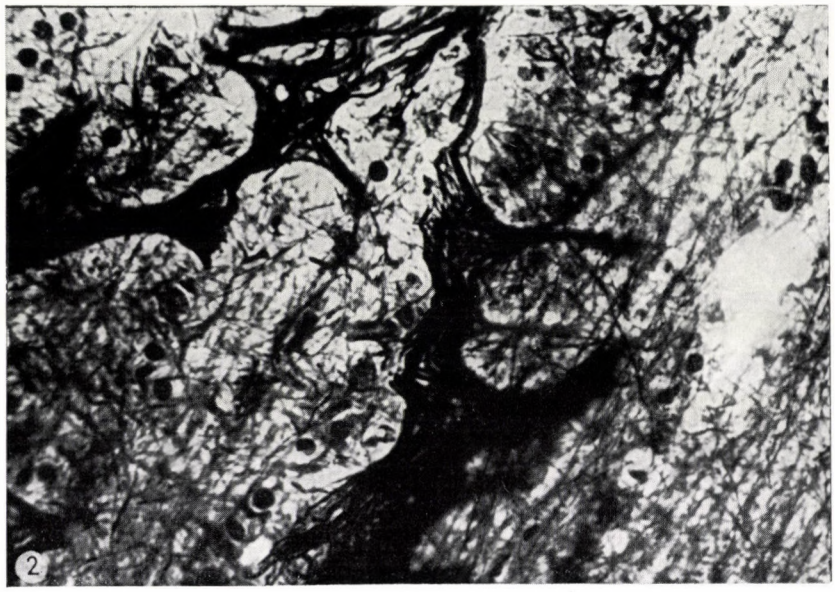


Fig. 2. Retrobulbar part of optic nerve. Note marked gliosis between widened connective tissue septa. Silver-carbonate impregnation. Eyepiece 6×, Lens, 24/0, 42

The neuroglia of mixed composition (oligodendroastrocyte) (Fig. 4), as seen in the incipient phase, was in the course of demyelination (Fig. 5) gradually replaced by typical astrocytes which formed compact glial scars at the level of and next to the lamina cribrosa.

(c) *Chiasm and optic tract.* Changes here were less marked, myelin lesions — precursors of demyelination — were of reversible character, sclerotic foci were scattered and appeared later (Fig. 6). The interfascicular neuroglia showed hyperplasia, sometimes with the predominance of oligodendroglial cells and sometimes with that of astrocytes.

(d) The *lateral genicular body* showed in some cases marked hyperergic perivasculitis (with or without bleeding) which was usually confined to earlier foci of demyelination or sclerosis.

(e) *Optic radiation.* Exudative perivascular foci, frequently accompanied by microscopic haemorrhages, were practically constant phenomena. Beside haematogenic and adventitial elements, microglial cells were also involved in the perivascular infiltrations (Fig. 7), and this to a greater extent than in the other parts of the optic apparatus. Predominance of microglial elements was found to be a constant phenomenon also in foci of demyelination formed in the acute phase of the disease. Microglial reaction was weakest in the optic nerve where the majority of cellular elements appeared to be of oligodendroglial origin.

Fig. 3. Sclerotic focus with proliferation of neuroglial elements. Silver-carbonate impregnation. Eyepiece, 6×, Lens, 24/0, 42

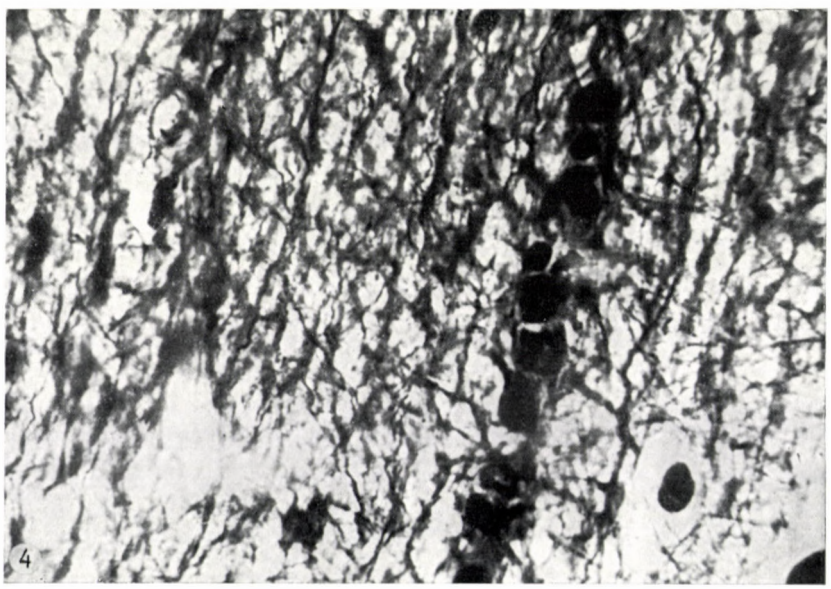


Fig. 4. Reactive transformation of oligodendroglial cells, suggestive of astrocytes. Silver-carbonate impregnation. Eyepiece 6×, Lens, 24/0, 42

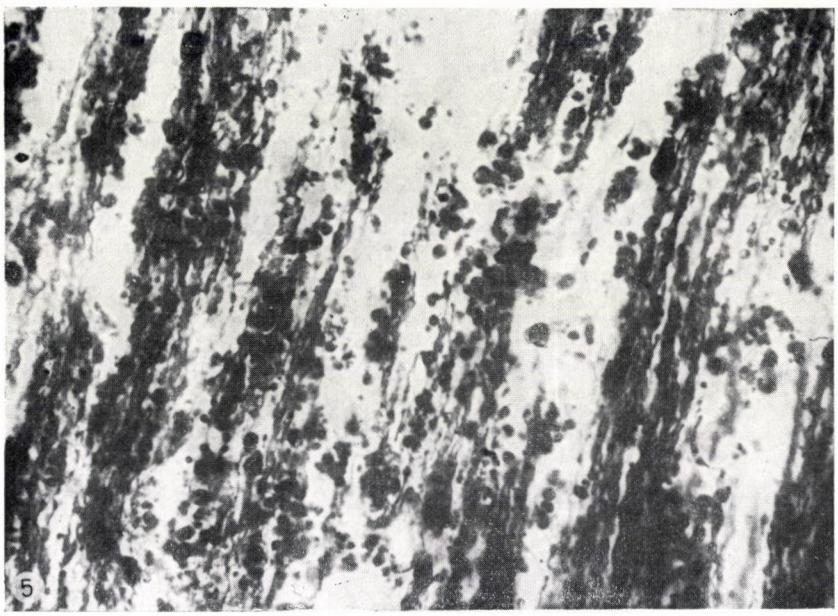


Fig. 5. Middle portion of optic nerve. Abundant myelin globules indicative of demyelination. Myelin stain. Eyepiece, 6×, Lens, 24/0, 42

Fig. 6. Optic tract near to the chiasm. Weak staining reaction indicates advanced demyelination. Myelin stain. Magnif.: Eyepiece 6×, Lens, 6/0, 16

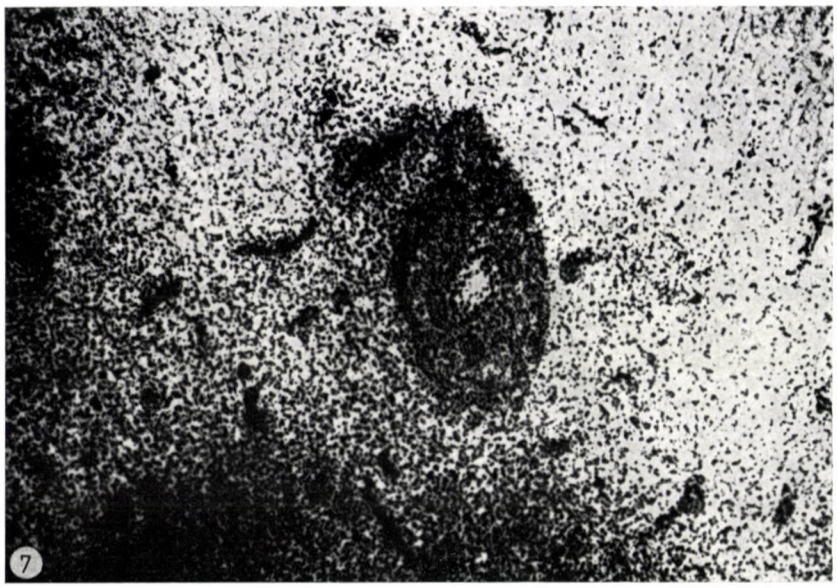


Fig. 7. Occipital pole, white matter. Perivascular infiltrates alternating with zones of diffuse cell infiltration. Silver-carbonate impregnation. Eyepiece, 6×, Lens, 6/0, 16

Zones of glial reaction were larger during the reparative phase in the optic radiation than elsewhere. Polynuclear giant macroglial structures were frequently seen in the zones around the circumscribed or diffuse foci.

Changes in the optic nerve were often accompanied by hyperergic — frequently necrotizing — processes occurring in the spinal cord or in the brain stem. Involvement of the occipital pole was usually associated with changes in the other parts of the hemispheres.

Discussion

The fact that both the optic nerve and the spinal cord are damaged in EAE — a feature characteristic also of human optic neuromyelitis [24] — might be due to the abundance of mesenchymal elements in these organs. A common factor is presumably responsible for the simultaneous occurrence of hyperergic processes in the spinal cord and the optic nerve. Structural properties of the perivascular connective tissue and the neuroglial limiting membranes are probably in the background of the phenomenon. This assumption seems to be supported by the observation that the pathologic processes take preferably place in the retrobulbar segment of the optic nerve and in the vicinity of the lamina cribrosa, areas rich in tissue elements of mesenchymal origin.

The peculiar reactivity of the optic neuroglia and its connection with the connective tissue is made obvious by some phylogenic and ontogenic data. The neuroglial apparatus undergoes notable changes during the development of the connection tissue system of the optic nerve. The most significant change consists in the appearance of increasingly numerous differentiated (fibrous) glial elements. For instance, the optic nerve of *Petromyzon* is devoid of supporting connective tissue, and the neuroglia is represented only by axially arranged ependymal cells. In amphibians and reptiles, astrocytes appear with the increase in perivascular connective tissue [10, 27], to be followed by intrafascicular plasmatic astrocyte structures [2, 25]. The interfascicular rows of neuroglia in birds consist almost exclusively of oligodendroglia [1]. Interfascicular connective tissue septa make the glial structure of the optic nerve still more intricate in mammals [7]. The connective-tissue apparatus in the optic nerve of mammals of the higher orders is even more complicated and abundant [32] which leads to an overwhelming predominance of astrocytes in man [17, 31], with an almost complete absence of microglia [3].

The development of the optic nerve presents a number of further interesting features. In the human newborn the neuroglial apparatus in the optic nerve consists almost entirely of oligodendroglia, and it is in the course of connective tissue growth that astrocytes emerge in increasing numbers [3]. They appear first at the level of the lamina cribrosa [4, 29, 30].

While, according to our observations, oligodendroglia is predominant in the optic nerve of young dogs, astrocytes and transitory forms appear in increasing numbers with advancing age.

Similar transitory structures have been observed in man not only from the optic nerve but from the optic chiasm and the optic tract as well [3, 17, 31]. We are in agreement with other authors [23] that oligodendroglia and astrocytes of the optic nerve are but two varieties of the same neuroglia.

The neuroglial structure, the development of which is correlated with the growth of the supporting connective tissue is in our opinion a decisive factor in the optic (retrobulbar) localization of the process. To the same factor might be due the fact that sclerotic foci develop along the subpial connective tissue and around the connective tissue sheath of the central artery of the retina.

The neuroglial reaction leading to astrocytic gliosis and then to sclerosis is presumably induced by the inflammatory process in which the mesenchymal elements are involved; this is particularly probable the optic system where the interconnection between neuroglia and adventitial connective tissue is especially close [5].

Some further factors may also have a part in the retrobulbar localization of demyelinating processes, thus a hyperaesthesia of the myelin, reactive changes of which occur sooner near the lamina cribrosa than elsewhere. Ear-

lier studies have shown [13, 22] that the myelin sheath showed the earliest changes at the junction of myelinated and unmyelinated segments of the nerve fibre.

Reactive cell types of the interfascicular neuroglial rows may play a significant role in processes occurring independently of the connective tissue septa and the blood vessels [11, 12]. These foci, precursors of sclerosis, remain long reversible, especially in the chiasm and the optic tract. The regenerative power of the optic apparatus, observed in lower animals [26, 28] may likewise be due to the same structural properties, i.e. the absence or low number of connective tissue septa and astrocytic elements.

Functional changes in local circulation, too, seem to play an important part in the localization of allergic and hyperergic processes. There are data concerning the existence of a correlation between the localization of the said processes and functional factors [8]. It is perhaps owing to a similar mechanism that circulatory disorders such as hyperaemia, haemorrhage and inflammatory processes are marked in the optic apparatus. The functional stresses to which the human optic system is more exposed than that of other species may be regarded as another reason why multiple sclerosis so frequently occurs in the optic apparatus.

Conclusions

The optic system is nearly always affected in the experimental encephalomyelitis of dogs. Microscopic changes are usually demonstrable even if the optic apparatus shows no pathological signs. Lesions appear in most cases in the optic nerve and the occipital pole, but the retina and sometimes even the optic chiasm, the optic tract and the lateral geniculate body may also be involved. The process mostly resembles an optic neuromyelitis.

A correlation presumably exists between the site of the changes and the morphological properties of the affected areas. The fact that pathologic processes arise early and frequently in the retrobulbar segment of the optic nerve may be due to the close connection between the richness of supporting connective tissue and the rows of interfascicular neuroglia. Reactive and astrocytic oligodendroglial elements are presumably important factors in early demyelination and sclerosis, and it is the optic system that seems to sustain the earliest damage.

The frequency of perivascular processes in the occipital pole may be due to increased vasomotor activity in connection with functional stresses. This might be even more significant in human pathology than in animal experiments.

REFERENCES

1. BAIRATI, A., BARTOLLI, E.: (1955) Ricerche morfologiche ed istochimiche sulla glia del nevrasse di vertebrati II. Uccelli. Z. Zellforsch. **42,** 275. — 2. BAIRATI, A., TRIPOLI, G.: (1954) Riserche morfologiche ed istochimiche sulla glia del nevrasse di vertebrati. Z. Zellforsch. **39,** 392. — 3. CASASCO, E.: (1952) La glia nel nervo ottico e nel chiasma. Riv. Pat. nerv. ment. **73,** 238. — 4. CONE, W., MCMILLAN, J. A.: (1932) The Optic Nerve and Papilla. In: Cytology and Cellular Pathology of Nervous System. Penfield ed. Vol. 2., P. 839. — 5. ERSKINE, C. A.: (1958) Capillary–Glia Relationships in the Human Optic Nerve, Chiasma and Tract as Shown by Phase Colour Contrast and Microdissection. Brit. J. Ophthal. **42,** 81. — 6. FOG, T.: (1963) Den disseminerede skleroses patologiskanatomiske grundlag. Periphlebitis cerebrospinalis et retinealis. Ugeskr. Laeg. **125/38,** 1291. — 7. FRANZ, V.: (1934) Vergleichende Anatomie des Wirbeltierauges. In: Handbuch der vergleichenden Anatomie der Wirbeltiere. Eds: Bolk, L., Göppert, E., Kallius, E., Lubosch, W., Vol. III/2, Urban und Schwarzenberg, Berlin. — 8. GOLDSTEIN, J. E., COGAN, D. G.: (1964) Exercise and the Optic Neuropathy of Multiple Sclerosis. Arch. Ophth. **72,** 168. — 9. HAARR, M.: (1964) Changes of the Retinal Veins in Multiple Sclerosis. Acta neurol. scand. **40**/10, 17. — 10. HORSTMANN, E.: (1960) Die Neuroglia und ihre physiologische Bedeutung. 1st European Anatomical Congress, Strasbourg. Anat. Anz. Suppl. **109.** — 11. LÁZÁR, L., MAROS, T.: (1967) Au sujet des formes réactives de l'oligodendroglie. Revue Roumaine d'Embryologie et de Cytologie **4,** 133. — 12. LÁZÁR, L., MAROS, T., KOVÁCS, V. V.: Morphologische Veränderungen der Oligodendroglia in der experimentellen allergischen Enzephalomyelitis (EAE) beim Hund. In press. — 13. LÁZÁR, L., MAROS, T., KREPSZ, I.: (1960) Структуральные изменения нервных волокон и окончаний под воздействием рентгеновских лучей. Revue des Sciences Médicales **3—4,** 195. — 14. LÁZÁR, L., MAROS, T., KOVÁCS, I.: (1966) Über gewisse Eigentümlichkeiten des physikalischen Abbaus der Markscheide bei der experimentellen allergischen Enzephalomyelitis der Hunde. Acta morph. Acad. Sci. hung. **14,** 87. — 15. LEHOCZKY, T.: (1962) Développement des recherches anatomopathologiques sur la sclérose en plaques de 1957 à 1960. Acta neurol. belg. **62,** 719. — 16. LEHOCZKY, T., SÓS, J., HALASY, M.: (1964) Animal Experiments on the Aetiology of Myelopathy. Akadémiai Kiadó, Budapest. — 17. LISS, L.: (1956) The Astroglia of the Human Optic Nerve, Chiasma and Tract. A Study with Silvercarbonate. J. comp. Neurol. **105,** 151. — 18. MAROS, T., LÁZÁR, L., KOVÁCS, V., FÓRIKA, M. M.: (1966) Considerations Regarding the Possible Reproduction of Experimental Allergic Encephalomyelitis in Dogs. Confin. neurol. (Basel) **28,** 18. — 19. MAROS, T., LÁZÁR, L., KOVÁCS, V. V.: (1966) Caracterele morfologice ale encefalomielitei alergice experimentale la cîini. Rev. med. (Tîrgu-Mureș) **4,** 149 and 155. — 20. MAROS, T., LÁZÁR, L., KOVÁCS, V. V., MARINICA, D.: (1967) Contributions au problème de l'influence des facteurs météorologiques sur l'évolution de l'encéphalomyélite allergique expérimentale. J. neurol. Sci. (Amsterdam) **4,** 217. — 21. MAROS, T., LÁZÁR, L., KOVÁCS, V. V., FÓRIKA, M. M.: (1965) Model experimental pentru reproducerea encefalomielitei alergice la cîini. S. Cerc. neurol. **10/5—6,** 517. — 22. MAROS, T., LÁZÁR, L.: (1959) Zur Frage der postmortalen Veränderungen in peripheren Nerven. Acta anat. (Basel) **38,** 34. — 23. PESSACQ, T. P.: (1964) L'astroglie et l'oligodendroglie constituent-elles deux catégories cellulaires indépendantes ou différents degrés d'imprégnation d'un seul type cellulaire? Arch. Anat. micr. Morph. exp. **53,** 169. — 24. PETERS, G.: (1958) Neuromyelitis optica. In: Handbuch der speziellen pathologischen Anatomie und Histologie, eds. O. Lubarsch, F. Henke, R. Rössle. Vol. 13, Springer, Berlin. — 25. PRINCE, J. H.: (1955) The Origin and Pattern of Oligodendroglial Cells in the Vertebrate Optic Nerve. J. comp. Neurol. **103,** 541. — 26. RASQUIN, P.: (1949) Regeneration of the Optic Nerve Fibres after Section with Return of Vision in the Astyanax mexicanus. Physiol. Zool. **22,** 131. — 27. ROHEN, J. W.: (1964) Das Auge und seine Hilfsorgane. In: Handbuch der mikroskopischen Anatomie des Menschen, eds. W. Möllendorf, W. Bargmann, Springer, Berlin. — 28. TANDON, K. K., SHARMA, S. CH.: (1964) On the Degeneration and Regeneration of Optic Nerve Fibres with Return of Vision in Danio rerio (Hem.) Proc. Ind. Acad. Sci. **60,** 287. — 29. VALU, L., KALAPOS, S.: (1961) Die argyrophilen Fasern des gesunden Corpus ciliare. Albrecht v. Graefes Arch. Ophthal. **163,** 226. — 30. VALU, L., KALAPOS, S.: (1963) Über die Silberaffinität der Bindegewebéfasern des Sehnerven und der Lamina cribrosa. Albrecht v. Graefes Arch. Ophthal. **165,** 510. — 31. WOLTER, J. R.: (1961) Silver Carbonate Technique for a Demonstration of Ocular Histology. In: The Structure of the Eye. Ed. G. Smelser, Academic Press, New York. — 32. WUNDER, W.: (1958) Biologie und Bau der Netzhaut beim Rotbarsch (Sebastes marinus). Zool. Anz. **160,** 94.

BETEILIGUNG DES NERVUS-OPTICUS-SYSTEMS BEI EXPERIMENTELLER ALLERGISCHER ENZEPHALOMYELITIS VON HUNDEN

T. MAROS und L. LÁZÁR

Die morphologischen Veränderungen des Nervus-opticus-Systems bei experimenteller allergischer Enzephalomyelitis von Hunden wurden mit lichtmikroskopischen Methoden untersucht. Das Nervus-opticus-System nimmt am Prozeß ebenso häufig teil, wie bei der akuten demyelinisierenden Enzephalomyelitis und der Sclerosis multiplex des Menschen. Es scheinen dabei auch symptomfreie morphologische Veränderungen frühzeitig aufzutreten.

Für die häufige und frühe Lokalisation des pathologischen Prozesses im Nervus-opticus-System werden die im retrobulbären Abschnitt des Sehnerven wahrnehmbaren intimen Beziehungen zwischen der Glia und dem Bindegewebe verantwortlich gemacht. In der frühzeitigen Entstehung der Demyelinisations- und Skleroseherde wird den reaktiven und astrocytoiden Oligodendroglia-Elementen der interfaszikulären Glia-Reihen eine bedeutende Rolle zugeschrieben. Die auffallend häufig vorkommenden perivaskulären Prozesse des okzipitalen Pols werden mit der funktionellen Vasomotorik in Zusammenhang gebracht.

УЧАСТИЕ СИСТЕМЫ ЗРИТЕЛЬНОГО НЕРВА В ЭКСПЕРИМЕНТАЛЬНОМ АЛЛЕГРИЧЕСКОМ ЭНЦЕФАЛОМИЕЛИТЕ У СОБАК

Т. МАРОШ и Л. ЛАЗАР

Обычными микроскопическими методами исследования были изучены морфологические изменения системы зрительного нерва при экспериментальном аллергическом энцефаломиелите у собаки. Система зрительного нерва участвует в процессе так же часто, как и при острых демиелинизирующих энцефаломиелитах человека. Кажется, что здесь сравнительно рано развиваются также бессимптомные морфологические изменения.

Как предположительную причину частой и рано развивающейся локализации патологического процесса в системе зрительного нерва, авторы выдвигают роль тесных связей между глией и соединительной тканью, наблюдаемых в ретробульбарном участке зрительного нерва. В раннем развитии очагов демиелинизации и склероза значительная роль приписывается реактивным и астроцитоидным элементам олигодендроглии межфасцикулярных рядов глии. Периваскулярные процессы, поразительно часто поражающие окципитальный полюс, авторы приводят в связь с функциональной вазомоторикой.

Dr. Tibor MAROS
Dr. László LÁZÁR
Tîrgu-Mureş (Roumania)
Gh. Marinescu str. 38.

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 265—269 1968*

Department of Pathology (Director: Prof. Gy. Romhányi),
University Medical School, Pécs

DEGRANULATION OF TISSUE EOSINOPHIL LEUKOCYTES

G. Kelényi

(Received February 20, 1967)

(Preliminary report)

It is known that after phagocyting bacteria or ingesting antigen-antibody complexes [1—2] the eosinophil leukocytes may undergo degranulation. The submicroscopic features of this process are not known, although eosinophils with a decreased number of granules in the intestinal mucosa have been reported [3]. In electron micrographs of cells from pleural effusion, pemphigus blister and from tissues with a high number of eosinophil leukocytes such as lymph glands in Hodgkin's disease, gastric polyps, skin tissues in pemphigus, we have noted eosinophil leukocytes with a decreased number of granules, some of them containing no more than 4 to 10 granules per whole cell profile, i.e. 1 to 3 granules per 10 μ^2 of cytoplasm. Mature human blood and bone marrow eosinophils showed an avarage number of 7 to 15 specific granules per 10 μ^2 of cytoplasmic area.

When searching for the mechanism which leads to degranulation of the eosinophil leukocytes, we observed electron microscopic changes in the granules, suggesting that these alterations might be related to the degranulation of the cells.

The tissues were fixed in 1% osmium tetroxide in 0.15 M phosphate buffer pH 7.2, isotonized with 7.5% sucrose, and after dehydration in ethanol they were embedded in Araldite (Ciba, Basel). Ultrathin sections were stained either with uranyl acetate (saturated solution in 70% ethanol) for 15 seconds, or with potassium permanganate (5% solution in distilled water) for 5 seconds, and with lead citrate [4]. When examining a large number of tissue and exudate eosinophils we often found granules in which the externum or matrix seemed to be not so dense and compact as in intact forms, resulting in reversed densities of the granules, i.e., in contrast to uranyl acetate-stained normal granules, the crystalloids appeared to be denser than the matrices. In others the matrix had become looser and a fine granular-vesicular structure appeared. Seemingly more altered forms showed ruptures in the granule membrane with a scattering of some of the vesicular elements into the cytoplasm. The number of granular-vesicular and disrupted granules varied from cell to cell in the same tissue. Generally, of 3 to 5 adjacent eosinophils 1 or 2 exhibited a number of altered granules, whereas the granules in the others seemed to be intact. Parallel to these changes of the matrices, some of the crystalloids at first slightly increased in density as measured by densitometric tracings of the electron micrographs across crystalloids of neighbouring normal and altered granules. Later, during the complete disintegration of the granules, the crystalloids normally disappeared (Figs 1—3).

In some of the material, disruption of the eosinophils and ingestion by macrophages of the cell fragments containing a certain number of granules

were seen. In granulomatous tissues, in peritoneal exudate cells and in the oestrous uterus of rats similar phenomena were observed [5—7]. The number of altered forms was as high among the extracellular granules (Fig. 4) as in the seemingly intact, non-disrupted cells; the degree of their alteration, however, was slight when compared with the phagocyted granules which frequently showed granular-vesicular matrices and disintegration.

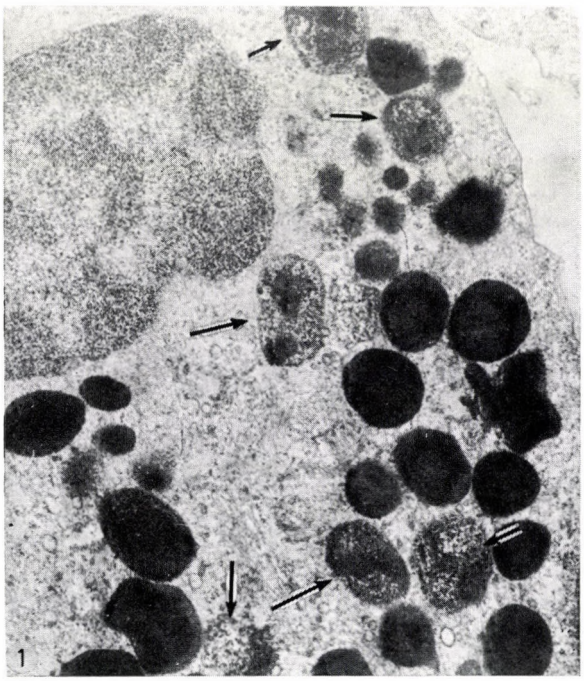


Fig. 1. In some of the specific granules (arrows) the crystalloids seem to be denser than the matrices, i.e., their densities are reversed when compared with normal granules. Magn.: approx. ×9000

Figs 1—4. Eosinophil granulocytes from pemphigus blister fluid (Fig. 1) and from a lymph gland in Hodgkin's disease (Figs 2—4). Osmium tetroxide–Araldite; stained with uranyl acetate (Figs 1—3) or potassium permanganate (Fig. 4) and lead citrate

In human and rat eosinophil leukocytes of the bone marrow and blood such changes occurred rarely. Postmortal alterations of the cells kept at room temperature for 1 to 12 hours after removal of the tissues were different.

Uranyl acetate-stained ultrathin sections, or tissue blocks stained with phosphotungstic acid [9] or uranyl acetate during dehydration, showed electron dense matrices and electron lucent crystalloids. In lead citrate or potassium permanganate-stained ultrathin sections of osmium tetroxide-fixed material reversed densities were observed, viz. electron dense crystalloids

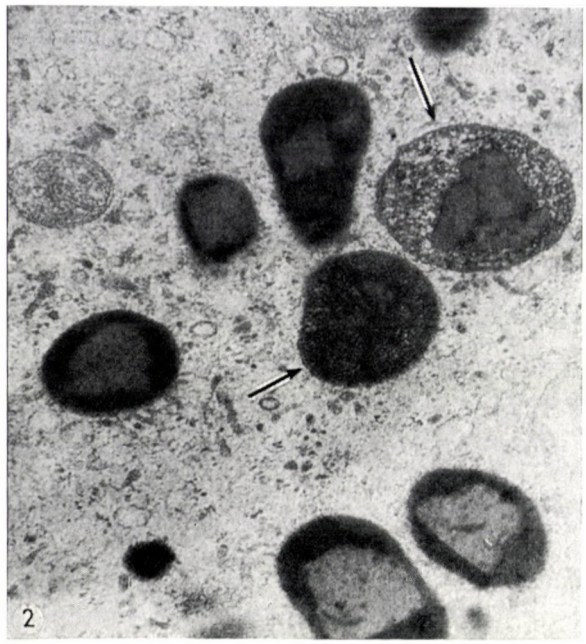


Fig. 2. Two granules (arrows) show loose, granular-vesicular matrices; their membranes seem to be intact. Magn.: approx. ×15 000

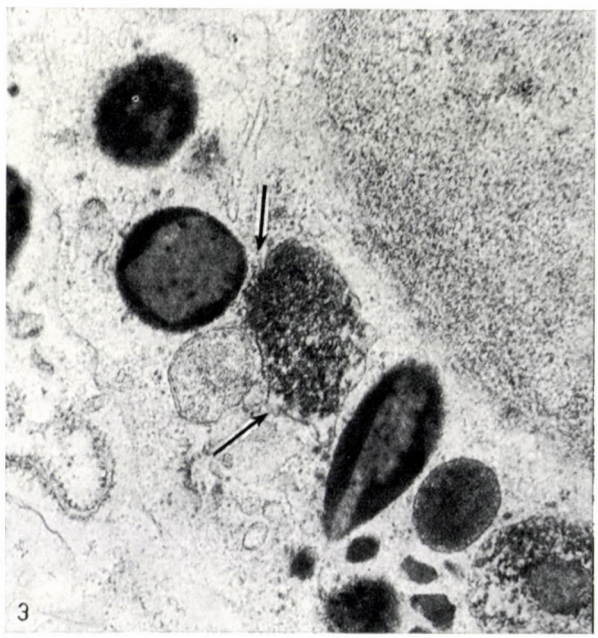


Fig. 3. Besides unaltered specific granules there is one in the centre of the field with granular-vesicular matrix and loosened membrane. At arrow, discontinuity of the granule membrane may be seen. Magn.: approx. ×14 000

and electron lucent matrices. However, the granular alterations, although less pronounced, could still be recognized.

In the light of these staining characteristics of the eosinophils, the two different types of densities observed in the granules of one and the same material by WELSH and GEER [10], GHADIALLY and PARRY [11] and HUDSON [8] might be indicative of different functional states or are the result of fixation and staining procedures. In our study the reversal of granule densities was the sign of an alteration leading to the disintegration of the granules.

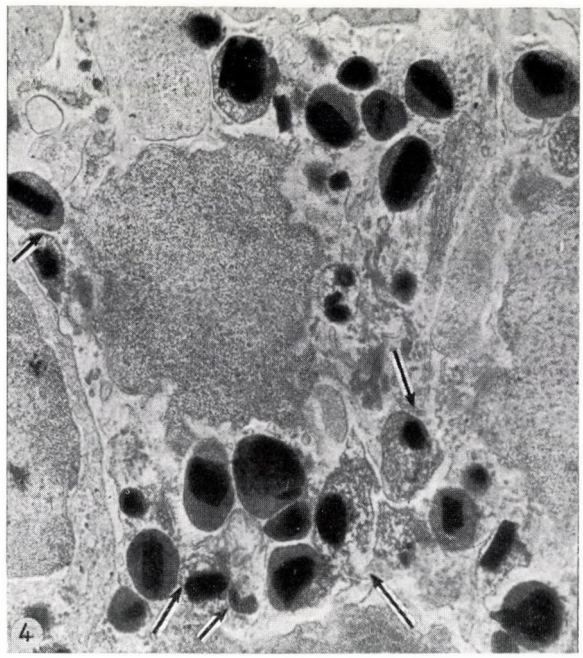


Fig. 4. Disrupted eosinophil granulocyte. Some of the granules (arrows) show granular-vesicular matrices and disrupted membranes. Magn.: approx. ×9000

As to the possible functional significance of the changes of the eosinophil granules, it may be assumed that during their disintegration, which may be initiated by intragranular peptidases, active substances, enzymes are liberated. The observation of SEEMAN and PALADE [12] that acid phosphatase was inactive in seemingly intact granules with dense and compact matrices but active in granules whose matrices were slightly less dense and compact, support this assumption.

REFERENCES

1. Archer, G. T., Hirsch, J. G.: (1963) Motion Picture Studies on Degranulation of Horse Eosinophils during Phagocytosis. J. exp. Med. **118,** 287. — 2. Litt, M.: (1964) Studies in Experimental Eosinophilia. VI. Uptake of Immune Complexes by Eosinophils. J. Cell Biol. **23,** 335. — 3. Teir, H.: (1966) Neubildung und Abbau der Granulozyten. Verh. dtsch. Ges. Path. **50,** 219. — 4. Venable, J. H., Coggeshall, R.: (1965) A Simplified Lead Citrate Stain for Use in Electron Microscopy. J. Cell Biol. **25,** 407. — 5. Gusek, W.: (1960) Zur Ultrastruktur und Natur der Granula in Fibrozyten und "pseudoeosinophilen" Leukozyten im faserbildenden Granulationsgewebe. Frankf. Z. Path. **70,** 523. — 6. Bo-Rasmussen, F., Egeberg, J.: (1966) The Ultrastructure of Eosinophilic Granulocyte in the Peritoneal Cavity of Rats Following Injection of Ferritin. Scand. J. Haemat. **3,** 257. — 7. Ross, R., Klebanoff, S. J.: (1966) The Eosinophil Leukocyte. Fine Structure Studies of Changes in the Uterus during the Estrous Cycle. J. exp. Med. **124,** 653. — 8. Hudson, G.: (1967) Eosinophil Granules and Uranyl Acetate. An Electron Microscopic Study of Guinea Pig Bone Marrow. Exp. Cell Res. **46,** 121—9. Kelényi, G., Zombai, E., Németh, Á.: (1965) Histochemische und elektronenmikroskopische Beobachtungen an der spezifischen Granulation der eosinophilen Granulozyten. Acta histochem. **22,** 77. — 10. Welsh, R. A., Geer, G. C.: (1959) Phagocytosis of Mast Cell Granule by the Eosinophilic Leukocyte in the Rat. Amer. J. Path. **35,** 103. — 11. Ghadially, F. M., Parry, E. W.: (1965) Possible Significance of Some Morphological Variations in the Eosinophil Granule Revealed by the Electron Microscope. Nature (Lond.) **206,** 632. — 12. Seeman, P. M., Palade, G. E.: (1967) Acid Phosphatase Localization in Rabbit Eosinophils. J. Cell. Biol. **34,** 745.

Dr. Gábor Kelényi; Pécs, Dischka Győző u. 5., Hungary

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 271—283, 1968*

Institute of Cytology (Director: Prof. Dr. A. S. Troshin) of the Academy of Sciences of the USSR, Leningrad

THE ULTRASTRUCTURE OF DIFFERENTIATING CELLS OF THE HEART MUSCLE IN THE STATE OF MITOTIC DIVISION

P. P. Rumyantsev and E. S. Snigirevskaya

(Received April 15, 1967)

An electron microscopic analysis of the mitotic figure in the developing rat myocardium (3, 5, 15, 19 day embryos and 2—3 day old rats) has revealed in all phases of the division of muscle cells the presence of bundles of hexagonally arranged myofilaments and specialized contact structures (intercalated discs and/or desmosomes). There is a partial disappearance of Z-discs and disorientation of myofilament bundles after onset of the metaphase, but no complete dedifferentiation of dividing myocardial cells.

Hence the current theory of myogenesis stating that DNA synthesis and mitosis are inhibited after the onset of elaboration of contractile proteins is not applicable to the cardiac muscle cells. These results are in agreement with the previously obtained evidence of 3H-thymidine incorporation into the nuclei of the heart muscle cells containing some myofibrils.

Myogenesis attracts the attention of investigators not only as a special problem but as one of the models for the analysis of cytodifferentiation as a whole [19, 25, 30].

In the recent decade the concept concerning the way of nuclear multiplication [4, 7, 9, 45] and its relation to synthesis of cell specific proteins [4, 19, 25, 30, 37, 45] in the course of myogenesis has been intensively revised using modern methods of cytological analysis. The following statements have been formulated.

1. DNA synthesis and mitoses occur only in non-differentiated separate myoblasts; 2. the onset of synthesis of contractile proteins is accompanied by a stable repression of genes controlling the multiplication of the muscle cell; 3. the pictures previously regarded as numerous amitoses are found to be in fact dependent on the shifting, clumping, rotation of neighbouring nuclei, on the folding of their membranes, etc.

The above conclusions have been derived solely from the investigation of somatic muscles in vitro, during regeneration or in the course of their normal histogenesis.

It must be stressed that no peculiarities concerning the relationship of proliferation and differentiation processes in the developing heart muscle cells have been taken into account.

Since the publication of the works by Hoyer [20], Godlewski [14] and Schockaert [36] many investigators have, however, described and illustrated

mitotic figures frequently observed in the fibres of the growing heart muscle [27, 33, 34, 39].

The evidence yielded by light microscopy shows that in the myocardial fibres division takes place in centrally located muscle nuclei; a certain number of myofibrils are usually observed in the surrounding of the chromosomes [34].

In full accord with these facts the analysis of ^{3}H-thymidine incorporation has shown that in the myocardium the DNA synthesizing nuclei are located within the myofibril containing muscle fibres. The index of labelled nuclei reaches 10—12% even in newborn rats after a single administration of precursor and up to 40—50% if repeated injections of ^{3}H-thymidine are given. Only for 15-day-old rats does the index practically attain the zero level [33, 34].

The above data evidently contradict the concept of absolute competition and mutual exclusivity of auto- and heterosynthesis in muscle cells [4, 19, 25, 30, 37]. It should, however, be noted, that this statement may only be convincing if confirmed by ultrastructural data, light microscopy being unable to resolve the membranes of individual cells and to prevent errors concerning the tissue nature of proliferating elements [30]. Thus, the nuclei in the S phase which seem to be localized "inside" muscle fibres containing myofibrils, may in fact belong to a non-differentiated myoblast or even to a connective tissue cell pressed between the processes of muscle fibres.

The purpose of the present work was to study the ultrastructure of muscle fibres containing mitotic figures in the growing myocardium and to determine whether chromosomes and myofibrils are actually localized within the boundaries restricted by a common plasma membrane of the same cell.

Among the numerous works dealing with the electron microscopy of a developing [18, 28, 29, 35, 40, 41, 42) or cultivated [8, 22, 43, 44] myocardium only WOHLFARTH-BOTTERMAN [44] gave a picture of the dividing cell, which he regards as a myoblast despite the absence of any specific morphological traits (e.g. myofibrils). MUIR [29], makes a causal mention of mitoses of myocardial cells which contain myofibrils giving no illustration whatever. It is even doubtful whether this statement of MUIR reflects electron microscopic observations. On the contrary, WAINRACH and SOTELO [42] describe numerous mitoses in heart myoblasts of young chick embryos stressing the fact that no myofibrils could be found in such cells. WEISSENFELS [43] states that during division of cardiac muscle cells around the mitotic figure one can see only the sarcoplasmic masses, the myofibrils being completely dissolved. In spite of the fact that this paper is dealing with the submicroscopic structure of the heart muscle in vivo and in vitro WEISSENFELS presents only light microscopic pictures of dividing myocardial cells saying that the rare occurrence of mitoses in the heart muscle makes the finding of mitotic figures under the electron microscope practically impossible. Indeed, in the chick embryo myocardium the mitotic index does not exceed 3.15% for all the stages of development [15].

In the course of myocardial histogenesis in the rat the index is reduced from 2.5% to 1.3% during the period from the 15th day of embryonal life up to birth [34]. This low incidence of mitoses in ultrathin sections is the reason why in electron microscopic investigations into the myogenesis of skeletal muscles [1, 2, 10, 13, 17, 31] the question of mitotic figures has never been discussed and no illustrations have been presented.

Material and method

Pieces of heart ventricle (1—2 cu. mm) from 13—15—19-day white rat embryos and newborn rats (2—3 days after birth) were fixed with cold 1% OsO_4 at pH 7.4. After dehydration in alcoholic series the pieces were embedded in butyl-methyl-metacrylate (8 : 1) and in some cases in Araldite. Staining was done during dehydration by the addition of uranyl acetate and phosphotungstic acid to 96% alcohol, and in sections with uranylacetate and lead citrate solutions. Sections on copper grids covered with formvar film were examined under the JEM-5g electron microscope. Micrographs were taken at 3000—12,000 magnification and subsequently enlarged to the desired size. In the majority of cases, with 3—4 hrs of work we could reveal no more than one or at most two mitotic figures. The present paper deals with the observation of several dozens of mitotic figures located within cardiac muscle fibres in all the stages of myocardial histogenesis.

Results

The most conspicuous trait of mitotic figures as seen under the electron microscope is the presence of chromosomes the sections of which appear as moderately contrasted granulated filamentous masses devoid of any membrane coating (Figs 1, 2, 3). A separate distribution of individual chromosomes (Fig. 2) occurs as well as their superimposition (Fig. 3) in which case there are extended masses of chromosomal material. In longitudinal and tangential sections the chromosomal substance often looks like a bundle of innumerable spiralized threads. Taking into account some typical changes of the cytoplasmic structure during cell division (rounding and swelling of the cell body, slightly increased density of cytoplasmic matrix as compared with non-dividing cells, etc.) often helps to reveal mitotic figures under the electron microscope. In some cases it is much more difficult to determine precisely the phase of division in ultrathin sections than in the case of light microscopic preparations.

Early prophase cells until the onset of nuclear membrane desaggregation can be detected owing to the appearance inside the nucleus of numerous chromosomes (Fig. 1) which differ in size and are randomly outlined masses of chromatic material present in some non-dividing nuclei. The late prophase, meta- and anaphase cells may be identified in the case of a well-oriented section due to the typical distribution of chromosomes in the cytoplasm (Figs 2, 3).

Whatever the plane of section it is easy to reveal telophase cells. The electron microscope permits to visualize not only the "packing" of chromo-

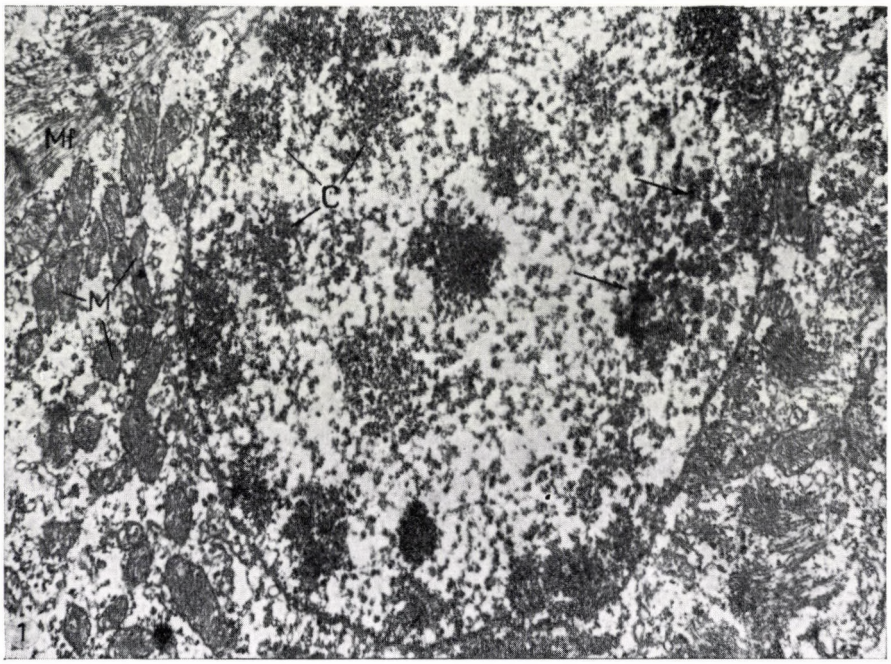


Fig. 1. Nucleus of heart muscle cell of 19-day-old rat embryo at the stage of early prophase. C — chromosome, Mf — myofibrils, M — mitochondria. The arrows mark a group of electrodense structures, probably indicating the onset of nucleolar desaggregation. ×10 700

somes which form compact masses, but also the reconstruction process of the nuclear membrane of which flattened vesicles and cisternes originating probably from elements of the endoplasmic reticulum and chromosomal cluster surface (Fig. 4) are quite typical. The components of the mitotic apparatus, centrioles and spindle filaments, are seldom revealed as the former is usually beyond the plane of section, and the latter is well preserved only after some special procedures (see ref. 3, 16). Anyhow, lead citrate staining in some cases reveals spindle filaments in the form of typical microtubules [3, 16, 26, 32] about 200 Å in diameter (insertion in Fig. 4). In a great number of mitotically dividing cells observed at different stages of myocardial development, the cytoplasm contained bundles of typical myofilaments among which thick myosin threads about 150 Å in diameter and sometimes thin filaments of 50 Å were clearly visible (Figs 2, 3, 4). Cross sections of such bundles reveal a hexagonal pattern of individual filaments (Fig. 3) characteristic of myofibrils [21]. The larger bundles of myofilaments are frequently located at the polar region of dividing muscle cells (Fig. 3). Closer to the equatorial zone, the accumulation of sarcoplasmic masses pushes the myofilament bundles towards the surface membrane. As a result, they are distributed in this zone as a very thin discontinuous layer and in the section only a few myofilaments (Figs 2, 3)

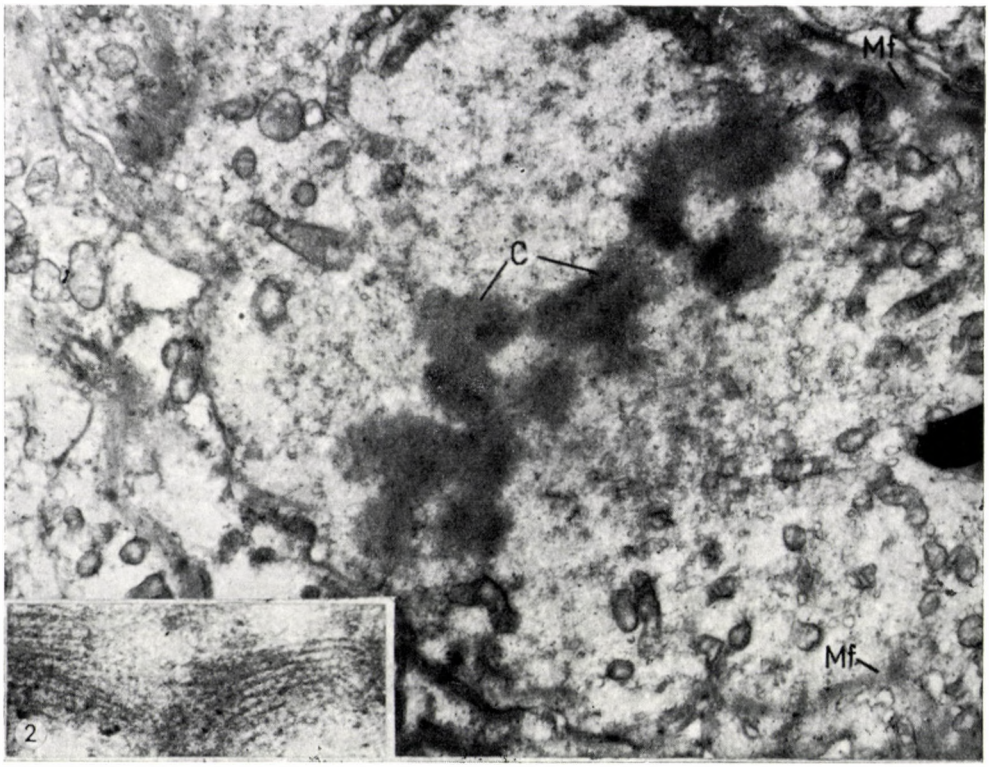


Fig. 2. Metaphase in heart muscle cell of 13-day-old rat embryo. Mf — myofibrils; C — chromosomes some of which appear as paired structures; M — mitochondria. ×9700. Insertion enlarged portion of myofibril seen at right upper corner of Fig. 2. ×40 000

can be seen usually. In many a case one may observe myofilaments in the close proximity of the chromosomal substance, this may be due to the tangential plane of the section. Unlike the non-dividing heart muscle cells the mitosis is characterized as a rule by a more disordered orientation of myofilament bundles which results in the transverse, oblique as well as longitudinal sectioning of such bundles in the adjacent regions of the cytoplasm (Figs 3, 4). Moreover, the subdivision of myofilament bundles into sarcomeres by contrasting Z-lines was unfrequent during mitosis, it mostly occurred before the metaphase. It must, however, be stressed that in the early prophase (Fig. 1) no appreciable change in myofibrillar structure can be detected. In the late telophase we hardly ever found typical Z-discs (Figs 2, 3, 4), a fact strikingly distinguishing the myofibrillar apparatus of the dividing cells from that of interkinetic ones where Z-discs are usually prominent. The partial disappearance of Z-discs including their membranous component [12], is indicated by the fact, too, that in the metaphase the myofilament bundles sometimes are to be

Fig. 3. Anaphase in heart muscle cell of 19-day-old rat embryo. C — chromosomes; M — mitochondria. In the left upper corner myofibrils of one adjacent cell are seen. ×16 000. Insertion: detail of the polar part of an anaphasic cell, representing disordered myofilament (Mfl) bundles which are sectioned longitudinally, transversally (arrows) and obliquely. ×37 000

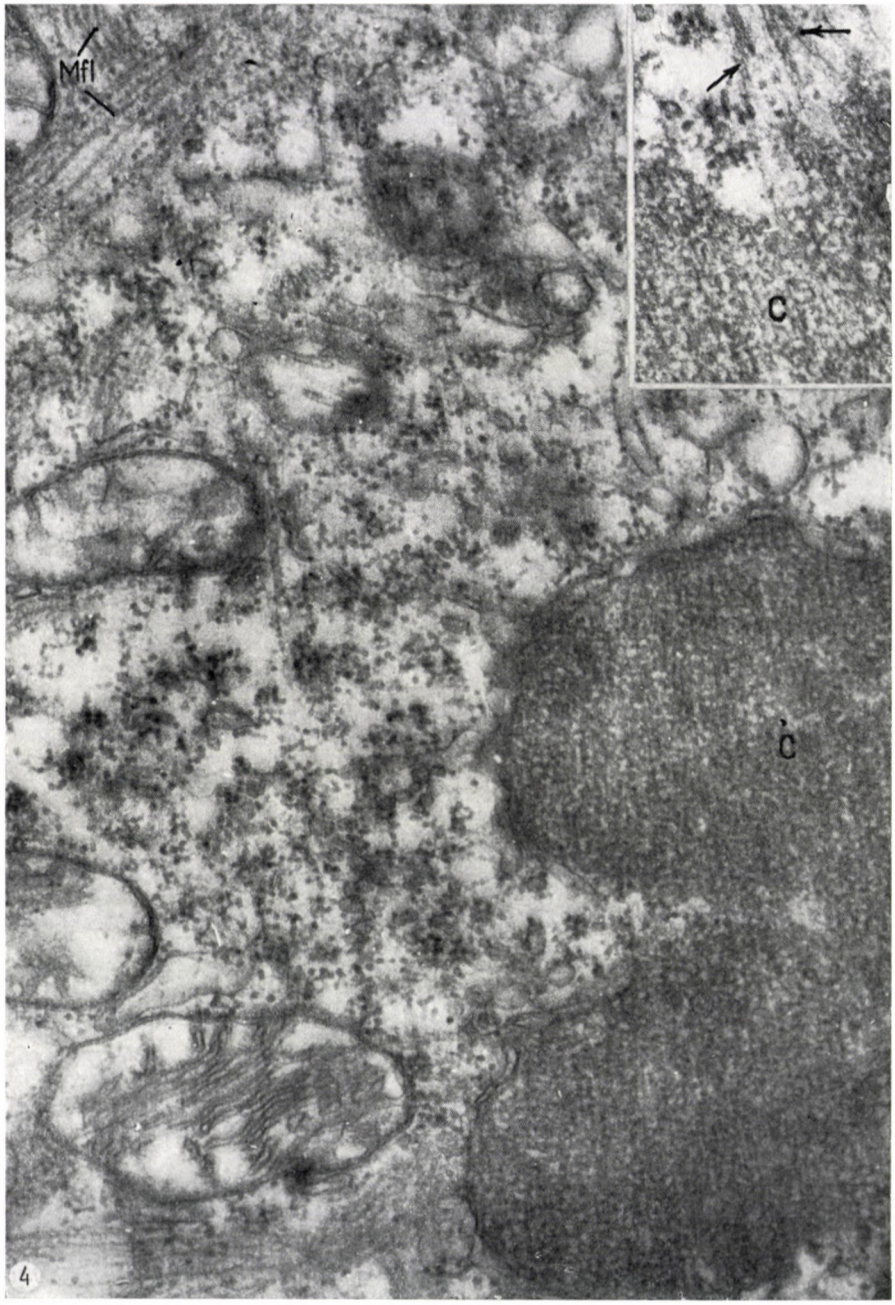


Fig. 4. Telephase chromosomes (C) in heart muscle cell of a 2-day-old rat. On the surface of the chromosomes, membranous coating reconstituting the nuclear envelope is visible. Mfl — myofilament bundles. ×45 700. The figure inserted at the upper right represents another portion of the same mitotic cell where double profiles of spindle filaments (arrows) near the chromosomal (C) material are seen. ×56 200

subdivided into sections of about the same length as that of sarcomeres in myofibrils of adjacent non-dividing cells; some rarefications instead of absent Z-lines were seen here. The partial or even complete disappearance of some Z-lines may play an important role in the above-described disorientation of myofilament bundles.

The second typical trait enabling us to identify the tissue nature of dividing heart muscle cells is the persistence at the boundary of a great number of such cells, special contact structures, desmosomes and/or primitive intercalated discs. This persistance of desmosome-like structures prevents the mitotic cell from a complete rounding off and from the loss of its association with the neighbouring ones. The tendency for such a separation is shown by the frequent divergence particularly in the equatorial region of membranes of mitotic and adjacent muscle cells, resulting in the formation of some kinds of boundary lacuna. Sometimes such lacunae are found to be crossed by cytoplasmic bridges rich in myofilaments which belong to dividing or on the contrary, to the adjacent non-dividing cells. A similar persistence of desmosomes preserving the zones of close contact between dividing and interkinetic cells, was observed in the epidermis [6].

The fact that dividing muscle elements are frequently considerably larger than mesenchymal mitotic cells is used as an auxiliary criterion helping to distinguish the two kinds of cell. The ultrastructure of mitochondria in mitotic and interkinetic myocardial cells seems to be rather similar; the mean size of organelles, the number of their cristae, and the degree of matrix contrast are typical for the given stage of histogenesis. By the time the chromosomes have settled in the equatorial plane the mitochondria become located as two separate complexes on both sides of the metaphase plate closely to the polar zones of the cell (Fig. 2). In ana- and telophase cells, many mitochondria move toward the equatorial zone (Fig. 3) wherein they are distributed between daughter cells after the end of division. The elements of endoplasmic reticulum to which vesicles originating from the dissolved nuclear membrane are probably added [32] form in the metaphase and the early anaphase two "caps" on both sides of the equatorial plate. The walls of these vesicles are as a rule smooth membranes not covered with ribosomes. We also found in some of dividing cardiac muscle cells, small isolated tubuli lying beyond the "caps", which belong to the "rough" type of endoplasmic reticulum observed in a number of interkinetic embryonic myocardial cells [28]. Smooth membrane vesicles forming sometimes small isolated groups might originate from the Golgi elements. More than once, in meta- and anaphase cells, we observed lysosome-like granules with the contrast content surrounded by membranes.

Large lipid drops frequently observed in myocardial cells usually were not present during mitosis. Randomly distributed in cytoplasmic granules

150—250 Å in diameter may represent both free ribosomes and glycogen; sometimes it is rather hard to distinguish them.

The comparison of mitotic figures of heart muscle cells at different stages of myocardial histogenesis has shown that all the above-mentioned traits may occur irrespective of a differentiation level of the muscle cells beginning to divide. At later stages of development a somewhat increased number of myofilaments and hence of their bundles has only been observed.

Discussion

Ultrastructural analysis of mitotic figures in the developing myocardium has wholly confirmed the evidence of light microscopy [33, 34, 36] showing that karyokinetic division is accomplished in cardiac muscle cells which have accumulated some amount of myofibrils. The data obtained are in full accord with our previous observations concerning the incorporation of ^{3}H-thymidine into the nuclei of cardiac muscle cells in the course of differentiation [33, 34]. Taking into account the current concept of myogenesis [4, 19, 25, 30, 37], it would be reasonable to assume that ^{3}H-thymidine is incorporated not into the nuclei of partially differentiated cardiac muscle cells but into those of persisting primitive myoblasts intimately connected with the cells containing myofibrils. In such a case, however, it is difficult to understand the way by which the numerous myofilaments, desmosomes and cardiac muscle cells may be elaborated during a period G_2 lasting in the embryonal myocardium 2—2.5 hours [33, 34]. Moreover, at the investigated stages of histogenesis we found no cells the ultrastructure of which would have made us to regard them as primitive non-differentiated myoblasts without any myofibrils. All these data have made us to discard the hardly probable suggestion that DNA synthesis and mitoses take place in different categories of heart myogenic elements and to state that both these fundamental acts of the cell cycle are well compatible with a certain degree of differentiation of myocardial cells.

The obtained evidence permits of quite a different interpretation of the concepts developed by some investigators [5, 19, 25, 37] concerning the competition of contractile protein synthesis and reduplication of DNA and division of nuclei during myogenesis. This competition was commonly interpreted as an absolute one, i.e. complete cessation of DNA synthesis and mitosis in the myogenic cell just after it begins producing myosin and actin [19, 25, 30, 37]. This view was based solely on the analysis of skeletal muscle histogenesis. The above electron microscope evidence and the previously obtained autoradiographic data [33, 34] have shown that in the developing myocardium there is no similar "absolute" form of competition between cell reproduction and differentiation. The interference of these processes may be revealed during myocardial histogenesis only as a step by step withdrawing from the mitotic

cycle of an increasing number of muscle cells which have accumulated some "critical mass" of contractile proteins [33, 34]. The gradual increase in the duration of the mitotic cycle periods in more differentiated cardiac muscle cells [33, 34] shows that these cells must overcome some difficulties when providing for multiplication processes simultaneously with a progressive accumulation in the cytoplasm of specific proteins. Perhaps the different forms of competition between the processes of cell reproduction and differentiation are not necessarily based solely on the repression of genes controlling the former of these processes (the synthesis of DNA polymerase, proteins of mitotic apparatus, etc.) just after the onset of elaboration of specific proteins as it is suggested by HOLTZER et al. [19, 30, 37]. It is quite possible that the cell is not able to supply with sufficient energy both these processes simultaneously, especially if differentiation proceeds very rapidly. Thus, may be the case in skeletal muscle histogenesis as compared with that of the myocardium the cells of which accumulate myosin and actin much more gradually.

The structure of tissue elements may also play a significant role in the determination of the degree of competition between the processes of differentiation and reproduction. In the course of formation of giant plasmodial fibres of skeletal muscles, these processes may easily be separated spatially, the non-differentiated myoblast being the cell for reduplication, while the multinucleated fibre formed by fusion of myoblasts is limited to the accumulation of specific proteins [19, 25, 30]. However, according to ZHINKIN and ANDREEVA [45] and KITIYAKARA and ANGEVINE [24], 1—2 hrs after ^{3}H-thymidine injection one can see labelled nuclei even in myofibril-containing multi-nucleated fibres of developing skeletal muscles. These data call for a revision of current views concerning the relationship between the proliferation processes and the synthesis of specific proteins during histogenesis of that type of muscle. An indispensable condition of such a revision is the analysis of skeletal muscle histogenesis at the ultrastructural level which is the only way to exclude errors concerning the presence or absence of protoplasmic continuity between the proliferating cell and the multinucleated fibre.

It is premature to decide if one can interpret as a sign of "dedifferentiation" the changes in the structure of specialized components of the heart muscle cell which accompany mitosis such as the partial disappearance of Z-lines, desorientation and possible dissolution of a part of myofilament bundles, and some simplification of intercalated disc structures. A brief note in the abstract of the paper of KASTEN et al. [23], stating that cultured rat heart muscle cells continue to contact during mitosis, indicates that if some "dedifferentiation" would indeed take place, it is not enough pronounced. In any case, the preservation during division of a great number of myofilament bundles and of specialized contact structures, even if simplified and disordered to some degree, must facilitate the rapid redifferentiation of postmitotic cells.

Owing to the difficulties in finding mitotic figures in ultrathin sections, further investigations are necessary to elucidate many important details of karyokinetic division in myocardial cells (such as nuclear membrane desaggregation in the prophase, cytokinesis in the telophase, partial disappearance and restoration of Z-discs, etc).

Studies on the ultrastructure of the developing myocardium failed to present reliable suggestions concerning amitoses of its muscle nuclei observed by a number of investigators mainly during the postnatal histogenesis [27, 39]. A rarely observed occurrence of two close "paired" nuclei in the same cell may be interpreted as a result of sectioning of an infolded nuclear membrane or of the absence of plasmatomy in the late telophase, especially if the numerous recent data speaking against amitoses in the developing skeletal muscle fibres are taken into account [4, 7, 9, 25, 38, 45].

REFERENCES

1. Allbrook, D.: (1962) An Electron Microscopic Study of Regenerating Skeletal Muscle. J. Anat. **96,** 137—152. — 2. Allen, E. R., Pepe, F. A.: (1965) Ultrastructure of Developing Muscle Cells in the Chick Embryo. Am. J. Anat. **116,** 115—148. — 3. Barnicot, N. A., Huxley, H. E.: (1965) Electron Microscope Observations on Mitotic Chromosomes. Quart. J. micr. Sci. **106,** 197—214. — 4. Bassleer, R.: (1963) Application des techniques cytologiques modernes à l'étude du problème des amitoses dans les bourgeons musculaires. Symp. int. Soc. for Cell Biology **2,** 289—312. New York. — 5. Betz, E. H., Firket, H., Reznik: (1966) Some Aspects of Muscle Regeneration. Int. Rev. Cytol. **19,** 203—227. — 6. Buck, R. C., Krishan, A.: (1965) Site of Membrane Growth during Cleavage of Amphibian Epithelial Cells. Exp. Cell Res. **38,** 426—428. — 7. Capers, C. R.: (1960) Multinucleation of Skeletal Muscle in vitro. J. biophys. biochem. Cytol. **7,** 559—566. — 8. Cedegren, B., Harary, J.: (1964) In vitro Studies on Single Beating Rat Heart Cells. VII. Ultrastructure of the Beating Cell Layer. J. Ultrastruct. Res. **11,** 443—454. — 9. Cooper, W. G., Königsberg, R.: (1961) Dynamics of Myogenesis in vitro. Anat. Rec. **140,** 195—200. — 10. Dessouky, D. A., Hibbs, R. G.: (1965) An Electron Microscope Study of the Development of the Somatic Muscle of the Chick Embryo. Amer. J. Anat. **116,** 523—566. — 11. Firket, H.: (1958) Recherches sur la synthèse des acides désoxyribonucléiques et la préparation à la mitose dans des cellules cultivées in vitro (Étude cytophotométrique et autoradiographique). Arch. Biol. **69,** 1—166. — 12. Franzini-Armstrong, C., Porter, K. R.: (1964) The Z-disc of Skeletal Muscle Fibrils. Z. Zellforsch. **61,** 661—672. — 13. Гилёв В. П.: (1959) Изучение регенерации поперечнополосатой мышечной ткани в электронном микроскопе. ДАН СССР, **124,** 485–489. —14. Godlewski, E.: (1902) Die Entwicklung des Skelett und Herzmuskelgewebes der Säugetiere. Arch. mikr. Anat. **60,** 111—157. — 15. Grohmann, D.: (1961) Mitotische Wachstumsintensität des embryonalen und fetalen Hühnchenherzens und ihre Bedeutung für die Entstehung von Herzmißbildungen. Z. Zellforsch. **55,** 104—122. — 16. Harris, P.: (1962) Some Structural and Functional Aspects of the Mitotic Apparatus in Sea Urchin Embryos. J. Cell Biol. **14,** 475—487. — 17. Hay, E. D.: (1963) The Fine Structure of Differentiating Muscle in the Salamander Tail. Z. Zellforsch. **59,** 6—34. — 18. Hibbs, R. I.: (1956) Electron Microscopy of Developing Cardiac Muscle in Chick Embryos. Amer. J. Anat. **99,** 17—51. — 19. Holtzer, H.: (1961) Aspects of Chondrogenesis and Myogenesis. In: Synthesis of Molecular and Cellular Structure. New York, Pp. 35—87. — 20. Hoyer, H.: (1899) Über die Struktur und Kernteilung der Herzmuskelzellen. Bull. int. Acad. Sci. Cracoviae **9,** 487—490. — 21. Huxley, H. E.: (1957) The Double Array of Filaments in Cross-striated Muscle. J. biophys. biochem. Cytol. **3,** 631—648. — 22. Jonek, J., Stockinger, L.: (1964) Die Ultrastruktur von Langzeit-Kulturen embryonaler Hühnchenherzen. Protoplasma **58,** 641—662. — 23. Kasten, F. H., Bovis, R., Mark, G.: (1965) Phase Contrast Observations and Electron Microscopy of Cultured Newborn Rat Heart Cells. J. Cell Biol. **27,** 112A (Abstr.) — 24. Kitiyakara, A., Angevine, D. M.: (1963) A Study of the Pattern of Postembryonic Growth of M. gracilis in Mice. Develop. Biol. **8,** 322—340. — 25. Königsberg, J. R.: (1965) Aspects of Cytodifferentiation of Skeletal Muscle. In: Organogenesis. Winston, New York. — 26. Королев,

М. Б., Шестопалова, Н. М., Чумакова, М. Я.: (1966) Электронномикроскопическое изучение делящихся клеток трансформированной культуры тканей. ДАН СССР. **166,** 716–718.–27.– 27. Кочетов, Н. Н.: (1959) О сходстве структур, выявляющемся при сопоставлении гистогенеза миокарда человека и строения сердца в ряду позвоночных. ДАН СССР. **128,** 202–204. –28. Leak, L. V., Burke, J. F.: (1964) The Ultrastructure of Human Embryonic Myocardium. Anat. Rec. **149,** 623—650. — 29. Muir. A. R.: (1957) An Electron Microscopic Study of the Embryology of the Intercalated Disc in the Heart of the Rabbit. J. biophys. biochem. Cytol. **3,** 193—202. — 30. Okazaki, K., Holtzer, H.: (1965) An Analysis of Myogenesis in vitro Using Fluorescein-labelled Antimyosin. J. Histochem. Cytochem. **13,** 726—739. — 31. Price, H. M., Howes, E. L., Blumberg, J. M.: (1964) Ultrastructural Alterations in Skeletal Muscle Fibres Injured by Cold. II. Cells of the Sarcolemmal Tube: Observations on "Discontinuous" Regeneration and Myofibril Formation. Lab. invest. **13,** 1279—1302. — 32. Robbins, E., Conatas, N. K.: (1964) The Ultrastructure of a Mammalian Cell during the Mitotic Cycle. J. Cell Biol. **21,** 429—263. — 33. Rumyantsev, P. P.: (1963) A Morphological and Autoradiographical Study of the Peculiarities of Differentiation Rate, DNA Synthesis and Nuclear Division in the Embryonal and Postnatal Histogenesis of Cardiac Muscles of White Rats. Folia histochem. Cytochem. **1,** 463—471. — 34. Румянцев, П. П., Соколовская, И. А.: (1964) Синтез ДНК и кинетика пролиферации ядер в ходе дифференцировки мышцы сердца. В сб.: Исследование клеточных циклов и метаболизма нуклеиновых кислот при дифференциации клеток, 71—82, М.—Л. — 35. Schiebler, T. H., Wolff, H. H.: (1966) Elektronenmikroskopische Untersuchungen am Herzmuskel der Ratte während der Entwicklung. Z. Zellforsch. **69,** 2240. — 36. Schockaert, A.: (1909) Nouvelles recherches comparatives sur la texture et le développement du myocarde chez les vertébrés. Arch. Biol. (Liège) **24,** 277. — 37. Stockdale, F., Holtzer, H.: (1961) DNA Synthesis and Myogenesis. Exp. Cell Res. **24,** 508—520. — 38. Strehler, B. L., Konigsberg, I. R., Kelley, J.: (1963) Ploidy of Myotube Nuclei Developing in vitro as Determined with a Recording Double Beam Microspectrophotometer. Exp. Cell Res. **32,** 232—241. — 39. Törő, I.: (1939) Über die Kernteilung im Herzmuskel. Verh. Anat. Ges. **47,** 230—241. — 40. Virágh, Sz.: (1964) The Cardiac Mesenchymatous Cells and their Transformation into Myoblasts. Proceedings of the Third European Regional Conference on Electron Microscopy. Prague. B, 95—96. — 41. Virágh, S., Porte, A.: (1963) Electron-microscopic Studies of Myocardial Development in Embryo. Acta morph. Acad. Sci. hung. Suppl. **12,** 32. — 42. Wainrach, S., Sotelo, J. R.: (1961) Electron Microscope Study of the Developing Chick Embryo Heart. Z. Zellforsch. **55,** 622—634. — 43. Weissenfels, N.: (1962) Der Einfluß der Gewebezüchtung auf die Morphologie der Hühnerherzmyoblasten. IV. Über Differenzierungs- und Abbauvorgänge an den Muskelelementen. Protoplasma **55,** 99—113. — 44. Wohlfarth-Botterman, K. E.: (1959) Gestattet das elektronmikroskopische Bild Aussagen zur Dynamik in der Zelle? Cytologische Studien VI. Z. Zellforsch. **50,** 1—27. — 45. Zhinkin, L. N., Andreeva, L. F.: (1963) DNA Synthesis and Nuclear Reproduction during Embryonic Development and Regeneration of Muscle Tissue. J. Embryol. exp. Morph. **11,** 353—367.

ULTRASTRUKTUR DER DIFFERENZIERENDEN MYOKARDZELLEN WÄHREND DER MITOSE

P. P. RUMJANZEW und E. S. SNIGIREWSKAJA

Die elektronenmikroskopische Analyse der Kernteilung im Myokard von 3, 5, 15 und 19 Tage alten Rattenembryonen und 2—3 Tage alte Ratten zeigte in sämtlichen Teilungsphasen der Muskelzellen das Vorhandensein von Bündeln aus hexagonal geordneten Muskelfasern und spezifischen Kontaktstrukturen (Interkalarscheiben und/oder Desmosomen). Nach Einsetzen der Metaphase läßt sich ein teilweises Schwinden der Z-Scheiben und eine Desorientation der Muskelfaser-Bündeln, jedoch keine vollkommene Dedifferentiation der in Teilung begriffenen Myokardialzellen beobachten.

Die übliche Theorie der Muskelgenese, nach der die DNA-Synthese und die Mitose nach dem Einsetzen des Aufbaus kontraktiler Proteine eine Hemmung erleiden, läßt sich daher auf die Myokardzellen nicht anwenden. Diese Ergebnisse stimmen mit der früheren Beobachtung überein, daß in die Myofibrillen enthaltenden Zellkerne des Myokards 3H-Thymidin inkorporiert wird.

УЛЬТРАСТРУКТУРА ДИФФЕРЕНЦИРУЮЩИХСЯ КЛЕТОК МИОКАРДА ВО ВРЕМЯ МИТОЗА

П. П. РУМЯЩЕВ и Е. С. СНИГИРЕВСКАЯ

Электронномикроскопический анализ деления клеток в развивающемся миокарде (3, 5, 15 и, 19-дневные зародыши и 2—3-дневные крысы) показал во всех фазах деления мышечных клеток наличие пучков из гексагонально размещенных мышечных волокон и специфических контактных структур (интеркалярных дисков и/или десмосом). После начала метафазы выявляемо частичное исчезновение Z-дисков и дезориентация пучков мышечных волокон, однако полной дедифференциации делящихся миокардиальных клеток не наблюдается.

Следовательно, обычная теория о миогенезе, согласно которой синтез ДРК и митоз после начала производства контрактильных белков потерпевают торможение, в отношении миокардиальных клеток несостоятельна. Эти результаты соответствуют прежним наблюдениям, что в ядра клеток миокарда, содержащие миофибриллы, встречвается ЗН-тимидин.

P. P. Rumyantsev
E. S. Snigirevskaya
Leningrad, F-121, pr. Maklina, 32, USSR

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 285—293, 1968*

Department of Anatomy (Director: Prof. J. SZENTÁGOTHAI),
University Medical School, Budapest

MONOAMINERGIC INNERVATION OF EXTRA- AND INTRACEREBRAL VESSELS

T. DONÁTH

(Received May 30, 1967)

As judged from the complete lack of surrounding nerve elements that would give fluorescence effect with the Falck-Hillarp technique, intracerebral vessels have no adrenergic innervation. The sparse adrenergic nerve plexus of the pial vessels gives few side branches to the initial portion of the intracerebral vessels, which can be traced only for about a few tens of microns as far as the vessels are ensheathed by the leptomeninx. A cellular sheath having a strong autofluorescence of unknown nature continues along the intracerebral course of the vessels.

Literature on cerebral vessels is highly controversial. Early observers (KOELLIKER, 1896; HUNTER 1900, LAPINSKY, 1913) described nerves joining vessels that enter into the brain tissue, while others (BERGER, 1924; HASSIN, 1929) failed to find nerves along the intracerebral vessels. Later CLARK [1929, 1934], PENFIELD [1932], CHOROBSKI—PENFIELD [1932], GRIGORIEVA [1932], HUMPHREY [1939], BAKAY [1941], HADJIOLOV et al. [1954] and HAGEN [1955] demonstrated pictures of an abundant cerebral vascular innervation. On this basis, however, STÖHR [1957] took for granted the direct nervous regulation of intracerebral vessels. More recent studies using the electron microscope (PEASE—MOLLINARI [1960], SAMARASINGHE [1963, 1965]) did not give any support to the assumption of an innervation of the intracerebral vessels. While HAGEN reported nervous elements to penetrate into the media and even to reach the intima forming a terminal reticulum, the electronmicroscopical pictures did not reveal nerves in the adventitia of the intracerebral vessels.

The problem of the tonic regulation of extra- and intracerebral vessels is of crucial importance for cerebral blood circulation. It would be, therefore, decisive to show, whether the cerebral vessels have any vasomotor nervous regulation, or whether, as found recently in the retina, the tonus of the vessels lacking innervation might be regulated by chemical mechanisms (HOGAN—FEENEY [1963]; or, alternatively, as surmised by FOG [1939]), by simple autoregulation of the contractile elements through mechanical forces.

The usual way of investigating the innervation of intracerebral vessels was to examine silver stained sections. Impregnation, however, seems not to be an appropriate method for the detection in the cerebral tissue of the perivascular nerve fibres. This is on the one hand due to the fact that the nerve

cannot be differentiated with certainty from the argyrophilic connective tissue, and on the other hand, that the intensely argyrophile central nervous tissue appears to consume all the silver available for impregnation, so that nothing is left for the delicate and less argyrophilic vasomotor fibres. Thus, identification of the nerves depends mainly on the judgement of the investigator. Furthermore, silver stains do not permit any conclusion concerning the functional character of the fibre.

Method

The present study being concerned exclusively with the monoaminergic fibre system, the highly sensitive and specific fluorescence method was used. Instead of the usual histological sections it was found advantageous to study the innervation on vessels extracted from the cerebrum. Blocks having a surface area of approximately 0.25 sq.cm were excized from the basis and from the convex lateral surface of the rat and cat brain. Starting from the midline (Fig. 1) the cerebral substance was scraped off from the blocks that were placed on the slides with their meningeal surface. Vessels entering from the meningeal fragment into the cerebrum became thus attached to the slide extending in centipede-like manner in lateral direction. This technique allowed to study the extracerebral vessels of the pia mater, their branches entering into the cerebral tissue, and also the intracerebral parts. The nerve elements of the basilar artery and of the large cerebral arteries were also examined in cryostat and frozen-dried sections. For the examination of the monoaminergic nerves of the vessels the method of FALCK et al. (1962) was applied, using the condensation of paraformaldehyde gases with catecholamines, i.e. noradrenaline, dopamine or 5-hydroxytriptamine, in the form of a specific highly fluorescent substance (3—4 dihydroxyisoquinoline) (CORRODI, FUXE, DAHLSTRÖM, NORBERG, MALMFORS). Using 5 rats for each variation, 4 and 12 hours prior to sacrifice MAO inhibition was induced with 100 mg/kg nialamide and monoamine depletion with 5 mg/kg reserpine.

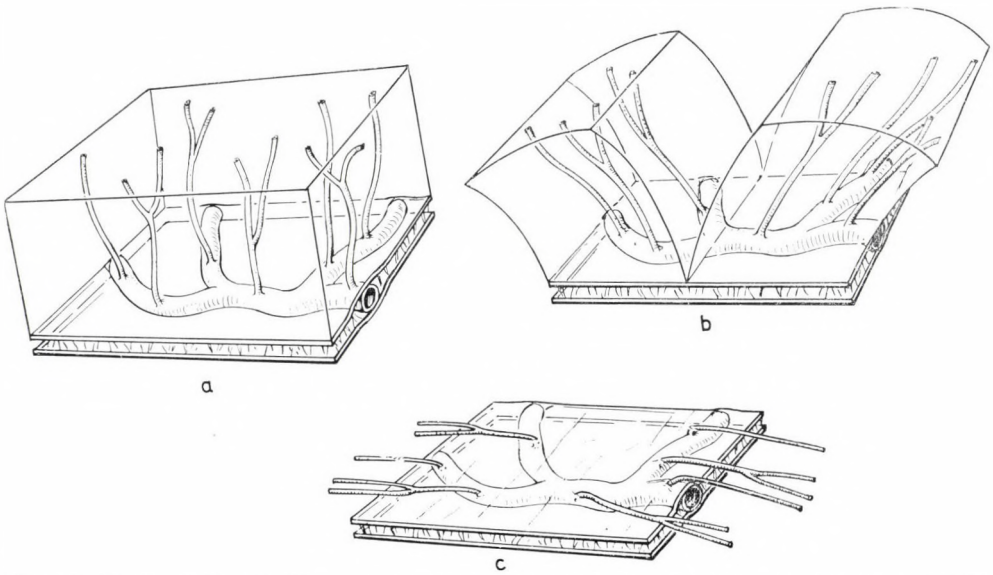


Fig. 1. Diagram of method for removing brain tissue from extra- and intracerebral vessels. The pial surface of excised blocks is placed on the surface of the slide (a), the brain tissue is removed as indicated in (b) and the preparation dried as a membrane (c)

Results

Large and medium sized arterioles in the pia mater are surrounded in their whole course by a nerve plexus showing a strong specific green fluorescence. The sparse reticulum of this plexus consists of wavy varicose fibres, with more intensive fluorescence at the sites of the varicosities (Fig. 2). The fibres forming the plexus are nearly identical in thickness. Density of the plexus is more marked in the angles of ramifying vessels (Fig. 3). The number of nerve elements showing fluorescence is significantly smaller than in vessels from other regions of the same character and size. This difference is well seen in Figs 4 and 5 comparing two small arteries of the same caliber (50 μ) having the same source of sympathetic supply (Sup. cervical ganglion) in the pia (Fig. 4) and a ciliary artery (Fig. 5).

The nerve plexus is situated on the outer surface of the muscular layer beneath the thin adventitia characteristic of pial vessels. The nerve fibres do not seem to enter the deeper strata of the media, nor do they show specific terminations on individual muscle cells. As shown also by electron microscopy, the neuromuscular contact is effected simply by not too intimate contacts between fibres emerging from the plasma of the Schwann cells and muscle

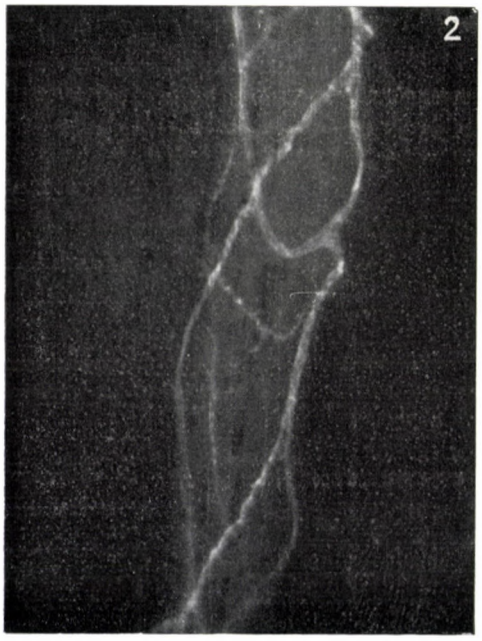


Fig. 2. Sparse plexus of varicose fibres exhibiting characteristic green fluorescence. Cat. Paraformaldehyde reaction. 200 ×

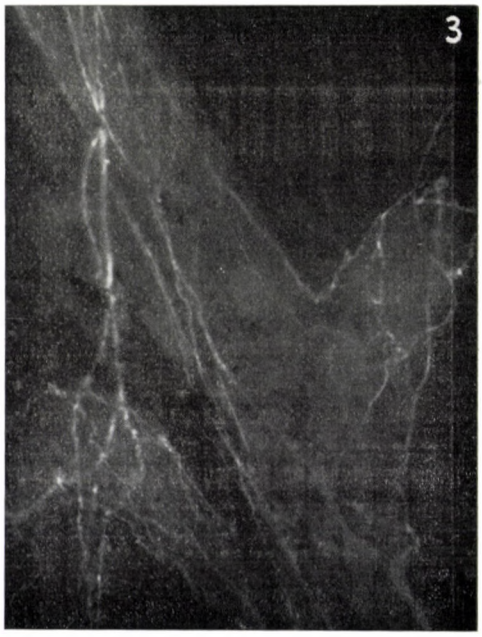
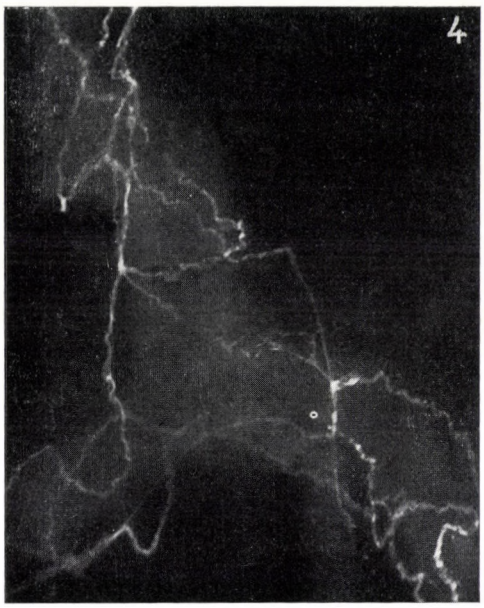
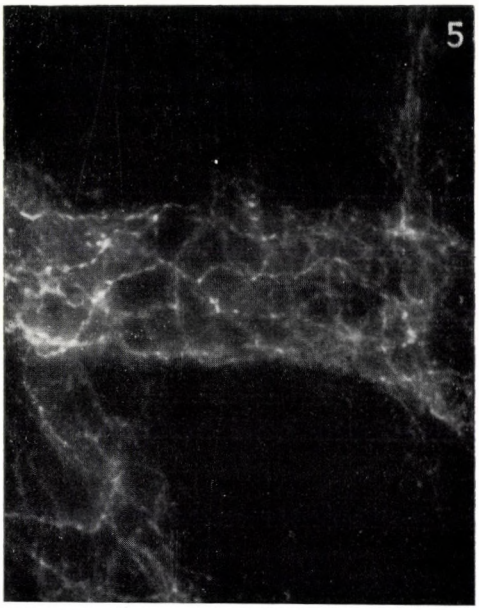


Fig. 3. Monoaminergic nerve plexus is somewhat denser at the angles of vascular ramifications. Pia mater. Rat. Paraformaldehyde reaction. 140 ×





Figs 4—5. Vegetative plexus of pial (Fig. 4) and ciliary artery (Fig. 5) of identical calibre (50 μ in diameter). Adrenergic nerve supply of the pial vessel is remarkably poor as compared with other vessel of similar calibre. Rat. Paraformaldehyde reaction. 140 ×

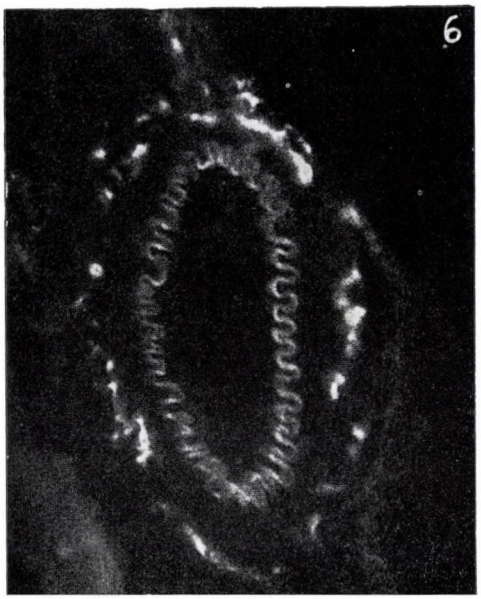


Fig. 6. Cross section of basilar artery. Transected monoaminergic nerve fibres running between the outer muscle layer and the adventitia. Rat. Cryostat section. Paraformaldehyde reaction. 200 ×

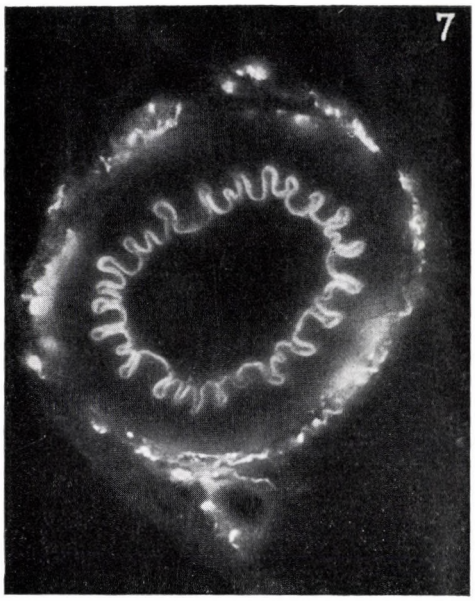


Fig. 7. Cross section of basilar artery. After treatment with monoaminooxidase inhibitor (nialamide) the fluorescence becomes considerably stronger and causes much scattering of light. Rat. Cryostat section. Paraformaldehyde reaction. 200 ×

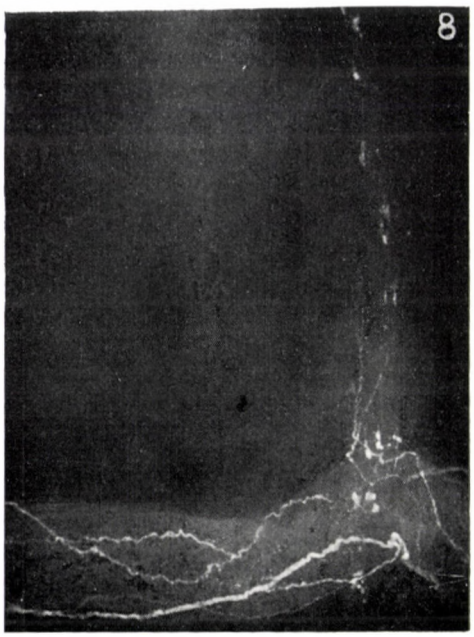


Fig. 8. The vessel entering into the cerebrum is joined for a few tens of microns by 1—2 nerve fibres detached from the vascular plexus and seemingly having free terminations. In the further intracerebral portion, lacking monoaminergic innervation, there are cells showing an intensive orange fluorescence. Cat. Paraformaldehyde reaction. 140 ×

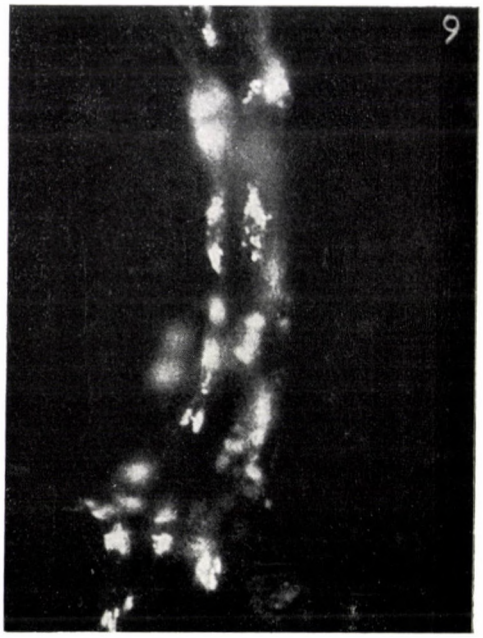


Fig. 9. Intracerebral vessel, lacking monoaminergic innervation with elongated cells having ovoid nuclei, arranged parallel to the vessel longitudinal axis. The coarse plasma granules of the cells show a vivid fluorescence. Cat. Paraformaldehyde reaction. 400 ×

cells crossed by the fibres. There are also individual fibres which have no direct relation to vessels and which terminate freely in the pial tissue.

In the cross-section pictures of the basilar and middle cerebral arteries the cross sections of 10—20 adrenergic fibres or perhaps fibre bundles can be seen (Fig. 6) corresponding to vasomotor nerves that run predominantly longitudinally along the outer surface of the media.

In animals pretreated with MAO inhibitor, the nerve cross sections show a strong diffuse scattering of fluorescence, obviously due to accumulations of larger amounts of catecholamines (Fig. 7).

The arterioles entering into the cerebrum are for only about 1—3 mm joined by 1—2 fibres detached from the nerve plexus of the pial vessel, which then soon taper off and are lost from sight. Whether they terminate freely or only lose their catecholamine contents cannot be judged on the basis of this material. On the further intracerebral portion of the vessels no nerve fibre giving a specific fluorescence is discernible.

From the very place of the cessation of innervation, however, cells are seen having an orange fluorescence (Fig. 8) around the arterioles about 15—30 μ in diameter. Morphologically, these cells, having an elongated form and ovoid nuclei, are arranged parallel to the vessels. Fluorescence is due to the coarse granulation of the plasma, the nuclear area appears empty. This granular fluorescence, an autofluorescence present also in untreated preparations, does not show any changes after administration of either reserpin or nialamide (Fig. 9).

Discussion

From the above findings it seems that the adrenergic innervation of the pial vessels is rather poor if compared with that of other vascular regions. It seems nevertheless to be sufficient to induce, experimentally, vasoconstriction by direct stimulation. On the other hand, long-distance effects, such as transection of the cervical sympathetic, injection of procaine into the superior cervical ganglion, etc., do not seem to cause any significant change in cerebral circulation.

The number of nerves around the main arterial stems of the brain is much larger and only slightly less than in other arteries of similar size. As the nerves are in immediate contact only with the outer muscle cells, for the media as a whole there is no possibility of direct neural transmission. On the basis of neuropharmacological experiments it may be assumed that deeper situated muscle cells may be reached by the transmitter substance through diffusion. This diffusion theory of chemical transmitter has been advanced for the femoral artery by DOLEZEL (1966). Alternatively, excitation exercized by the nerve elements on the outer layer of muscle cells may directly propagate to the deeper rows. As shown in the semi-diagrammatic Fig. 10 by the adrenergic fibres, the vessels entering into the cerebrum are joined up to the point where the vessel is still surrounded by its leptomeningeal sheath. According to the electronmicroscopical findings of SAMARASINGHE [1965] this portion — in spite of being situated in the brain tissue — should be considered essentially still of extracerebral character. In this region the nerve fibres are found between the adventitia consisting of a single cellular layer and the outer muscle cell-layer. Between the adventitia and the leptomeningeal sheath, consisting similarly of a single cellular layer, there is a cleft, the Virchow–Robin space.

Onwards from the site where the two cellular layers merge into one single cellular coating and the intracerebral portion proper begins, no adrenergic nerve fibre has been observed. The single cellular coating of the vessels shows a strong autofluorescence orange in colour. Whether this cell row belongs to the adventitia or to the leptomeninx and whether the cells with respect to their origin are transformed glial cells or of mesenchymal character (mast cells,

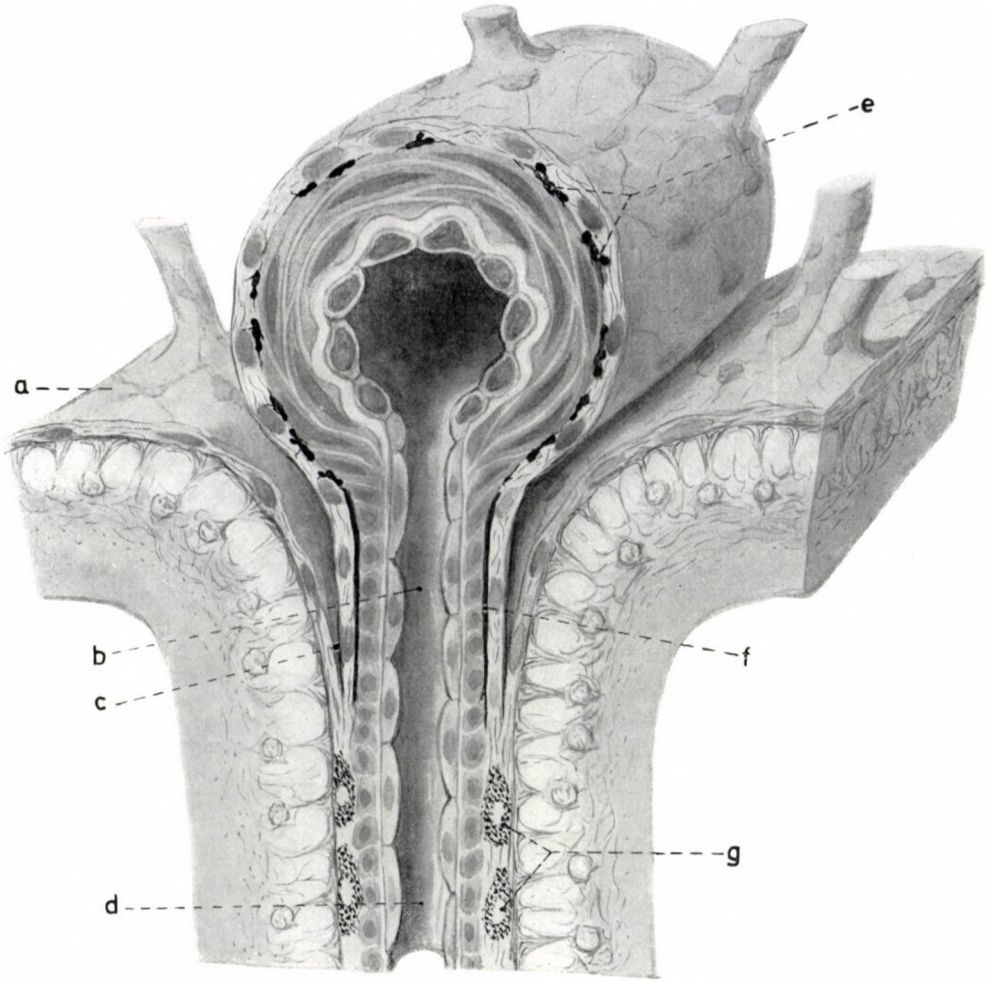


Fig. 10. Semi-diagrammatic stereoscopic view of the monoaminergic innervation of extra- and intracerebral vessels. *a)* Leptomeninx; *b)* Extracerebral portion of vessel; *c)* Virchow–Robin's space; *d)* Intracerebral portion of vessel; *e)* Transections of adrenergic nerves; *f)* Adrenergic fibre; *g)* Intensive fluorescence of cytoplasm

macrophages) cannot be decided. Nor has been elucidated the chemical nature of the autofluorescent granules (FLEISCHHAUER, 1964).

The lack of an adrenergic innervation of the intracerebral vessels corroborates the physiological and clinical observations according to which no direct neurogenic constrictor effect seems to exist. The only possible involvement of the sympathetic innervation in the regulation of cerebral circulation is a remote and not very significant effect exercized through the extracerebral (pial) vessels. The contractile cells of the true intracerebral vessels appear to rely mainly on direct chemical and humoral stimuli and other local factors.

REFERENCES

1. BAKAY, v. L. JR.: (1941) Die Innervation der Pia mater, der Plexus chorioidei und der Hirngefässe, mit Rücksicht auf den Einfluß des sympathischen Nervensystems auf die Liquorsekretion. Arch. Psychiat. Nervenkr. **113,** 412—427. — 2. BERGER, H.: (1924) Zur Innervation der Pia mater und der Gehirngefässe. Arch. Psychiat. Nervenkr. **70,** 216—220. — 3. CHOROBSKI, J., PENFIELD, W.: (1932) Cerebral Vasodilator Nerves and their Pathway from the Medulla Oblongata, with Observations on Pial and Intracerebral Vascular Plexus. Arch. Neurol. Psychiat. (Chic.) **28,** 1257—1289. — 4. CLARK, S. L.: (1929) Innervation of the Blood Vessels of the Medulla and Spinal Cord. J. comp. Neurol. **48,** 247—265. — 5. CLARK, S. L.: (1934) Innervation of the Chorioid Plexuses and the Blood Vessels within the Central Nervous System. J. comp. Neurol. **60,** 21—31. — 6. CORRODI, H., HILLARP, N. A.: (1963) Fluoreszensmethoden zur histochemischen Sichtbarmachung von Monoaminen. I. Identifizierung der fluoreszierenden Produkte aus Modellversuchen mit 6,7-Dimethoxyisochinolinderivaten und Formaldehyd. Helv. chim. Acta **46,** 2425—2430. — 7. CORRODI, H., HILLARP, N. A.: (1964) Fluoreszenzmethoden zur histochemischen Sichtbarmachung von Monoaminen. II. Identifizierung des fluoreszierenden Produktes aus Dopamin und Formaldehyd. Helv. chim. Acta **47,** 911—918. — 8. DAHLSTRÖM, A., FUXE, K.: (1964) Evidence for the Existence of Monoamine-containing Neurons in the Central Nervous System. I. Demonstration of Monoamines in the Cell bodies of Brain Stem Neurons. Acta physiol. scand. **62,** Suppl. 232. — 9. DOLEŽEL, S.: (1966) Monoamines in the Arterial Wall. Comm. Čs. Soc. Histochem. Cytochem. **1,** 31—33. — 10. FALCK, B., HILLARP, N. A., THIEME, G., TORP, A.: (1962) Fluorescence of Catecholamines and Related Compounds Condensed with Formaldehyde. J. Histochem. Cytochem. **10,** 348—345. — 11. FLEISCHHAUER, K.: (1964) Über die Fluoreszenz perivasculärer Zellen in Gehirn der Katze. Z. Zellforsch. **64,** 140—152. — 12. FOG, J.: (1939) Arch. Neurol. (Chic.) cit. 22. — 13. GRIGOREVA, T.: (1932) Histologische Untersuchungen über die Innervation der Hirngefässe. Z. mikr.-anat. Forsch. **28,** 418—426. — 14. HADJIOLOV, A., DOKOV, V. K., TSCHAKAROFF, E. L.: (1954) Contribution à l'étude de l'innervation des capillaires du cerveau. Acta morph. Acad. Sci. hung. **4,** 525—529. — 15. HAGEN, E.: (1955) Mikroskopische Beobachtungen über die Innervation der Gefässe in der Substanz des menschlichen Zwischenhirns und in der Pia mater. Z. Anat. Entwickl. Gesch. **118,** 223—235. — 16. HASSIN, G. B.: (1929) The Nerve Supply of the Cerebral Blood Vessels: A Histologic Study. Arch. Neurol. Psychiat. (Chic.) **22,** 375—391. — 17. HUNTER, W.: (1900) On the Presence of Nerve Fibres in the Cerebral Vessels. J. Physiol. (Lond.) **26,** 465—469. — 18. HOGAN, M. J., FEENEY, L.: (1963) The Ultrastructure of the Retinal Blood Vessels. 1. The Large Vessels. J. ultrastruct. Res. **9,** 10—28. — 19. HUMPHREY, S. P.: (1939) Anatomic Relations of Cerebral Vessels and Perivascular Nerves. Arch. Neurol. Psychiat. (Chic.) **41,** 1207—1221. — 20. KOELLIKER, A.: (1896) Handbuch der Gewebelehre des Menschen. Engelmann, Leipzig. — 21. LAPINSKY, L.: (1913) cit. 29. — 22. LASSEN, N. A.: (1959) Cerebral Blood Flow and Oxygen Consumption in Man. Physiol. Rev. **39,** 183—238. — 23. — MALMFORS, T.: (1965) Studies on Adrenergic Nerves. Almqvist and Wiksell, Uppsala. — 24. NORBERG, K. A., HAMBERGER, B.: (1964) The Sympathetic Adrenergic Neurons. Some Characteristics Revealed by Histochemical Studies on the Intraneuronal Distribution of the Transmitter. Acta physiol. scand. **63,** Suppl. 238. — 25. PEASE, D. C., MOLINARI, S.: (1960) Electron Microscopy of Muscular Arteries; Pial Vessels of the Cat and Monkey. J. ultrastruct. Res., **3,** 447—468. — 26. PENFIELD, W.: (1932) Intracerebral Vascular Nerves. Arch. Neurol. Psychiat. (Chic.) **27,** 30—44. — 27. SAMARASINGHE, D. D.: (1963) An Electron Microscope Study of the Arteries to the Brain of the Rat with Special Reference to the Accompanying Nerve Fibres. J. Anat. (Lond.) **97,** 311. — 28. SAMARASINGHE, D. D.: (1965) The Innervation of the Cerebral Arteries in the Rat: An Electron Microscope Study. J. Anat. (Lond.) **99,** 815—828. — 29. STÖHR, P. JR.: (1957) in Möllendorf: Handbuch der mikroskopischen Anatomie IV. Bd. 5. Teil.: Mikroskopische Anatomie des vegetativen Nervensystems. Pp. 472—478. Springer —, Berlin.

MONOAMINOERGISCHE INNERVATION DER EXTRA- UND INTRAZEREBRALGEFÄSSE

T. DONÁTH

Geurteilt nach dem totalen Fehlen von umgebenden Nervenelementen, die bei Untersuchung mit der Falck-Hillarpschen Methode eine Fluorescenz geben würden, besitzen die Intrazerebralgefäße keine adrenergische Innervation. Das spärliche adrenergische Nervengeflecht der leptomeningealen Gefäße gibt eine geringe Anzahl von Nebenästen zum Anfangsabschnitt der

Intrazerebralgefäße ab, die sich jedoch nur bis zu einer Entfernung von etwa einigen Zehntelmikronen verfolgen lassen, dort werden sie durch die Leptomeninx verdeckt. Längs der intrazerebralen Gefäße läßt sich eine zellenförmige Scheide mit starker Autofluoreszenz unbekannter Natur beobachten.

МОНОАМИНОЕРГИЧЕСКАЯ ИННЕРВАЦИЯ ВНЕ- И ВНУТРИМОЗГОВЫХ СОСУДОВ

Т. Донат

Судя по тотальному отсутствию окружающих нервных элементов, показывающих при исследовании методом Фальк—Хилларпа флюоресценцию, внутримозговые сосуды не имеют адренергической иннервации. Редкое адренергическое нервное сплетение сосудов твердой мозговой оболочки отдает немногочисленные боковые ветви к начальному участку внутримозговых сосудов, которые можно проследить на расстоянии лишь в несколько десятков микрона, где сосуды прикрываются твердой мозговой оболочкой. Вдоль внутримозгового хода сосудов выявляема клеточная оболочка с сильной автофлюоресценцией неизвестной природы.

Dr. Tibor Donáth; Budapest, IX., Tűzoltó u. 58, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 295—303, 1968*

Institute of Surgical Anatomy and Operative Surgery (Director: Prof. T. KARLINGER)
University Medical School, Pécs

OCCURRENCE OF SPECIFIC TISSUE ELEMENTS SEVERAL YEARS AFTER ALLOPLASTIC VASCULAR REPAIR

G. BARTOS

(Received July 6, 1967)

Vascular prostheses of various kinds have been applied to dogs in order to study the behaviour of vessel-specific tissue elements in the area of regeneration between the 1st and the 8th postoperative year. Smooth muscle cells, elastic fibres and endothelium were present in the adequately nourished parts of the regenerated area even after several years. Their number — especially that of elastic fibres — was found to diminish with advancing time, i.e. with the progress of fibrosis in the outer and inner envelope.

Literature on experimental vascular replacement contains few papers on the qualitative problems of morphogenesis. As regards the question as to whether structures characteristic of vascular tissue are formed in the area of the graft, VOORHEES et al. [10], the pioneers of vascular prosthetics, described that the inner sheath of prostheses was lined by endothelium-like cells, whereas they found no smooth muscles and elastic fibres either in fresh or in old preparations. These findings were subsequently confirmed by most authors, particularly MEIJNE [6], MACKENZIE [5] and STUMP et al. [8] who verified these observations, especially those concerning the endothelium, by silver nitrate impregnation.

PETRY and HEBERER [7] observed smooth muscle-like cells in the inner, and to a less extent also in the outer, layer enveloping the prostheses. They saw no elastic elements or saw them only in the form of poorly developed indistinct fibres. In addition to confirming these observations in respect of smooth muscles, JELLINEK et al. [3, 4] demonstrated the presence of elastic fibres in the area of regeneration. Apart from identifying smooth-muscle cells and elastic fibres by current staining procedures, they differentiated them by a special polarization method. FLOREY et al. [1] in experiments on monkeys demonstrated microscopically and submicroscopically the presence of all three vessel-specific tissue elements in the inner envelope.

Excepting the experiments of VOORHEES et al. the period of observation was less than a year in those of the aforesaid authors so that no convincing data are available as to the later survival of the specific elements formed in the graft-area. On the other hand, later authors [2, 9, 12, 13] deal only with the endothelium so that the last communication of VOORHEES [11] is unique in

this respect: examining an eight-year old graft, he found smooth muscle and elastic fibres in the newly formed intima at the boundary between the host vessel and the prosthesis.

Material and method

Employing various kinds of vascular prostheses (some of our own design), thoracic and abdominal aortic substitution and aorto-subclavian bypass were performed on 117 adult mongrel dogs. Details regarding surgical technique and vascular prostheses have already been described [14, 15]. The observation period lasted from one day to eight years. Sections 6 to 12 μ thick were embedded in paraffin or celloidin and paraffin, stained with haematoxylin-eosin, azan, orcein, orcein-ironhaematoxylin, further by the method of Endes and Masson.

Results

Group I (76 cases with observation periods of one day to one year).

Two to three-week-old preparations. In highly porous grafts an inner layer of young granulation tissue developed, in the newly formed intima elastic fibres had formed (Fig. 9). The inner surface was covered by a row of flat endothelioid cells, while other cells of the inner sheath were still undifferentiated. Many preparations displayed on the outside, at the boundary between media and neoadventitia as also in the latter, clearly visible bundles of sometimes undulatory elastic fibres.

Three-month old preparations. Besides elastic fibres, also the endothelium was well observable (Fig. 1). The superficial zone of the neointima contained moreover a large number of spindle-shaped cells [3] which stained like the smooth muscles of arterioles of the neoadventitia and those of the aorta.

Six-month old preparations. A network of elastic fibres was present in all three layers. Fig. 10/a presents the picture of a network of elastic fibres in the superficial zone of a highly porous prosthesis, while delicate elastic fibres in the media covering the inner aspect of the prosthesis can be seen in Fig. 10/b. A bundle of elastic fibres in the neoadventitia of a moderately porous prosthesis is shown in Fig. 11. With the use of loose prostheses, many smooth-muscle-like cells, intensively reacting with Endes' stain, could be seen in the neointima (Fig. 4). Similar cells were observed in the media on both sides of the prosthesis.

Group II (32 cases with observation periods from one to three years). Smooth-muscle cells and elastic fibres were found in this group also. Compared with Group I, the elastic fibres were less marked in the inner sheath. They could often be identified only after the inner envelope had been detached from the synthetic material. Development of elastic fibres was more pronounced in the neoadventitia of the outside. It contained an undulatory elastic

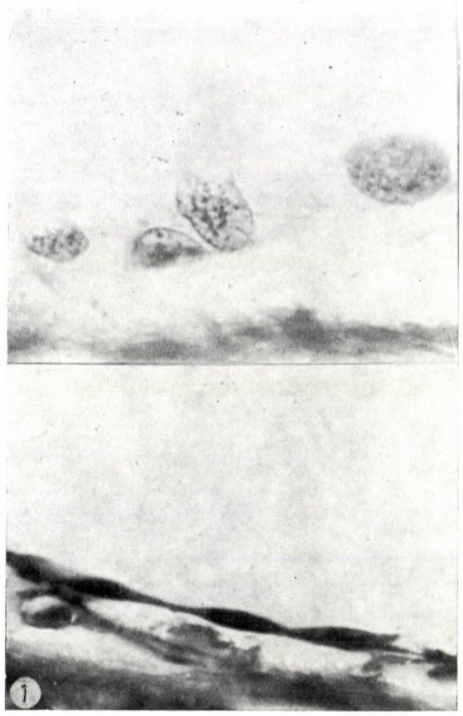


Fig. 1. Endothelial cells in a 3-month preparation. Haematoxylin-eosin. 630 ×

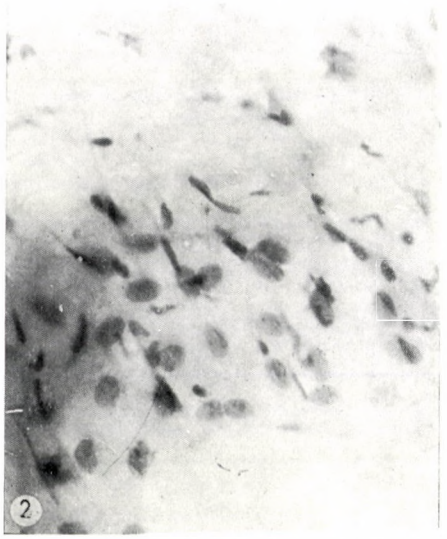


Fig. 2. Endothelium of neointima. Three-year preparation. Iron haematoxylin-eosin. 280 ×

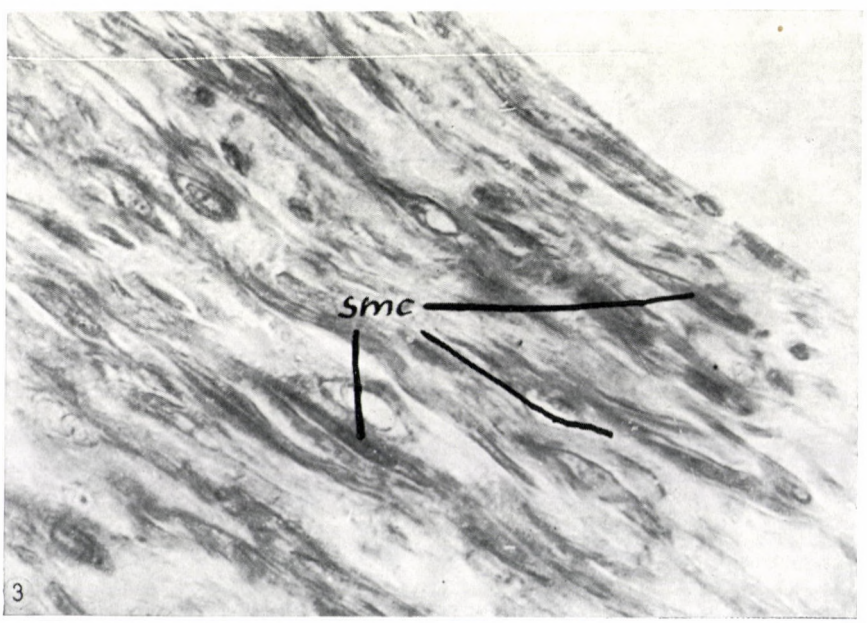


Fig. 3. Smooth muscles in the neointima (smc). Three-month preparation. Azan. 630 ×

Fig. 4. Smooth muscles in the neointima. Six-month preparation. Endes' stain. 280 ×

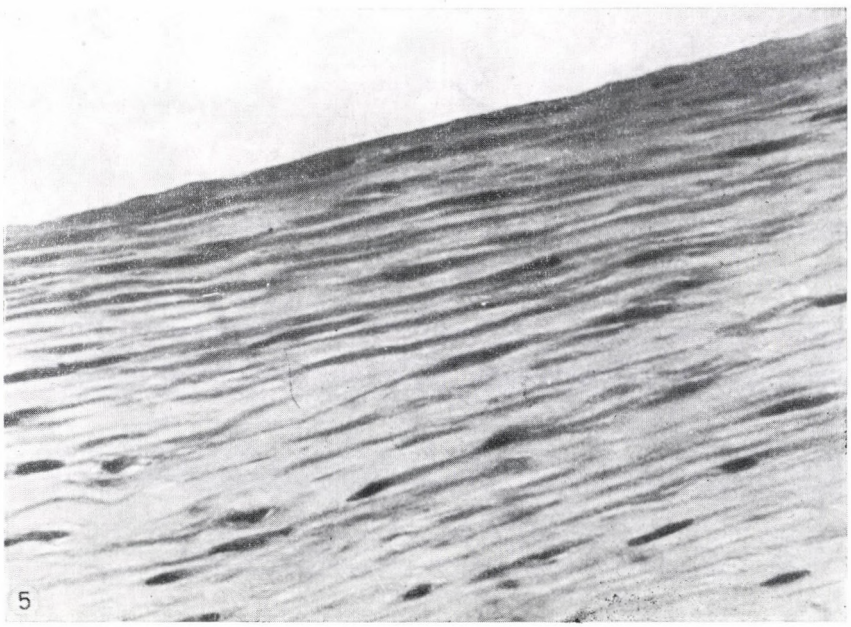


Fig. 5. Smooth muscles in the neointima. Three-year preparation. Haematoxylin-eosin. 450 ×

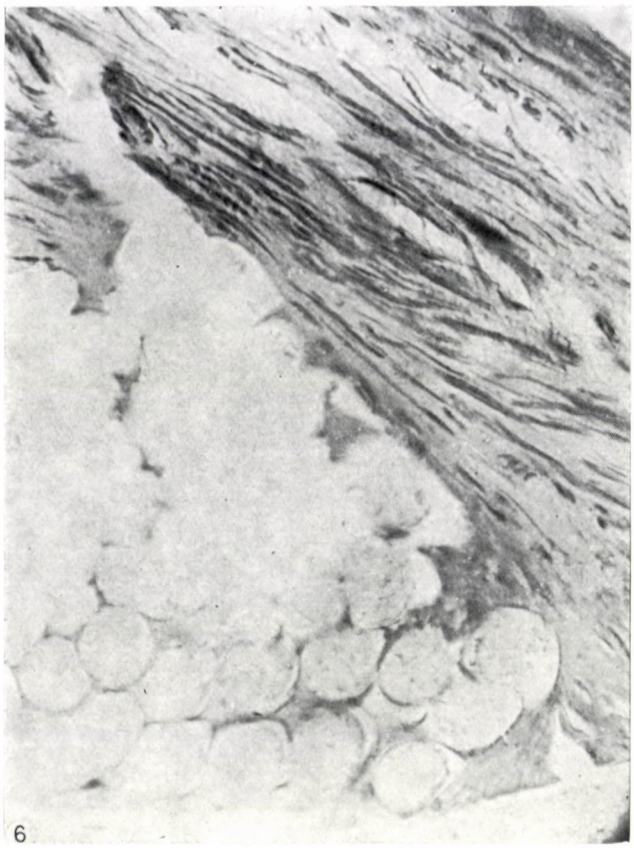


Fig. 6. Smooth muscles in the neointima. Seven-year preparation. Endes' stain. 280 ×

plate in a one-year preparation. In the same preparation, the superficial zone of the inner envelope contained a syncitium of smooth-muscle cells, similar to those of the host vessel.

In three-year old preparations the elastic fibres of the inner sheath were poorly developed. Highly porous prostheses were exceptions in this respect (Fig. 2). Conditions in the outer sheath were approximately the same as in one-year preparations. In contrast to elastic fibres, a fairly large number of smooth-muscle cells was found in the superficial zone of the neointima and a smaller amount in the media of all types of prosthesis (Fig. 5).

Group III (9 cases with observation periods of four to eight years). Elastic fibres in the inner sheath were still poorer and could be demonstrated only on the isolated lining as can be seen on the four-year preparation presented in Fig. 12. It was likewise in a deep layer of the isolated inner sheath that elastic fibres were observed around the nutrient vessels of a five-year preparation. Smooth-muscle cells were well visible in four-year preparations

Fig. 9. Elastic fibres in the neointima. Sixteen-day preparation. Orcein. 120 ×

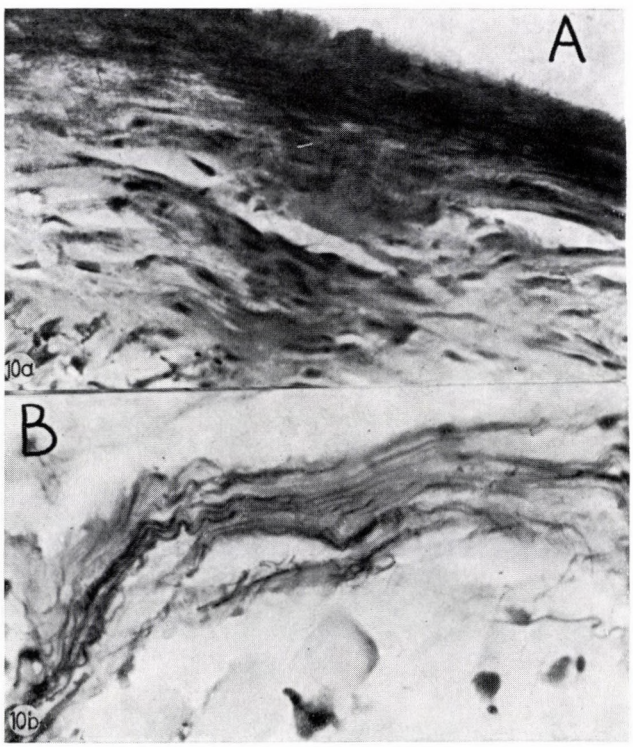


Fig. 10/a Elastic fibres in the neointima. Six-month preparation. Orcein-ironhaematoxylin. 280 ×

Fig. 10/b Elastic fibres in the inner longitudinal zone of the media. Six-month preparation. Orcein-ironhaematoxylin. 280 ×

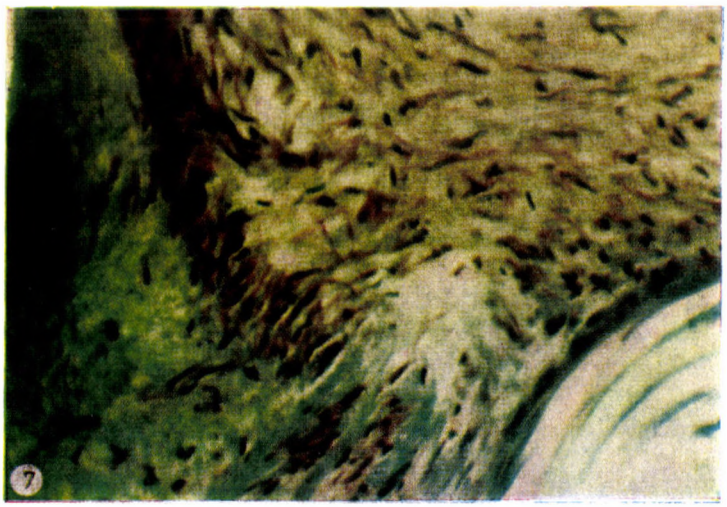


Fig. 7. Smooth muscles in the neointima. Four-year preparation. Masson's stain. 120×

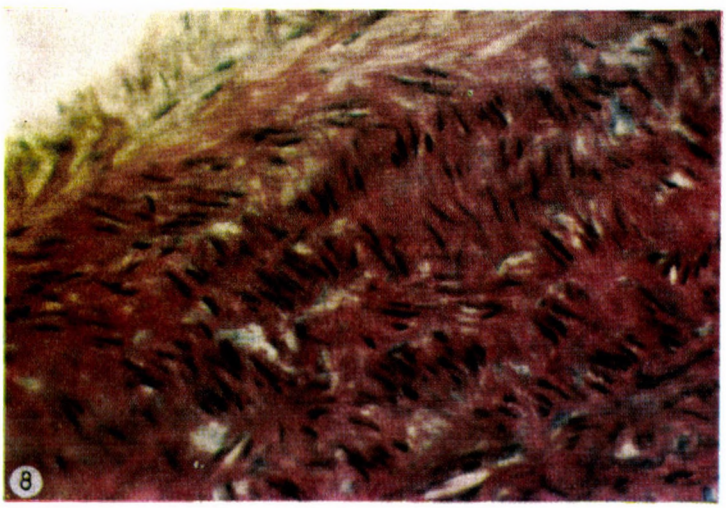


Fig. 8. Smooth muscles in the host aorta from Fig. 7. Masson's stain. 120×

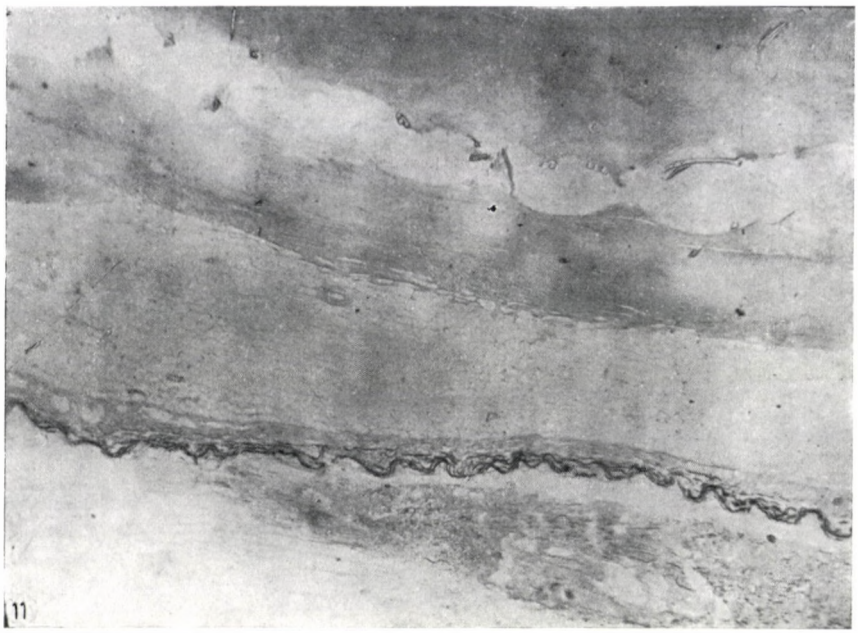


Fig. 11. Elastic fibres in the neoadventitia. Six-month preparation. Orcein. 15 ×

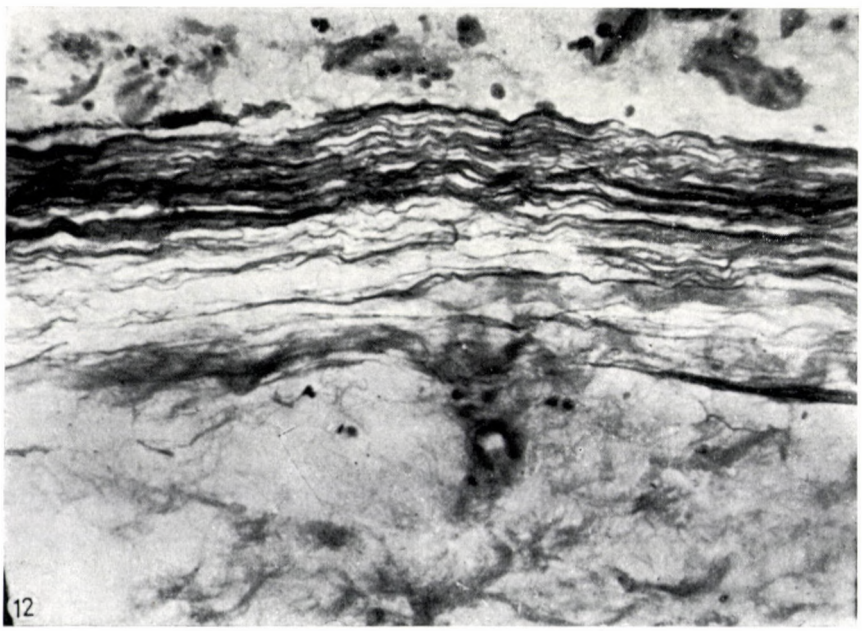


Fig. 12. Elastic fibres in the neointima (plane section from the isolated inner sheath). Four-year preparation. Orcein. 120 ×

(Fig. 7). As regards shape and staining, these cells were similar to the corresponding cells of the host vessel (Fig. 8).

In 7 to 8-year preparations, there were few elastic fibres in both the inner and the outer envelope, whereas smooth-muscle cells could be observed in the superficial zone of the inner sheath both in the middle and at the periphery of the prosthesis (Fig. 6).

Discussion

It is evident from the foregoing that vessel-specific tissues persist around vascular grafts for several years, although their amount diminishes with advancing time. This applies to elastic fibres in particular whose dwindling is presumably hastened by progressing fibrosis of the inner and outer sheaths. The observations have proved the presence of elastic fibres in the neoadventitia, a phenomenon to which no reference has been made in earlier communications which contain likewise no mention of the delicate elastic fibres sometimes present in the media. The number of smooth muscles and elastic fibres was highest in the well-nourished zones of the graft area, e.g. in the superficial layer of the inner sheath, in the outer zone of the media on the outside of the prostheses, further in the neoadventitia.

REFERENCES

1. Florey, H. W., Greer, S. J., Poole, J. C. F., Werthessen, N. T.: (1961) Brit. J. exp. Path. **42,** 236. — 2. Foster, J. H., Ekman, C. A., Scott, W. H.: (1960) Ann. Surg. **151,** 867. — 3. Jellinek, H., Csillag, I., Kádár, A.: (1960) Orv. Hetil. **101,** 950. — 4. Jellinek, H., Csillag, I., Kádár, A.: (1963) Acta chir. Acad. Sci. hung. **4,** 257. — 5. Mackenzie, D. C., Loewenthal, J.: (1960) Brit. J. Surg. **48,** 212. — 6. Meijne, N. G.: (1959) Arch. chir. neerl. **11,** 41. — 7. Petry, G., Heberer, G.: (1957) Langenbecks Arch. klin. Chir. **286,** 249. — 8. Stump, M. M., Jordan, G. L., DeBakey, M. E., Halpert, B.: (1962) Amer. J. Path. **40,** 487. — 9. Szilágyi, D. E., Pfeifer, J. R., DeRusso, F. J.: (1964) Surgery **55,** 165. — 10. Voorhees, A. B., Jaretzki, A., Blakemore, A. H.: (1952) Ann. Surg. **135,** 332. — 11. Voorhees, A. B.: In: Wesolowski, A. S., Denis, V. eds.: (1963) Fundamentals of Vascular Grafting. McGraw-Hill, New York — 12. Weber, W.: (1965) Langenbecks Arch. klin. Chir. **313,** 847. — 13. Wesolowski, A. S.: (1961) Evaluation of Tissue and Prosthetic Grafts. Thomas, Springfield. — 14. Szőllőssy, L., Bartos, G., Hübner, H.: (1958) Gefäßsubstitutionsversuche an mit autoplastischem Gewebe gefütterten Kunststoffröhren. Bruns' Beitr. klin. Chir. **197,** 295. — 15. Bartos, G., Karmos, V., Kustos, Gy., Szőllőssy, L., Török, B., Tóth, I., Temes, Gy.: (1965) Acta chir. Acad. Sci. hung. **6,** 109.

SPEZIFISCHE GEWEBSELEMENTE MEHRERE JAHRE NACH ALLOPLASTISCHER GEFÄSSTRANSPLANTATION

G. BARTOS

Verschiedene Gefäßprothesen wurden Hunden eingepflanzt und die Veränderungen der gefäßspezifischen Gewebselemente im Regenerationsbereich vom ersten bis zum achten postoperativen Jahr verfolgt. In den adäquat ernährten Teilen des regenerierten Bezirks waren sogar nach mehreren Jahren noch Muskelzellen, elastische Fasern und Endothel zu beobachten. Ihre Anzahl, insbesondere die der elastischen Fasern, nahm mit der Zeit, d. h. mit dem Fortschreiten der Fibrose der inneren und äußeren Gefäßhäute, ab.

СПЕЦИФИЧЕСКИЕ ТКАНЕВЫЕ ЭЛЕМЕНТЫ ЧЕРЕЗ НЕСКОЛЬКО ЛЕТ ПОСЛЕ АЛЛОПЛАСТИЧЕСКОЙ ТРАНСПЛАНТАЦИИ СОСУДОВ

Г. БАРТОШ

Авторы имплантировали собакам различные протезы сосудов и в области регенерации следили за изменениями сосудоспецифических тканевых элементов, наблюдаемых в течение восьми лет после операции. В частях с адекватным питанием в регенерированной области даже по истечении нескольких лет выявляемы мышечные клетки, эластические волокна и эндотелий. Их число, в частности, количество эластичных волокон, с течением времени, то есть, по мере прогрессирования фиброза во внешней и во внутренней оболочках сосудов, понижалось.

Dr. Gábor Bartos; Pécs, Széchenyi tér 5., II. Sebészeti Klinika, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 305—309, 1968*

Department of Dermatology and Venereology (Director: Prof. L. Szodoray), University Medical School, Debrecen

EFFECT OF ULTRAVIOLET LIGHT ON THE EPIDERMIS III.

HISTOCHEMISTRY OF CELL RESPIRATION AND PHOSPHORYLATION

Éva Szabó and Irén Horkay

(Received July 7, 1967)

Histochemical changes induced in the epidermis by exposure to UV-light, have been studied.

(1) Following the administration of a single large dose of UV-light, succinic dehydrogenase activity increases during the first hours in the basal-cell layer and the stratum granulosum. It disappears from the latter, but increases further in the prickle-cell and the basal-cell layers at the appearance of parakeratosis. Activity is vigorous in the entire epidermis, and especially so in the stratum granulosum, at the desquamation of parakeratosis.

Acid phosphatase activity increases during the first hours in the stratum granulosum and the stratum corneum, and becomes very intensive in the entire epidermis at the development of parakeratosis, to decrease during desquamation.

Non-specific esterase activity is intensive in the parakeratotic nuclei.

(2) Repeated administration of single erythema doses of UV-light moderately increases succinic dehydrogenase activity in the entire epidermis, especially in the stratum granulosum. Acid phosphatase activity is pronounced in the hyperkeratotic zone and in the stratum granulosum.

(3) Absorption of UV-rays by the stratum granulosum, by promoting cellular metabolism, induces quicker and imperfect nuclear degeneration and gives, thus, rise to the development of parakeratosis.

Small doses of UV-light induce, on the other hand, hyperkeratosis because the amount of imparted energy does not suffice for impairing cellular activity.

Irradiation of the skin by UV-light induces disturbances in the metabolism of the epidermis. The enzymatic phenomena in the background of such disturbances were studied in detail by Hanke [1] who examined the histochemical effect of UV-rays of different wavelengths and doses on the web of frogs and the skin of mice. He found that, after a certain period of latency, enzymatic activity was especially increased in the wavelength range between 280 and 320 mμ. He did not regard the reaction as specific since any impartment of energy gives rise to similar reactions in every cells. It was, on the other hand, emphasized by Daniels et al. [2] that exposure to UV-light induces cellular degeneration. Prior to degeneration they observed no histochemical change pointing to enzymatic disturbances. Once the process of degeneration had started, glycogen synthesis increased without a simultaneous increase in succinic dehydrogenase and acid phosphatase activities. Other authors [3, 4] studied the cellular metabolism of glycogen and proteins with a view

to making inferences concerning enzymatic changes in the skin due to UV-light.

In earlier communications [5, 6] we have quoted authors who have pointed out that irradiation by UV-light produces changes in cellular metabolism. The question has not been settled whether these changes are anabolic or catabolic in nature.

Cell respiration is one of the decisive factors influencing cellular metabolism. Different enzymes (cytochrome oxidase, dehydrogenase, etc.) constitute, each, a separate link in the chain of biological oxidation; they are most active where the processes of metabolism are most vigorous [7, 8].

Again, phosphatases play a significant role in intermediary carbohydrate metabolism, in the composition and decomposition of nucleic acid. Acid phosphatases are especially active in the stratum granulosum, a phenomenon justifying the conclusion that they are involved in physiological karyorrhexis. Their activity is enhanced under pathologic conditions such as the processes associated with parakeratosis [9, 10]. Nuclear degeneration is determined also by other enzymes, e.g. DNA-depolymerase, aminopolypeptidase, tyrosinase. These enzymes are responsible for the physiological degeneration, i.e. keratinization of the epidermis.

The present experiments were designed to study cell respiration and processes of phosphorylation by examinations of tissue enzymes in order to obtain data regarding the metabolic changes induced in the epidermis by exposure to UV-light.

Material and method

Obtaining and treatment of specimens has been described earlier [5, 6]. After preparing 3 to 5 μ unfixed frozen sections (partly in the cryostat) from the skin specimens, they were studied for succinic dehydrogenase [11], cytochrome oxidase [12], alkaline and acid phosphatase according to GÖMÖRI [13] and VADÁSZ [14], non-specific esterase [15], aminopolypeptidase [16], and tyrosinase [17].

Results

Group I. Irradiation with 4 erythema doses of UV-light

After 4 to 6 hours. Succinic dehydrogenase activity was increased in the basal-cell layer and in the stratum granulosum (Fig. 1) as compared with the normal skin's reaction (Fig. 2). No cytochrome oxidase activity was observed at this time. Acid phosphatase was increased in the stratum lucidum, and a narrow zone of increased activity was found in the stratum cornerum as well (Fig. 3). For comparison, normal acid phosphatase activity of the epidermis is shown in Fig. 4. Aminopolypeptidase activity was normal.

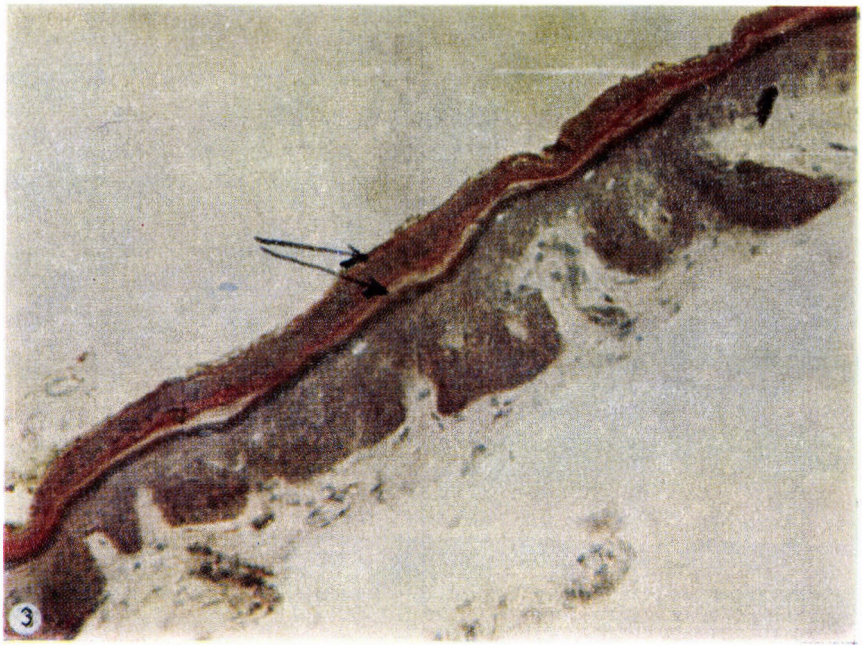


Fig. 3. Gluteal skin 4 hours after irradiation with 4 erythema doses. Acid phosphatase reaction, naphthol method. Activity appears only in the form of a double band in the stratum corneum and the stratum lucidum

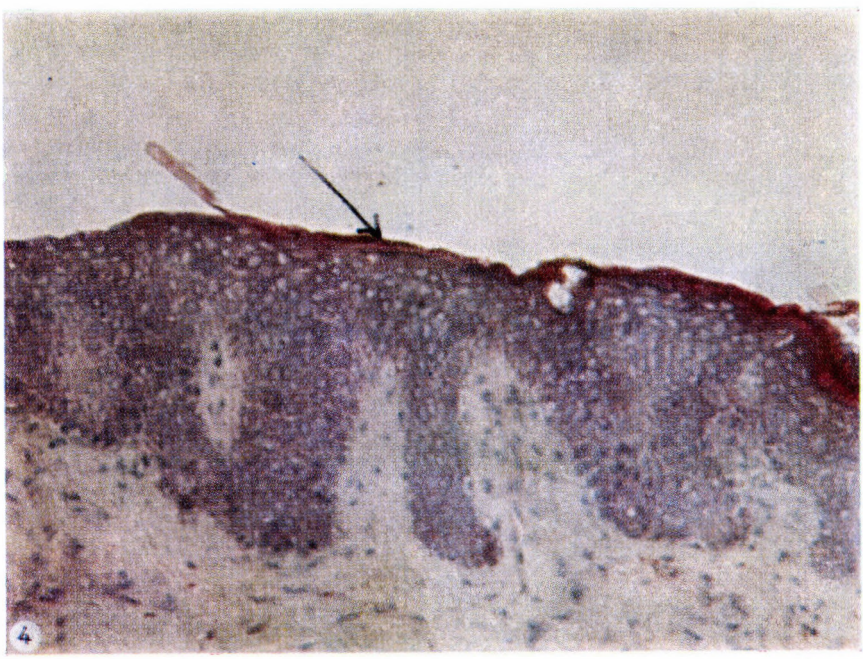


Fig. 4. Gluteal skin. Control. Acid phosphatase reaction, naphthol method. Activity is present only in stratum corneum

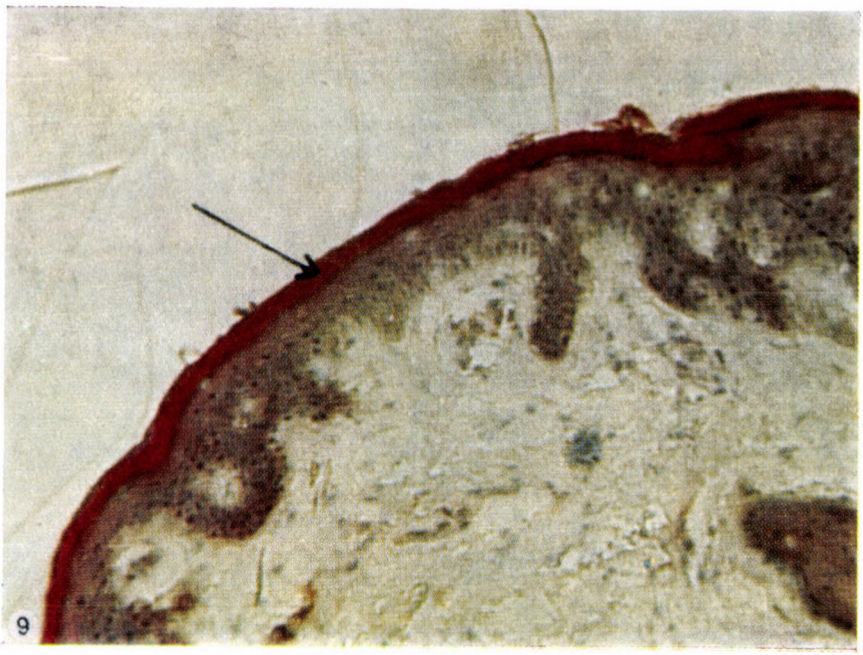


Fig. 9. Gluteal skin after eight irradiations with a single erythema dose. Acid phosphatase reaction, naphthol method. Increased activity in stratum granulosum and corneum

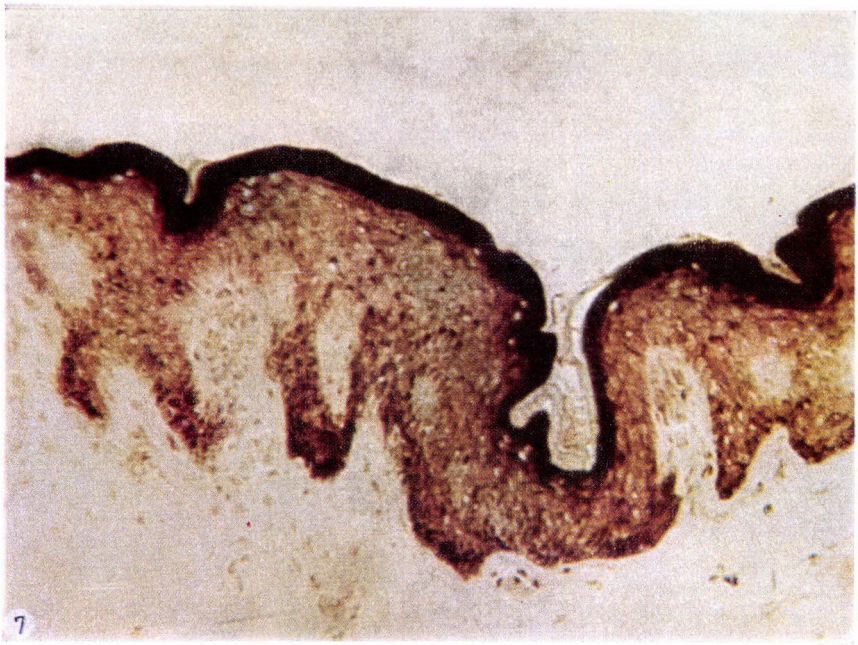


Fig. 7. Gluteal skin 24 hours after irradiation with 8 erythema doses. Alkaline phosphatase reaction, Gomori's method. Increased activity in stratum corneum and in the cytoplasm of certain cells in the deeper layers of the epidermis

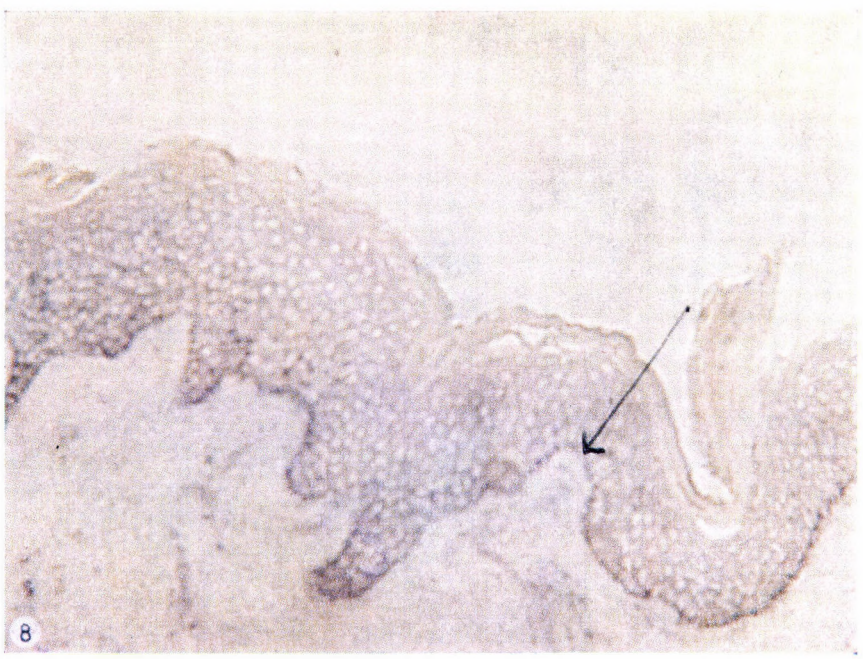


Fig. 8. Gluteal skin after eight irradiations with a single erythema dose. Succinic dehydrogenase reaction. Activity is displayed by the basal-cell layer only

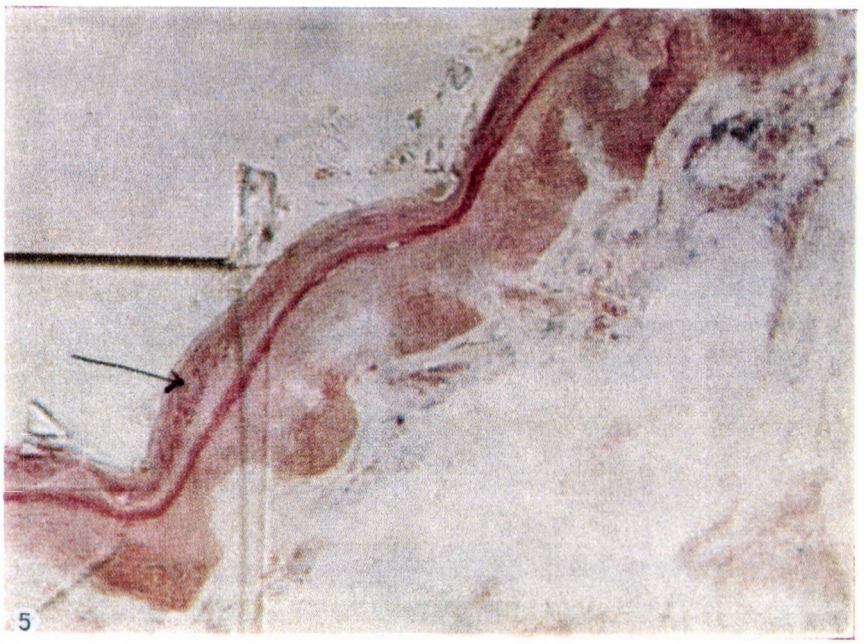


Fig. 5. Gluteal skin 48 hours after irradiation with 4 erythema doses. Intensive non-specific esterase activity in parakeratotic layer

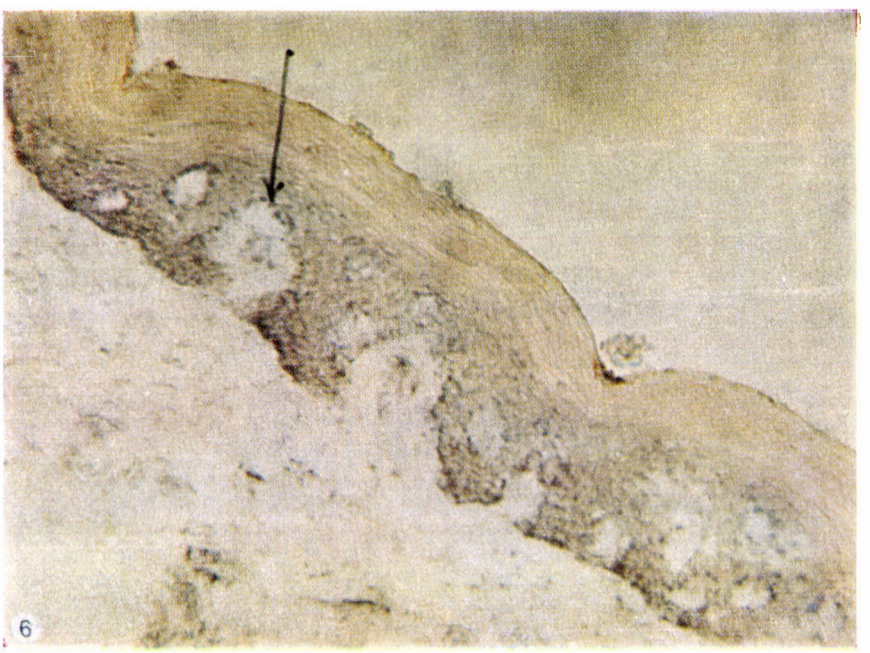


Fig. 6. Gluteal skin 8 days after irradiation with 4 erythema doses. Strong succinic dehydrogenase activity in basal-cell layer

Fig. 1. Gluteal skin 4 hours after irradiation with 4 erythema doses. Succinic dehydrogenase reaction. Increased activity in basal-cell layer and stratum granulosum

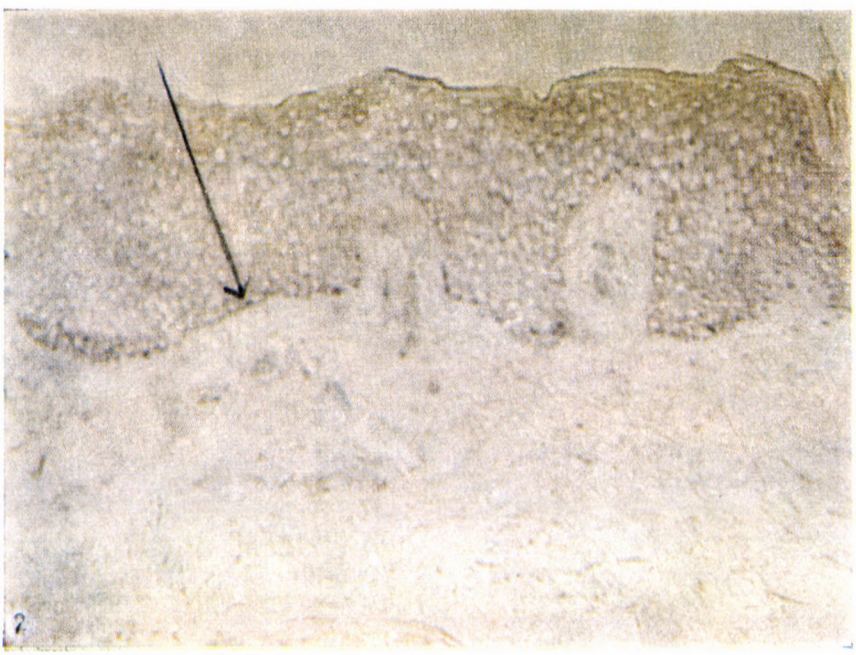


Fig. 2. Gluteal skin. Control. Succinic dehydrogenase activity in basal-cell layer

After 48 hours. Succinic dehydrogenase activity at this time was especially vigorous where parakeratosis had not yet developed. No cytochrome oxidase activity was observed. Acid phosphatase activity was intensive in the area of parakeratosis. Non-specific esterase activity was marked in parakeratotic nuclei (Fig. 5).

After 72 hours. Succinic dehydrogenase activity was increasing anew in the basal layer, and the activity of acid phosphatase involved also the prickle-cell layer.

After 5 to 6 days. Succinic dehydrogenase was most active in the basal layer, and — advancing upward — formazan crystals were practically no longer demonstrable in the stratum granulosum (Fig. 6). Acid phosphatase and non-specific esterase were most active in the stratum granulosum and the parakeratotic zone. No cytochrome oxidase activity was registered.

After 12 days. Neither acid phosphatase nor non-specific esterase activity was marked in the desquamating parakeratotic layer. On the other hand, succinic dehydrogenase in the stratum granulosum showed normal values.

Group II. Irradiation with 8 erythema doses of UV-light.

After 6 to 12 hours. Dehydrogenase of acid phosphatase activity was marked in the upper layers of the epidermis.

After 24 hours. Dehydrogenase was present in traces only while acid phosphatase activity increased further in all layers. Alkaline phosphatase activity was considerably increased (Fig. 7).

Group III. Irradiation with 8 × 1 erythema dose

Dehydrogenase activity increased almost imperceptibly in the basal-cell layer and in the lower part of the stratum granulosum (Fig. 8); increase in acid phosphatase activity was likewise modest in the stratum granulosum and the stratum corneum (Fig. 9). Non-specific esterase activity seemed to be increasing in the stratum granulosum.

Discussion

It has repeatedly been reported [18, 19, 20] that the activity of enzymes involved in processes of oxidation and phosphorylation is increased in conditions associated with parakeratosis and acanthosis. It has moreover been shown that thickening of the horny layer caused by UV-light is an aspecific change [20, 21]. Metabolic phenomena connected with these processes are, therefore, of the same nature as those observed in parakeratosis of a different aetiology.

Absorption of UV-light accelerates cellular activity in the epidermis, a phenomenon which — being determined by the special function of each layer — manifests itself differently in the various strata. Enzymatic activity, after

increasing during the hours following irradiation, disappears almost completely after the formation of the parakeratotic layer. Cell respiration, and consequently also cellular activity, are accelerated by the absorption of energy which leads to imperfect nuclear degeneration and parakeratosis. Thus, UV-light absorbed by the basal-cell layer increases cell respiration and accordingly promotes nuclear division. In the last phase of regeneration following UV-irradiation, i.e. in that of desquamation, the parakeratotic layer is thrown off by the newly formed hyperkeratotic scales since the formation of the hyperkeratotic layer requires an increased rate of nuclear degeneration in the stratum granulosum. As pointed out by MIESCHER [21], habituation to light, i.e. thickening of the keratotic substance, results if the epidermis is exposed to repeated small doses of UV-rays. Formation of the hyperkeratotic zone depends on a constant slight stimulation of epidermal metabolism.

The phosphorylation reactions are in harmony with those of oxidative enzymes. Acid phosphatase activity is intense in the keratogenic zone even before the development of parakeratosis, a phenomenon which admits of the assumption that UV-rays induce anabolism or catabolism in a direct manner. The appearance of the double band in the compact stratum corneum and the stratum granulosum seems to support this assumption. Subsequently, with the development of parakeratosis acid phosphatase activity is enhanced over the entire epidermis, a phenomenon which is likewise non-specific, it being present in other parakeratotic conditions, too.

Repeated administrations of small doses of UV-rays also increase epidermal phosphatase activity.

The present experiments justify the conclusion that UV-irradiation directly promotes cellular metabolism, both anabolic and catabolic reactions. These enzymatic reactions start those metabolic changes which result in the visible but no longer specific morphological picture.

REFERENCES

1. HANKE, W.: (1961) Strahlentherapie **115,** 124. — 2. DANIELS, F., BROPHY, D., LOBITZ, W. C.: (1961) J. invest. Dermat. **37,** 351. — 3. RABBIOSI, G.: (1962) Ann. ital. Derm. **16,** 23. — 4. NIEBAUER, G.: (1962) Ann. ital. Derm. **16,** 93. — 5. SZABÓ, É., HORKAY, I.: (1967) Acta morph. Acad. Sci. hung. **15,** 71. — 6. SZABÓ, É., HORKAY, I.: Acta morph. Acad. Sci. hung. **15,** 383-389 (1967) — 7. BRAUN-FALCO, O., RATHJENS, B.: (1954) Derm. Wschr. **134,** 1271. — 8. MONTAGNA, W., FORMISSANO, V.: (1955) Anat. Rec. **122,** 657. — 9. BRAUN-FALCO, O.: (1957) Klin. Wschr. **35,** 1182. — 10. SPIER, MARTIN: (1956) Arch. klin. exp. Derm. **202,** 120. — 11. PEARCE, A. G.: (1960) Histochemistry. Churchill, London. — 12. BURSTONE, M. S.: (1959) J. Histochem. Cytochem. **7,** 112. — 13. GÖMÖRI, G.: (1939) Proc. Soc. exp. Biol. (N. Y.) **42,** 33. — 14. VADÁSZ, GY.: Personal communication. — 15. DAVIS, B. J., ORNSTEIN, L. I.: (1959) J. Histochem. Cytochem. **7,** 297. — 16. PEARSON, B., WOLFF, P., ANDREWS, M.: (1962) J. Histochem. Cytochem. **10,** 673. — 17. LILLIE, R. D.: (1954) Histopathologic Technic and Practical Histochemistry. McGraw Hill, New York. — 18. GOTTRON, H. A., SCHÖNFELD, W.: (1962) Dermatologie und Venerologie. Thieme, Stuttgart. — 19. LOBITZ, W. C., HOLYOKE, J. B.: (1954) J. invest. Derm. **22,** 189. — 20. PINCUSS, H: (1952) Dermatologica (Basel) **105,** 85. — 21. MIESCHER, G.: (1940) Arch. Derm. Syph. **80,** 238.

DIE WIRKUNG DER UV-BESTRAHLUNG AUF DIE EPIDERMIS

III. Histochemie der Zellatmung und der Phosphorylationsprozesse

É. SZABÓ und I. HORKAY

Bei der Untersuchung einiger histochemischer Veränderungen der Oberhaut nach UV-Bestrahlung wurde festgestellt:

1. In den ersten Stunden nach einmaliger Bestrahlung mit größeren UV-Dosen erhöht sich die Succinodehydrogenase-Aktivität im Str. granulosum und im Str. basale; bei der Entstehung der parakeratitischen Hornschicht verschwindet sie aus dem Str. granulosum, während sie im Str. spinosum und Str. basale gesteigert ist. Nach Abschilferung der Parakeratose läßt sich in der gesamten Oberhaut, vor allem aber im Str. granulosum starke Aktivität nachweisen.

In den ersten Stunden nach der Bestrahlung steigerte sich die Aktivität der sauren Phosphatase im Str. granulosum und Str. corneum, beim Entstehen der Parakeratose war sie in der gesamten Oberhaut sehr erheblich und bei der Abschilferung nahm sie ab. In den parakeratotischen Kernen war eine starke nicht-spezifische Esterase-Reaktion nachweisbar.

2. Nach mehrfacher UV-Bestrahlung mit 1 ED war die Succinodehydrogenase-Aktivität in der Oberhaut mäßig und im Str. granulosum ausgeprägt gesteigert. Die Aktivität der sauren Phosphatase war dagegen in der hyperkeratotischen Hornschicht und im Str. granulosum intensiver.

3. Die im Str. granulosum absorbierten Strahlen induzierten durch die Steigerung des Zellstoffwechsels einen schnelleren und unvollkommenen Kernabbau und bewirkten das Entstehen der parakeratotischen Hornschicht. Auf Wirkung kleiner UV-Dosen erfolgte die Bildung des hyperkeratotischen Keratins aus dem Grunde, weil die Energievermittlung zu gering war um eine pathologische Veränderung der Zellfunktion herbeizuführen.

ДЕЙСТВИЕ УЛЬТРАФИОЛЕТОВЫХ ЛУЧЕЙ НА ЭПИДЕРМИС

Е. САБО и И. ХОРКАИ

При исследовании гистохимических изменений, возникающих в эпидерме после облучения ультрафиолетовыми лучами, было установлено:

1. В первые часы после однократного облучения более большой дозой ультрафиолетовых лучей активность сукцинодегидрогеназы повышается в области Str. granulosum и basale. При развитии паракератитического рогового слоя она исчезает из Str. granulosum а в области Str. spinosum и basale она усиливается. При шелушении паракератоза во всей надкожице, а особенно в Str. granulosum наблюдается сильная активность.

В первые часы после облучения реакция кислой фосфатазы усиливается в области Str. granulosum и corneum, при развитии паракератоза она во всей надкожице очень значительна, а при шелушении она уменьшается.

В паракератитических ядрах наблюдается очень сильная неспецифическая эстеразная реакция.

2. После повторного применения облучения ультрафиолетовыми лучами дозой, вызывающей эритему, во всей надкожице наблюдается незначительное усиление реакций сукцинодегидрогеназы, причем в области Str. granulosum ее активность более бы, ражена.

Реакции кислой фосфатазы более выражены в области гиперкератозного рогового слоя и Str. granulosum.

3. Лучи, поглощаемые в Str. granulosum, вызывают путем повышения клеточного обмена веществ более быстрый и неполный распад ядер и приводят к образованию перекератозного рогового слоя.

Образование гиперкератозного рогового слоя наступает под влиянием небольших доз ультрафиолетовых лучей потому, что передача энергии незначительна и недостаточна для того, чтобы вызвать патологическое изменения функции клеток.

Dr. Éva Szabó; Hospital of County Fejér. Székesfehérvár, Hungary
Dr. Irén Horkay; Debrecen, Bőrklinika, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 311—330, 1968*

Institute of Anatomy, University Medical School, Budapest

THE GOLGI ARCHITECTURE OF CLARKE'S COLUMN

M. RÉTHELYI

(Received September 22, 1967)

A Golgi study of Clarke's column in the kitten has confirmed the existence of two types of neurons viz. [1] *main cells*, giving rise to the dorsal spinocerebellar tract (DSCT) fibres, and [2] *border cells*, small interneurons with short axons and resembling Golgi II type neurons. Three kinds of dorsal funiculus afferents could be distinguished, all of which are primary sensory neuron collaterals. One group could be identified (a) as Ia (muscle spindle) afferent collaterals, another (b) as Ib (Golgi tendon organ) collaterals and a third (c) as probably of cutaneous origin. While groups (a) and (c) terminate in the larger dorsomedial sector of the column, group (b) terminates exclusively in a smaller ventrolateral sector. It is inferred that the Clarke neurons that convey muscle spindle information are different and differently located from those that carry Golgi tendon organ information. Mutual synaptic interrelationships of the Clarke column neurons, and some geometric patterns of synaptic arrangements are considered.

Apart from the early cytoarchitectonic descriptions our knowledge of the dendritic and axonal architecture of Clarke's column is mainly based on the classical Golgi descriptions of the spinal cord (v. LENHOSSÉK, 1895; RAMÓN Y CAJAL, 1909). The fundamental and rather peculiar structural properties of this nucleus — viz. (1) the "close" character, i.e. sharp restriction of the dendritic arborizations to the confines of the nucleus; (2) the predominantly longitudinal orientation of its dendrites; (3) the origin of axonal collaterals, terminating in this nucleus, from the medial part of the dorsal funiculus, whereas little if any connections are established between the general axonal neuropil of the other spinal grey matter and that of the column; and (4) the axons of the main cells running towards the dorsal superficial region of the lateral funiculus, where they form the dorsal spinocerebellar tract (DSCT) — emerge clearly from these descriptions. Interest has been aroused by the first electrophysiological studies on synaptic transmission in this nucleus (GRUNDFEST and CAMPBELL, 1942; LLOYD and MCINTYRE, 1950), which appeared to show a good agreement with the subsequent observations by SZENTÁGOTHAI and ALBERT (1955) on the peculiar synaptic arrangement between large calibre primary afferents and the Clarke neurons. A successive series of investigations into the electrophysiology of the DSCT by LUNDBERG and co-workers (LAPORTE et al., 1956; LAPORTE and LUNDBERG, 1956; LUNDBERG and OSCARSSON, 1956; HOLMQUIST et al., 1956; LUNDBERG and WINSBURY, 1960; LUNDBERG and OSCARSSON, 1960) using unit level recordings revealed that,

apart from a powerful synaptic articulation with Ia and partly also of Ib primary afferents, not only the afferent but also the intrinsic connections of the column are more complex than hitherto imagined. Cutaneous primary afferents are found to contribute presynaptic stimulation to these neurons.— Clear signs of interneuronal, partly inhibitory, activity indicate that either the column is entered by presynaptic axons other than primary afferents, or there are interneurons within the column itself. Small cells situated predominantly on the surface of the column have been described earlier (RAMÓN Y CAJAL, 1909) but nothing essential is known about the distribution of their axons. SZENTÁGOTHAI (1961a) made an attempt to explain the synaptic arrangement in the light of the new physiological observations with consideration to the postulate of having inhibitory synaptic connections. Anatomical information, however, particularly with respect to Golgi architecture, was still not at the level required for such an undertaking. Recent Golgi reinvestigations of various regions of the CNS, prompted by the increased demand for such data by the wealth of new physiological information and encouraged by the demands of electron microscopy, have brought forward a great abundance of new details of importance lacking from the classical descriptions. The present paper aims at a reinvestigation of the Golgi architecture with special consideration (1) of the cell types; (2) of the origin from the white matter and distribution within the column of various types of axons or collaterals; (3) of the initial course of Clarke cell axons, particularly possible initial collaterals and their synaptic relations; (4) of the synaptic interrelations of the Clarke neurons, and aims, finally (5) at an understanding of the geometric design of this region.

Material and methods

The spinal cord of newborn, 3 and 7 days old kittens was treated according to the classical rapid Golgi procedure with repeated impregnation. 60—70 micron thick sections from the lower thoracic and upper lumbar segments were cut in the transversal, the sagittal and the horizontal planes with reference to the natural posture of the cat. (These expressions are consequently used both in the text and the legends of the figures.) — For experimental degeneration purposes dorsal root ganglia L_3 or L_4 were removed in adult cats. Four or five days later the animals were killed, the brain and the spinal cord were fixed in 4% neutral formalin. Frozen sections were prepared from the upper lumbar and lower thoracic part of the cord in the three cardinal planes and the sections were subjected to the Nauta procedure.

Results

(1) The cell types of Clarke's nucleus

For the study of the cell bodies, their axons and dendritic arborization pattern sections in both longitudinal planes — sagittal and horizontal — are best suited, whereas little, if any, information of significance can be gained from transverse sections. Both cell types mentioned by RAMÓN Y CAJAL (1909) have been found in our sections.

a) *Focal cells* (*"cellules focales"* — the so-called Clarke or DSCT neurons). The cell body is rather large, of spheric or irregular ellipsoid form (Figs 1a, 1b, 1c). It appears from the sagittal as well as from the horizontal sections that the dendrites leave the soma in two opposite directions, i.e. cranial- and caudalwards, and after some initial deflections run parallel with the spinal cord axis. The length of the dendritic tree is roughly equal in the two directions. According to measurements of 14 focal cells, the whole span of the dendritic tree is 400—500 microns in length. As the dendrites are confined in width to a strip 70—80 microns wide, the average focal cells occupy longitudinally oriented cylindrical spaces. The dendrites are surrounded by thin axons running parallel to them, as pointed out by SZENTÁGOTHAI and ALBERT (1955) (Fig. 1d).

b) *Border cells (*"*cellules marginales ou limitantes*"*)*. These cells according to their names, are localized predominantly at the border of Clarke's nucleus (Figs 2a, 2b). The cell body is small, and as a rule fusiform with longitudinally oriented axis. The dendrites take their origin on the upper and lower poles of the cell with large trunks, which soon divide into two, three or occasionally more branches (Figs 2a, 2b, 3a, 3b). The dendrites have a much less regular course than the dendrites of the main Clarke cells making it difficult to reduce the space occupied by the dendritic tree to any simple geometrical form. The total dendritic span of these cells in longitudinal direction varies between 300 and 400 microns and the dendrites have spines of characteristic drumstick shape (Fig. 2c). The axons stain readily and originate from one of the dendritic trunks near the cell body, as has been described by RAMÓN Y CAJAL (1909). The axons immediately begin to arborize and cannot be traced outside the territory of the nucleus (Figs 2d, 3b).

(2) Origin from the white matter and distribution within the column of afferent axons or collaterals

Three different types of dorsal funiculus collaterals can be observed in the studied Golgi material viz. (a) collaterals (or axons) that appear to terminate exclusively in Clarke's column (b) collaterals to the intermediate region and to the ventral horn, giving only side branches to the column; and (c) collaterals to the dorsal horn yielding side branches to Clarke's nucleus. — No other preterminal axons arising from any other part of the grey matter that would enter the column have been observed. On transverse sections Clarke's nucleus appears as a round or sometimes as a somewhat elliptic territory. The fibres entering the nucleus, the majority of which are obviously collaterals of primary afferents, arise exclusively from the dorsal funiculus (Figs 4a, 4b). The three different sets of dorsal funiculus collaterals, and a fourth set of collaterals to the medial part of the ventral horn that are seen frequently and that

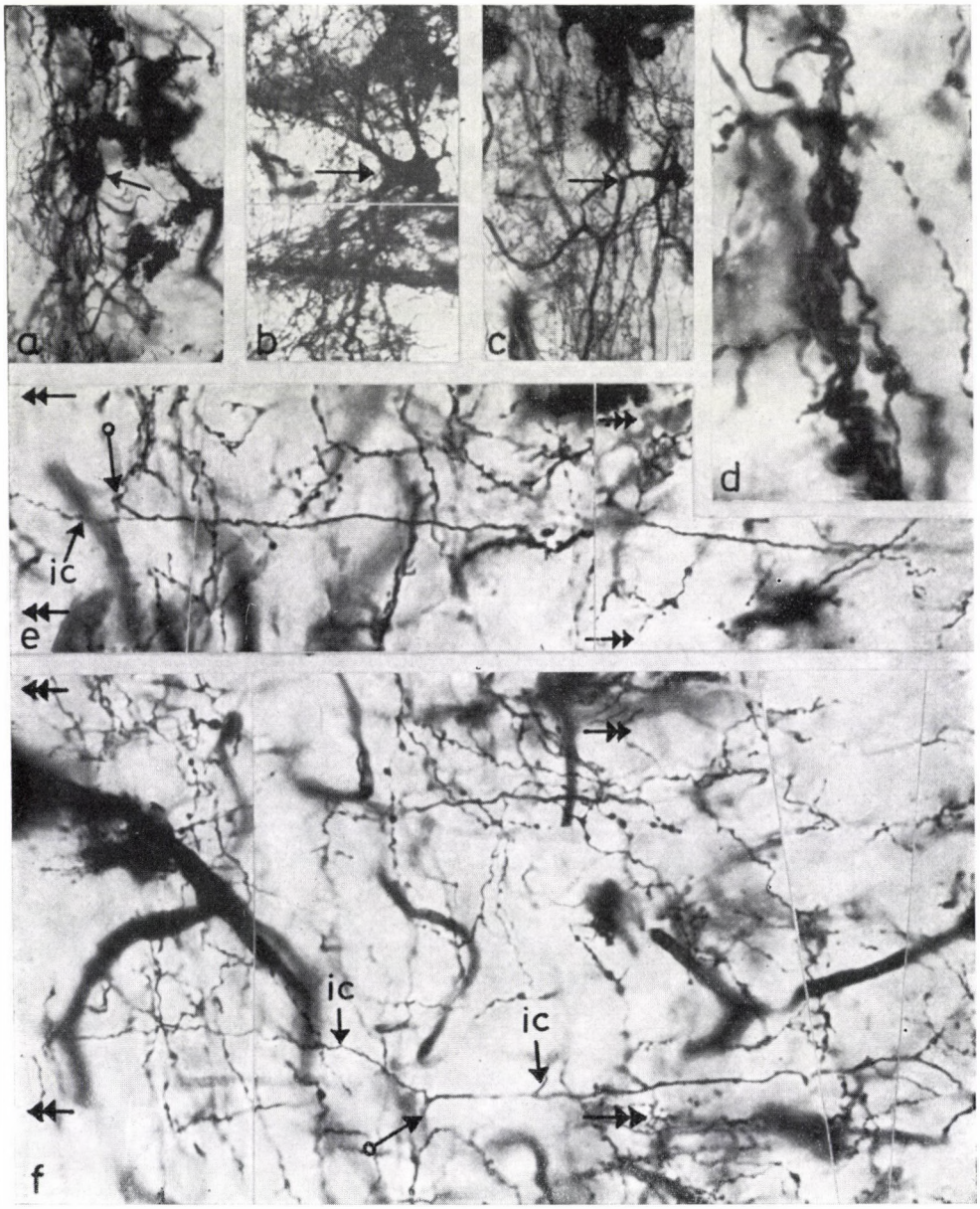


Fig. 1. The focal or main cells, their dendrites and supposed axons. Focal cells (indicated by arrows) are seen in horizontal (a) and in sagittal (b) and (c) sections, 180 × (d). Dendrite surrounded by a number of parallel axons, 1250 ×. (e) and (f) axons, running in transverse direction. Their beginnings are indicated by ringed arrows. ic = initial (recurrent) collaterals. The medial (left) and lateral borders (right) of Clarke's nucleus are marked by double arrows. Note the initial collaterals tending towards the borders of the column. 500 × — Rapid Golgi procedure, (a) — (d) 7-day-old, (e) and (f) 3-day-old kitten

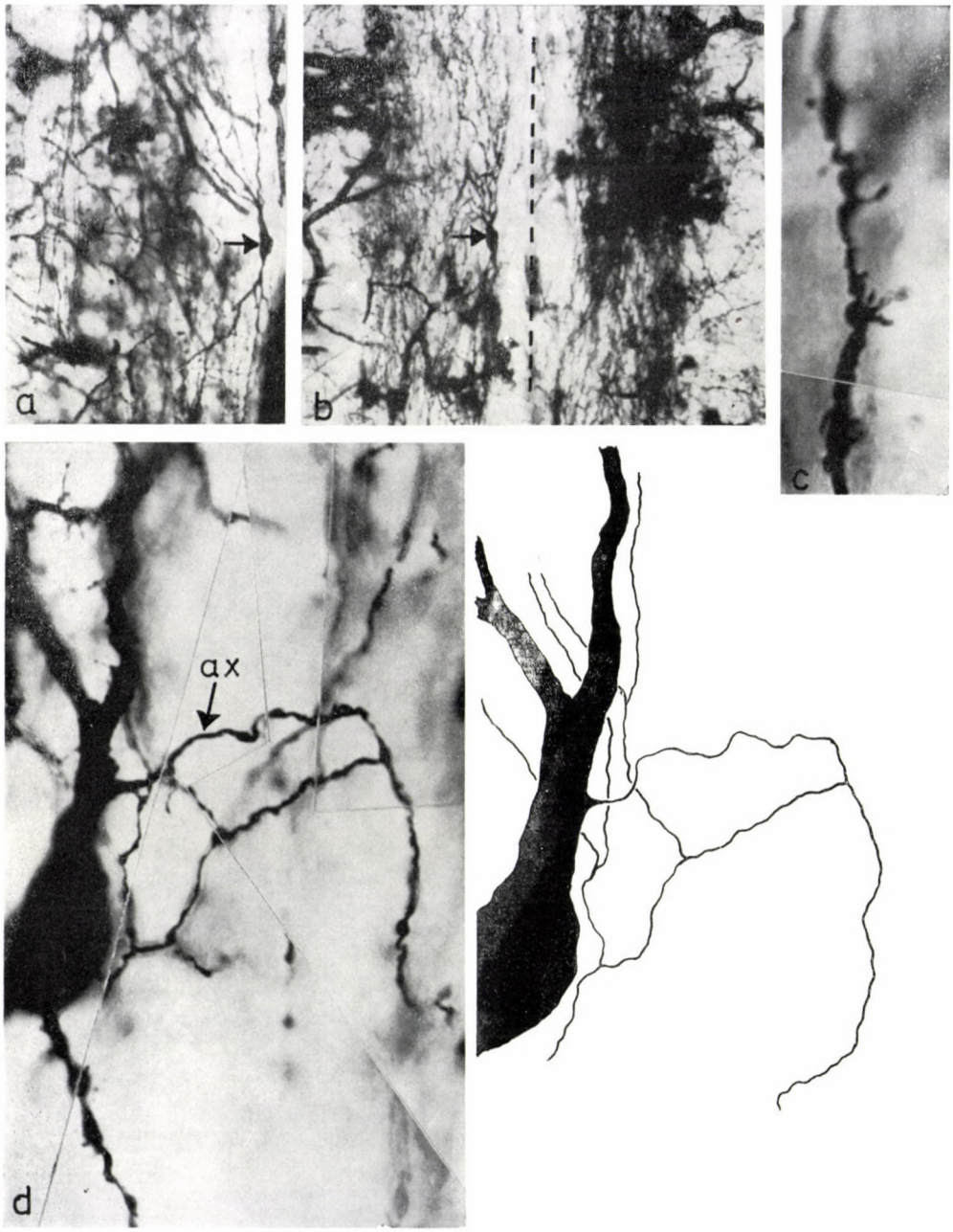


Fig. 2. Form and axonal arborization of the border cells (a) Horizontal section of Clarke's column. The right margin of the photograph corresponds to the midline. Border cell situated close to the medial border is indicated by arrow, 180×. (b) Horizontal section with Clarke's column of both sides. Midline is marked by dashed line, arrow indicates a border cell. 180× (c) Dendrite of border cell, note characteristic drumstick shape spines. 1250× (d) Axonal arborization of a border cell in photomicrograph (left) and in a camera lucida drawing (right); ax = axon. 1250× Rapid Golgi procedure; (a), (b) and (d) 7-day-old, (c) 3-day-old kitten

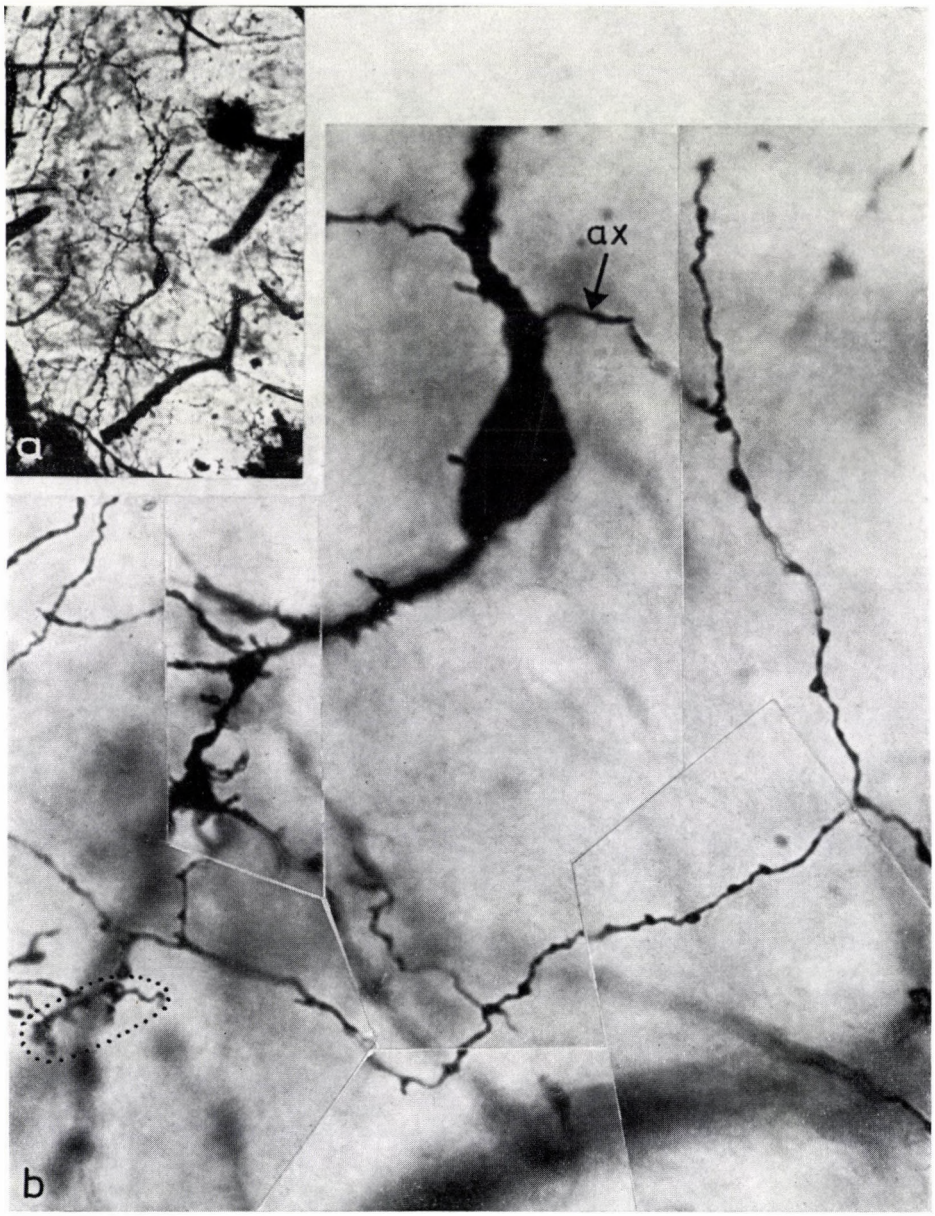


Fig. 3. Shape and axonal arborization of a border cell. (a) Sagittal section with border cell. 180 × (b) The same cell under higher power. Note axonal arborization (ax = axon) around the parent cell. Dotted line surrounds a terminal expansion belonging to the same axon. 1250 × — Rapid Golgi procedure, 3 day-old kitten

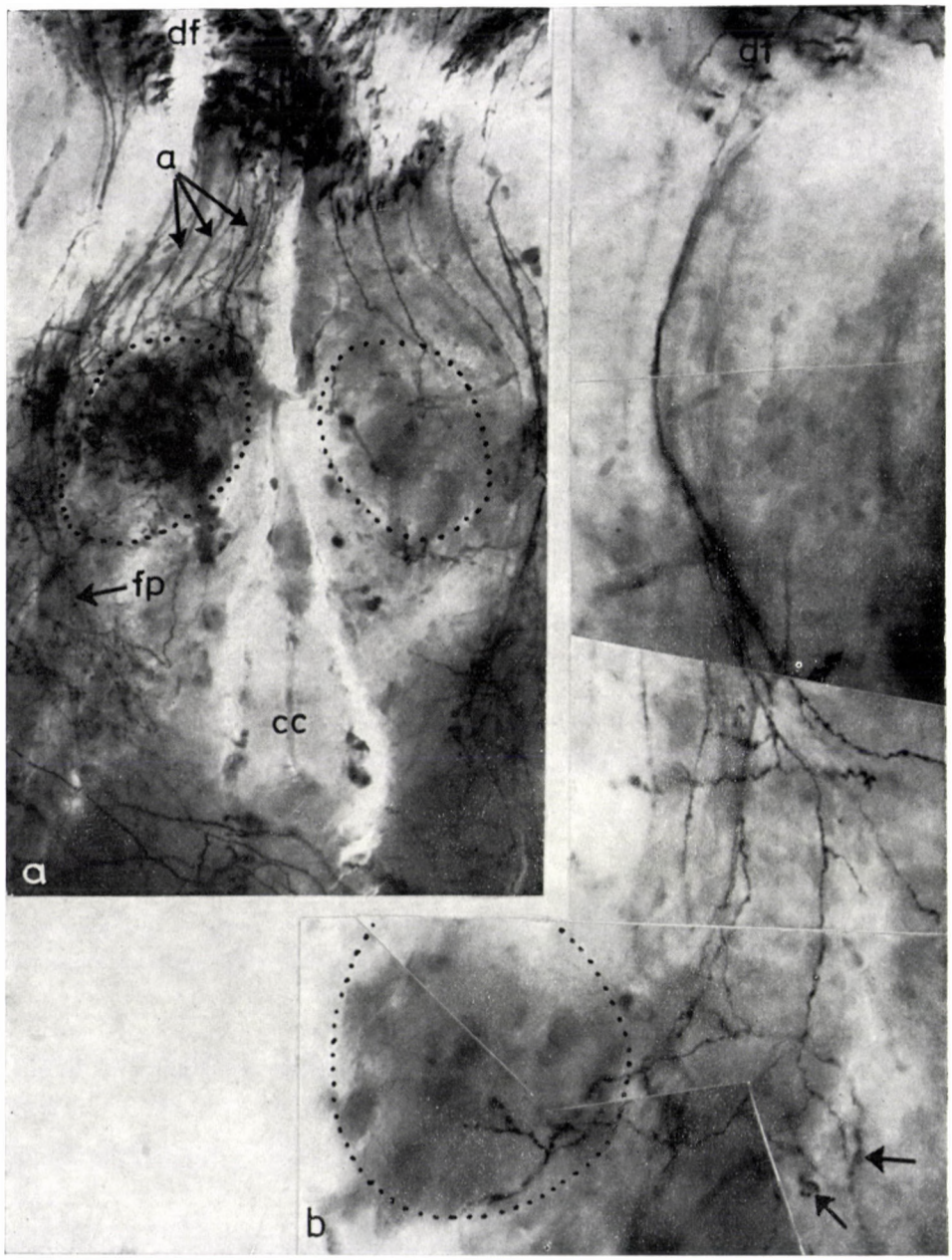


Fig. 4. Transverse section of the spinal cord with Clarke's nucleus (a) Clarke's nucleus is outlined by dotted line, a = set (a) of collaterals arising from the dorsal funiculus (df), some other fibres (fp) are passing by the lateral border of the nucleus. Note lack of stained nerve fibres and terminals in the ventrolateral sector of the nucleus on the left side; cc = the ependymal wall of the central canal. 180 × (b) Same as (a) at higher magnification (500 ×) A bundle of collaterals arising from the dorsal funiculus (df) are giving terminal expansions to the ventrolateral part of Clarke's nucleus and to the adjacent grey matter. The latter are marked by arrows (at bottom right). These collaterals correspond to set (b). Rapid Golgi procedure, newborn kitten

strictly avoid giving any connections to the column are shown in Fig. 5. All collaterals and branchings shown in Fig. 5 are exact tracings of fibres from our preparations.

a) *Collaterals directed exclusively to Clarke's nucleus.* These collaterals arise from the medial and rather deep part of the dorsal funiculus. Upon reaching the dorsal border of the column the more lateral ones turn into lateral, the medial ones into medial direction and before giving penetrating branches to the nucleus run for some distance around its dorsal circumference (Fig. 5a). Collaterals arising from sites in between often give rise to two branches, from which one runs around the dorsal border of the column in medial and the other in lateral direction. The further course of these fibres being longitudinal, it cannot be traced in transverse sections, so that sections in the sagittal and horizontal planes have to be consulted. As seen from Fig. 7 there is good reason to assume that the large axons, having both themselves as well as their terminal branches a longitudinal course strictly within the column, belong to this group. The axonal neuropil of the column derives to considerable extent from these large longitudinal axonal branches that are mainly ascending, but to some extent also descending branches of the original dorsal funiculus collaterals mentioned at the beginning of this paragraph (Figs 7b, 7c). As seen from Fig. 8 these large, longitudinal preterminal axon branches can be traced along distances of 500—600 microns, so that their real length may be much greater. They are giving at frequent intervals side branches that terminate with multiple bulbous thickenings in close neighbourhood of the preterminal longitudinal axon. There is a remarkable topical arrangement of these collaterals in two respects: *(i)* the collaterals arising in lateral parts of the dorsal funiculus area are giving rise to this set enter the lateral parts of the column, whereas those arising from medial points enter predominantly medial regions of the nucleus; *(ii)* this set of collaterals terminates only in the dorsomedial $^2/_3$—$^3/_4$ of the column, whereas a ventrolateral sector of $^1/_4$—$^1/_3$ of the nucleus is left completely free (Figs 4a, 5a).

b) *Side branches of collaterals to the intermediate region and the ventral horn.* The second set of collaterals shown in Figs 4b and 5b differs entirely from those described under (a), but particularly in being parts of collaterals directed to grey regions other than Clarke's column. They arise from a region of the dorsal funiculus distinctly lateral from that giving rise to set (a). They curve around the lateral border of the nucleus and begin to break up into branches at the base of the dorsal horn. Some of these branches enter Clarke's nucleus from its ventrolateral or lateral side and terminate in its already mentioned ventrolateral sector. In sagittal or horizontal sections the longitudinal course of these branches can seldom be distinguished from other axonal branches, as shown in Fig. 7a. The branches entering Clarke's nucleus do not appear to represent the main termination sites of these collaterals as some

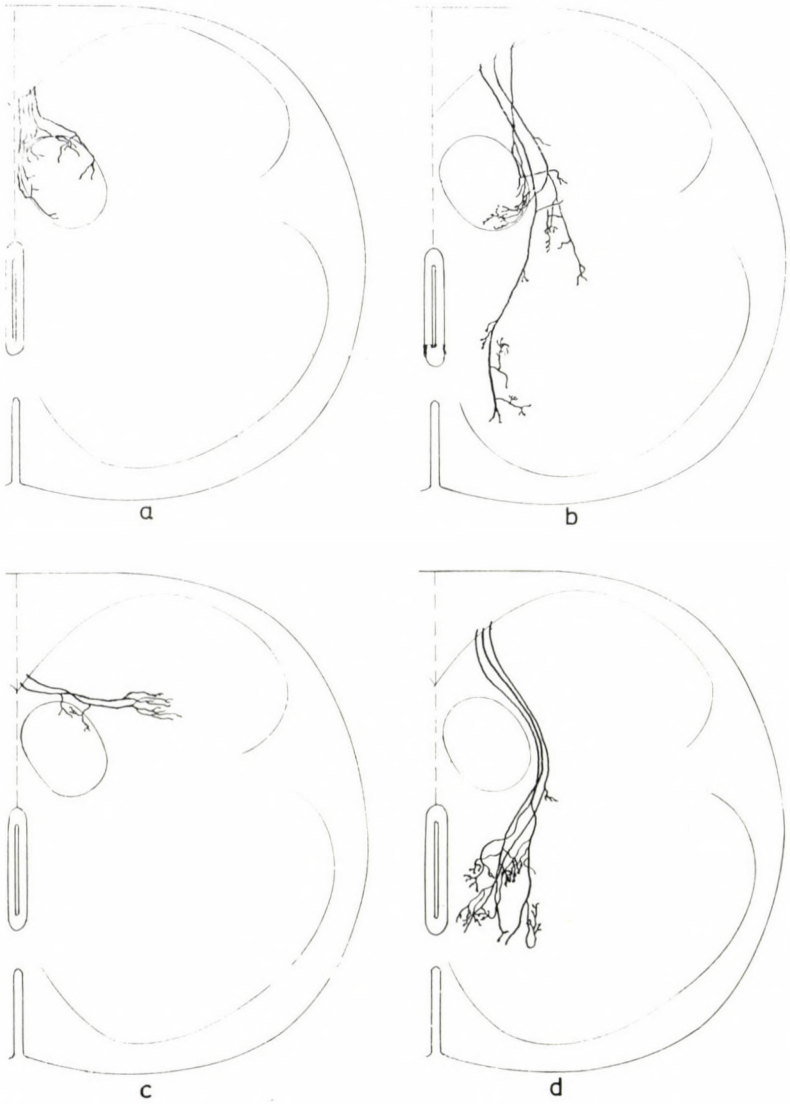


Fig. 5. Diagram showing the different sets — (a), (b) and (c) — of primary afferents which contact Clarke's column and a fourth set — (d) — passing by the column at its lateral side. Explanation in text

of them can be traced clearly to medial parts of the ventral horn. There is no convincing evidence of their having synaptic contacts with motoneurons. One may safely state that the bulk of synaptic contacts by these collaterals is established in laminae V, VI, VII and VIII of Rexed (1954), i.e. only in the medial parts of the first three of these layers. Quite similar results are gained from Nauta preparations. Fig. 6. shows a transversal section of the spinal cord

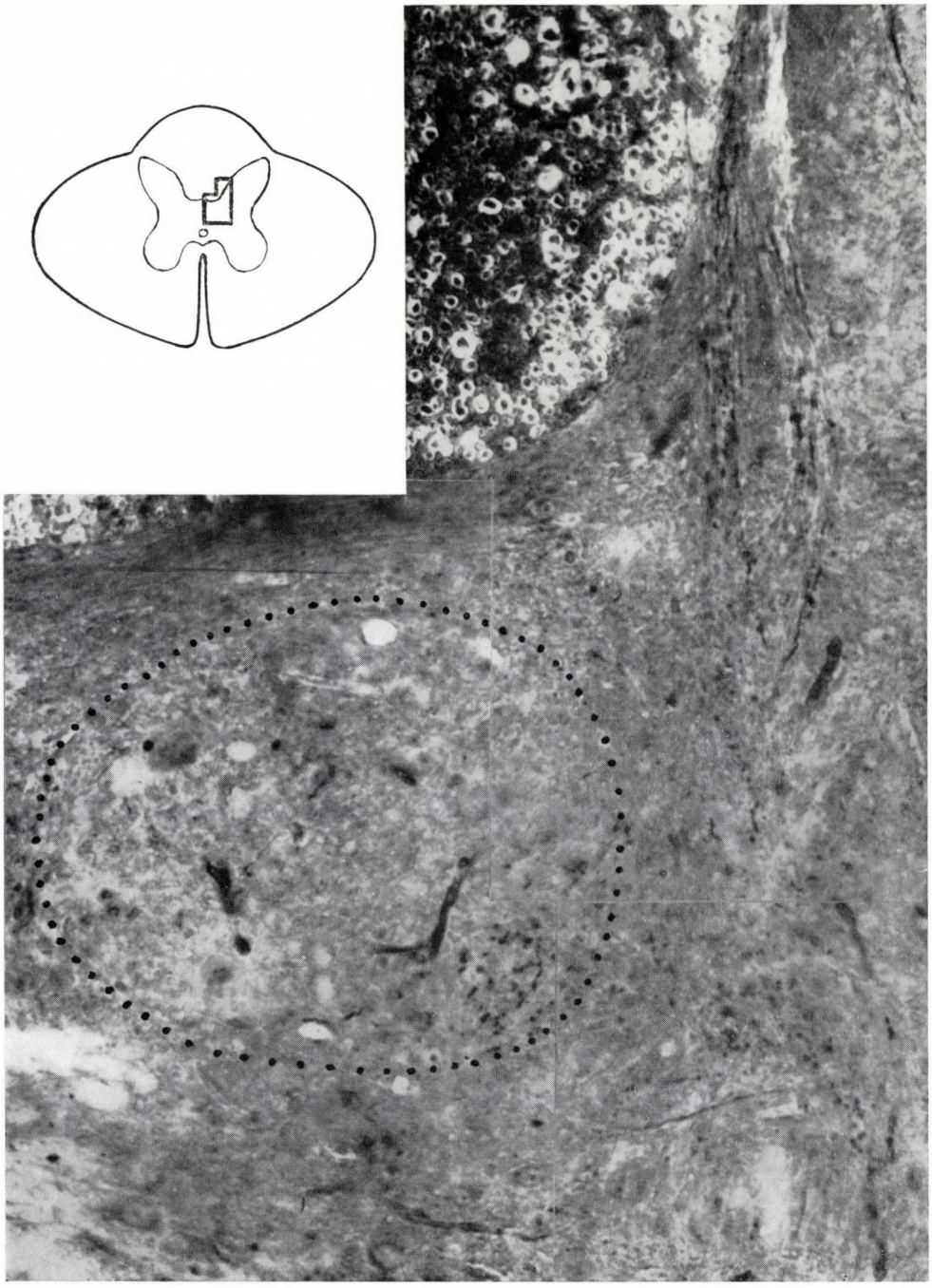


Fig. 6. Transverse section of the spinal cord at L_1 five days after having removed the ipsilateral spinal ganglion L_4 (for orientation see inset at left top). Degenerated fragments can be seen exclusively in the ventrolateral sector of Clarke's nucleus and in the grey matter lateral to the nucleus. Dotted line indicates the confines of Clarke's nucleus. 500 ×. — Adult cat, Nauta procedure

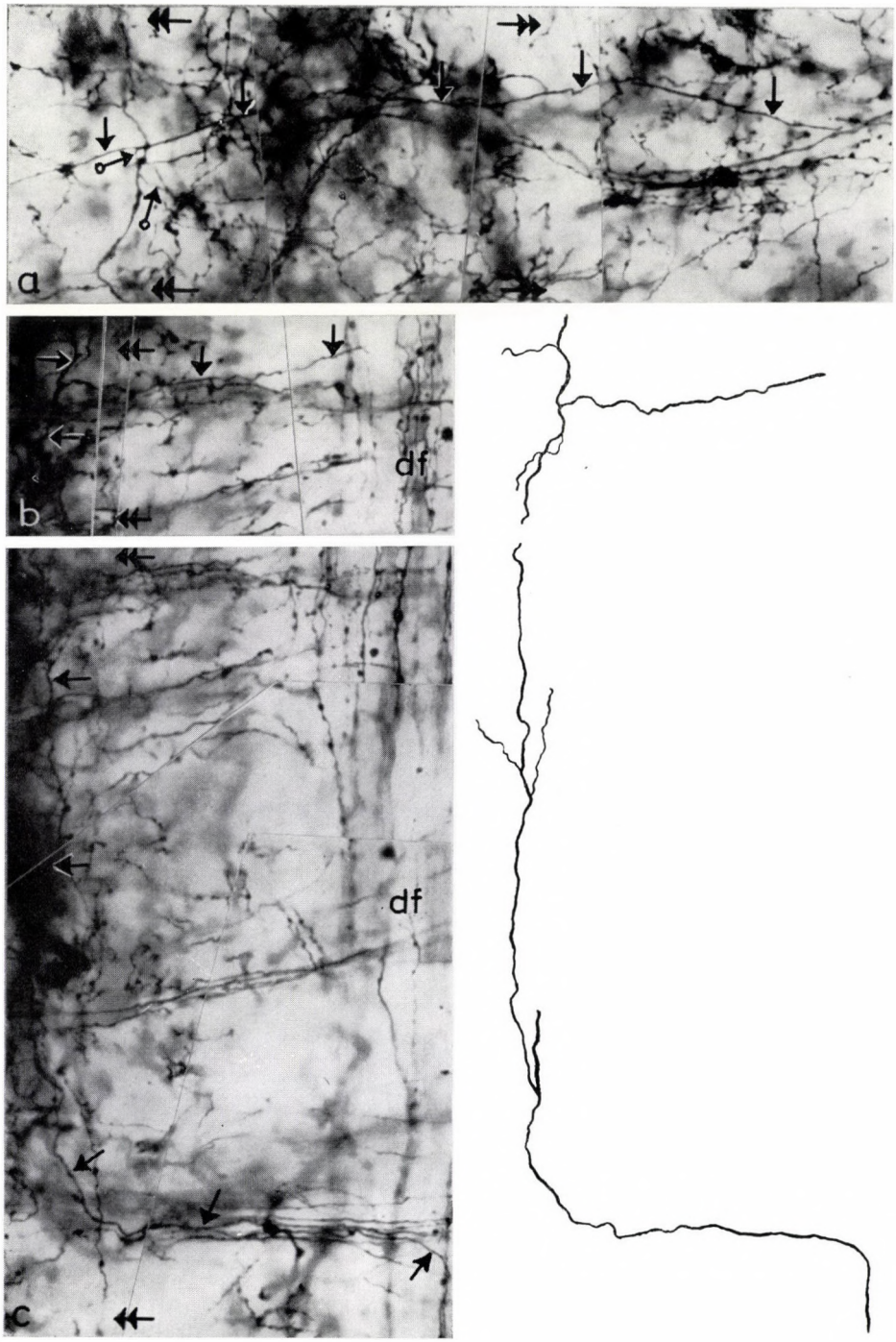
in L_1. Spinal ganglion L_1 was removed in this animal. Clarke's nucleus is present upwards from L_3, but degenerated fibres appear only at L_1. Degenerated fragments are seen on the ventrolateral part of Clarke's nucleus and on the adjacent grey matter. Comparing this picture with the corresponding Golgi preparation (Fig. 4b) the similarity is conspicuous. It emerged from all of our Nauta series — particularly well visible in horizontal sections — that preceding from aft to front the degenerated fragments appear first at the lateral border of the column and upwards gradually spread in medial direction, which would be in good agreement with the description given by SZENTÁGOTHAI (1961b). However, the exclusive occurrence within the ventrolateral sector of the column of degeneration fragments in the lowermost segment might indicate that set (b) of collaterals enters more caudally than set (a.)

In Fig. 5d a group of collaterals entirely similar in origin and initial course is shown having no connection with Clarke's nucleus. In the Golgi preparations, the two sets (b) and (d) might easily be mistaken for being one set. However, if, as it was done in Figs 5b and 5d, the collaterals are separated on the basis of the criterion whether or not they have side branches to Clarke's column the difference between the two sets becomes apparent. While set (b) has a rather indiscriminative mode of termination in a wide but still defined area, set (d) has a circumscript termination area in the intermediomedial nucleus of the earlier authors (medial part of lamina VII of Rexed). The degeneration pictures shown recently by SZENTÁGOTHAI (1966) doubtlessly prove that these collaterals derive from primary afferents.

(c) *Collaterals to the dorsal horn.* Although relatively rare in this material, one can find collaterals originating from the most medial portion of the dorsal funiculus that run horizontally towards the centre of the dorsal horn (lamina IV of Rexed). During their course through the dorsal border of the column they give off small side branches, which enter and — probably after some longitudinal course within the column — terminate in the dorsal sector of that nucleus. The course of these collaterals would be fairly characteristic of cutaneous primary afferents, if it were not for the extremely medial origin of the collaterals in the dorsal funiculus. But, as is the case also with the collaterals of set (a), they take origin from the ascending dorsal funiculus fibres only several segments above their entrance into the cord, which may account for their medial position in the dorsal funiculus.

(3) The initial course of Clarke cell axons

The origin of the main cell axons failed to show in the Golgi series. In horizontal sections rather coarse axons could, however, be traced running in transverse direction from the interior of Clarke's nucleus, towards its lateral border and further in the same direction towards the lateral funiculus. On the


a
b
df
df
c

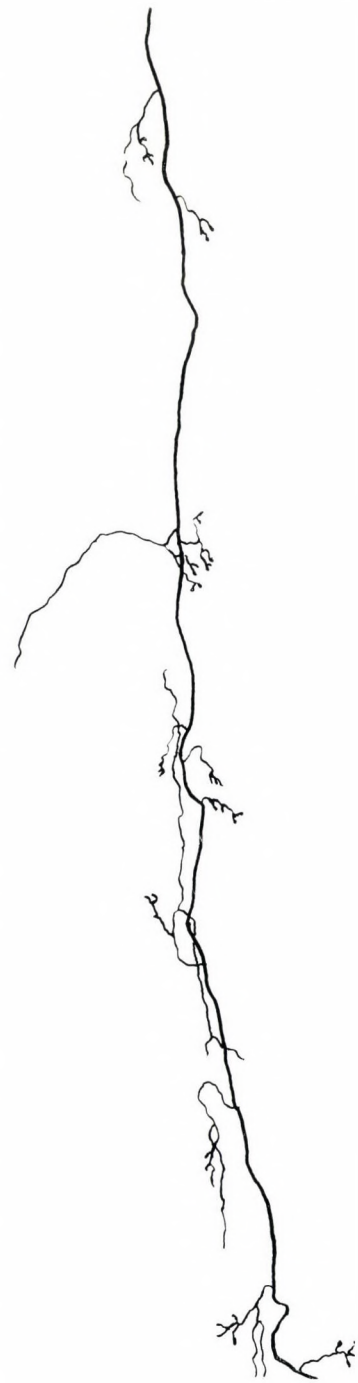
basis of their initial course these axons can be recognized with fair probability as the axons of the focal cells. They issue initial collaterals much thinner than the parent axons both inside and outside the column. Some of the former approach the margin of the column, where they turn into longitudinal direction. Their terminations could not be observed directly; they appear to contribute to the longitudinal axonal neuropil of the column (Figs 1e, 1f).

Origin, arborization and termination of the border cell axons are easier to observe. They originate from one of the main dendritic trunks near the cell body. After a short tortuous course — as already mentioned — they begin to arborize into branches of equal size, which soon divide repeatedly giving rise in a similar manner to an axonal arborization in the neighbourhood of the parent cell (Figs 2d, 3b). This arborization is oriented predominantly longitudinally and strictly confined to the territory of the column. The delicate terminal expansions of these axons can be seen in abundance within small distance from the cell of origin (Fig. 3b). This type of axonal arborization closely resembles that of Golgi II type nerve cells. It could of course be argued whether such cells can justly be called true Golgi II type neurons. They are certainly local internuncial cells without any indication of any axonal branch reaching other grey regions. Admittedly, the axonal arborizations of many true Golgi II type neurons, especially in the cerebral and cerebellar cortex, are denser and restricted to smaller territories or the arborization territory has a more strictly determined shape. These are, however, differences of degree only. Entirely similar local interneurons were described earlier and their significance has been recently brought into focus by the studies of SZENTÁGOTHAI et al. (1966), TÖMBÖL (1967) and SZENTÁGOTHAI (1967b) in the thalamus and geniculate bodies. They were referred to by these authors always as Golgi II type cells.

(4) The synaptic interrelations of Clarke column neurons

As Clarke's column consists of two types of nerve cells, both of which are giving off initial collaterals inside the nucleus, various possibilities of local neuronal coupling have to be considered. Quantitatively, there seems to be a considerable difference between the richness of arborization of the average

Fig. 7. Dorsal funiculus collaterals entering Clarke's column as seen in sagittal sections. In both photographs as well as in the drawing, dorsal is to the right and ventral to the left. The dorsal funiculus (df) is seen in (b) and (c) but to save space it had to be omitted in (a). (a) Dorsal funiculus collateral (indicated by arrows) which runs through Clarke's column giving off a thin secondary branch (indicated by ringed arrows) to its ventral part. This collateral is, therefore, supposed to belong to set (b). Double arrows indicate the dorsal and ventral borders of Clarke's column. 500 × (b) Dorsal funiculus collateral (left in photomicrograph — indicated by arrows — and right in drawing) divides on reaching the dorsal border of the column and the branches begin to run cranial- and caudalwards. The longitudinally oriented branches soon give off side branches. 500 × (c) Similar kind of collateral, having only an ascending course in the column. Having reached the column it yields terminal branches. 500 ×. Both in (b) and (c) double arrows mark the dorsal border of Clarke's column. Rapid Golgi procedure. 7-day-old kitten



border cell axon and the sparse branching of the main cell initial collaterals. However, if the larger number of the main neurons and the weak staining qualities of these axons are considered, the impression may be misleading. Exact cell counts of the two cell types might help to a better understanding of the possible mutual synaptic relationships.

Considering the mutual interconnections of the two types of neurons the following four possibilities have to be envisaged.

(a) The initial axon-collaterals of the main cells may terminate on the border cells. From the mentioned fact that most of these collaterals tend to arborize in the marginal zone of the column (Figs 1e, 1f) this proposition is most probable.

(b) These collaterals might contact other main cells. There is no way to exclude this possibility on the basis of the present material, and SZENTÁGOTHAI and ALBERT (1955) have in fact assumed this type of connection from their degeneration material. However, they then failed to consider the existence of local interneurons within the column, which could well explain the persistence of terminal knobs in completely deafferented columns. Although RAMÓN Y CAJAL advanced the theory that initial collaterals tend to terminate on the same kind of cell, this view has not been substantiated by recent histological studies (e.g. SZENTÁGOTHAI, 1967a) and also physiological evidence seems to favour the assumption that initial collaterals contact local interneurons (ECCLES et al., 1954; ANDERSEN et al., 1964; a general discussion of this question for the Purkinje cell has been given in Chapter IX by ECCLES et al., 1967).

(c) The axons of the border cells may contact the focal cells. This is not quite evident — although highly probable — from our results. The function of the border cells could be either inhibition or excitation within a restricted area around the nerve cell. Electrophysiological investigations (LAPORTE et al., 1956; HOLMQUIST et al., 1956; CURTIS et al., 1958., JANSEN et al., 1966) led to the assumption of excitatory or inhibitory interneurons intercalated between the primary afferents and the focal cells. The border cells would fit well into such a concept as they certainly receive synaptic contacts from primary afferents. As they are of Golgi II type, they could very well be, at least partly, of specific inhibitory character. As according to proposition (a) the border cells receive the initial collaterals of the main (focal) cells, the two propositions (a) and (c) together would involve the possibility of recurrent inhibition (or facilitation) as shown to exist in the dorsal column nuclei and in the thalamic nuclei.

Fig. 8. Long ascending collateral in the neuropil of Clarke's column. Longitudinally oriented axonal neuropil of Clarke's column. A single larger axon, probably a collateral of a primary afferent (set (a)) is shown in the drawing at right with its side branches and terminal knobs. Arrows indicate the point where the branches originate. Rapid Golgi procedure, 3-day-old kitten

(d) The axons of the border cells might terminate on other border cells. The histological data seem to support this assumption, although the evidence is not conclusive. On the analogy of the specific nuclei of the thalamus and especially of the lateral geniculate body (SZENTÁGOTHAI et al., 1966) the assumption holds a fair amount of probability. Mechanisms of disinhibition or disfacilitation, if they were present, could well be explained on this structural basis. Further electrophysiological and electronmicroscopic studies might help to clarify these interesting issues.

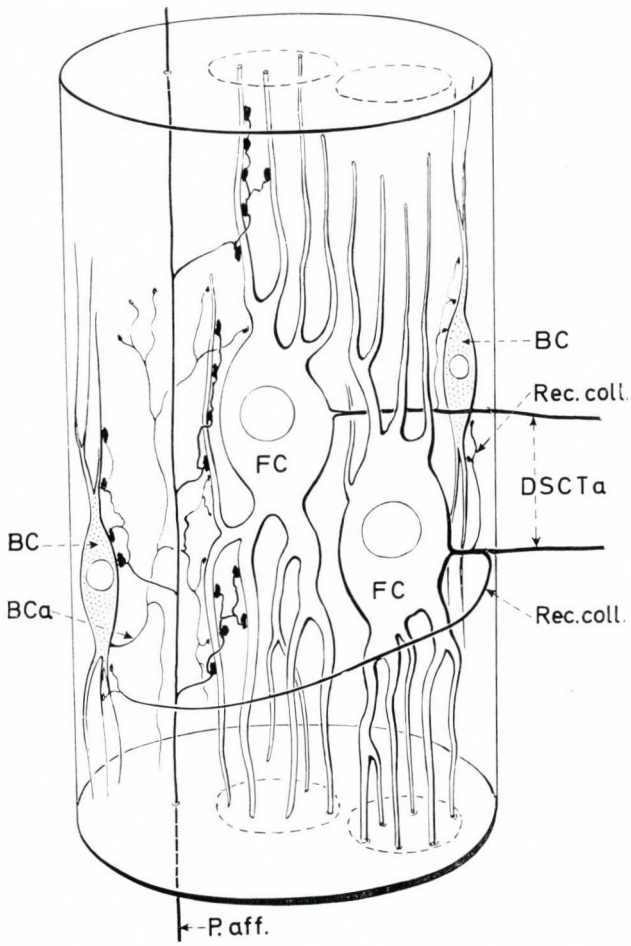


Fig. 9. Diagram illustrating the geometrical aspects of Clarke's column as discussed in Paragraph 5, with indication of synaptic interrelation between the two cell types. FC = focal cell; DSCTa = axon of focal cell composing the fibres of the dorsal spinocerebellar tract; Rec. coll. = recurrent collateral originating from the focal cell axon; BC = border cell; BCa = axon of the border cell; P. aff. = primary afferent axon. This diagram has been developed from the earlier one of Szentágothai (1961a), where the possibility of interneurons within Clarke's column itself was not taken into consideration

(5) The geometric design of Clarke's column

It has been mentioned that the form of the focal cell dendritic trees can be reduced to the shapes of cylinders with longitudinally oriented axis. As the column varies between 150 and 250 microns in diameter and as the main cell dendritic tree measures about 70—80 microns in transversal diameter in the ideal case seven such cylinders (one in the core and six in the surrounding mantle) could be accomodated within the column without major interlacing of the dendritic arborizations. The cells are shifted relative to each other in longitudinal direction in random fashion and there is also no continuity of the cylindric spaces belonging to several cells in the longitudinal direction. However, the predominantly longitudinal orientation of the axonal neuropil within the column permits to draw certain important conclusions on synaptic geometry. It has been recognized by SZENTÁGOTHAI and ALBERT (1955) and later again emphasized by SZENTÁGOTHAI (1961a) that the primary afferent axon collaterals ascend for considerable distance within the column and establish repeated and very large contacts with the dendrites they accompany in almost "climbing fibre" like fashion. This view is supported essentially by the Golgi observations, although certain smaller differences have to be mentioned. As shown in Fig. 8, a single primary afferent collateral can be traced for over 500 microns distance, and in reality it is obviously much longer. It gives off several preterminal branches of predominantly longitudinal orientation. From the position of the terminal thickenings of these preterminal branches it can well be imagined that they are in contact mainly with the same or with several longitudinal dendrites of the same focal cell. Assuming this to be the case with the collateral shown in Fig. 8 (right) one would arrive at around 50 or more boutons given to the same cell by a single primary afferent. Considering the density of primary afferent preterminal branches ascending through any cross section of the column—as it appeared also from the degeneration pictures of SZENTÁGOTHAI and ALBERT (1955) — one should assume each of the dendrites to be thoroughly buried in a tangle of preterminal branches running parallel and giving contacts of "boutons terminaux" and "boutons de passage". This then would correspond to the high power picture seen in Fig. 1d. The data available at present do not permit similar speculations to be made on the afferent and efferent contacts of the border cells. However, as their axonal arborizations are also longitudinally oriented, the synaptic relations between border cells and focal cells may be of essentially similar character. These main features of synaptic geometry are diagrammatically illustrated in Fig. 9 using also the information presented by SZENTÁGOTHAI and ALBERT (1955) and SZENTÁGOTHAI (1961a and b).

Discussion and conclusions

The general geometric features of synaptic connectivity having been discussed in paragraph (5) it now remains to make an attempt at correlating the structural observations with some of the more recent physiological findings concerning impulse transmission through Clarke's column. The general conclusions drawn by SZENTÁGOTHAI and ALBERT (1955) and SZENTÁGOTHAI (1961b) on a rather powerful kind of synaptic articulation between primary — probably mainly Ia — afferents that are in good agreement with physiological observations will not be discussed here. Very little if anything could be added from the histological point of view at the present stage and the author hopes to be able to return to this problem on the basis of EM and degeneration studies.

Perhaps the most relevant new observation, from the functional point of view, is the separation of two different territories in Clarke's column, receiving an entirely different input from primary afferents. The larger dorsomedial part of the column receives relatively coarse primary afferent collaterals (set (a) of collaterals) that are exclusively directed to Clarke neurons. As judged from their origin from a relatively medial region of the posterior funiculus, the primary afferents giving rise to these collaterals have entered the spinal cord several segments below the origin of the collaterals. In addition they are arranged approximately in the somatotopic relation described by SZENTÁGOTHAI (1961b) on the basis of secondary degeneration after cutting dorsal roots, which in itself is ample proof of these collaterals being of primary sensory origin. As the same sector of the column receives also side branches of collaterals directed to the centre of the dorsal horn (set (c) of collaterals), the most obvious explanation of this would be that set (a) are the collaterals of Ia primary afferents and set (c) the cutaneous afferents, both of which have been shown to contact Clarke neurons (LUNDBERG and OSCARSSON, 1960). The separation, however, of cells contacted by muscle spindles and cutaneous afferents cannot be deduced from the Golgi observations. That set (a) are obviously the Ia afferents follows also from the fact that this set contacts the considerable majority of Clarke neurons, as it emerges also from physiological experiments (LUNDBERG and OSCARSSON, 1956).

As little if any convergence is observable at the level of the Clarke neurons from Ia (muscle spindle) and Ib (Golgi tendon organ) primary afferents (LUNDBERG and OSCARSSON, 1956; JANSEN et al., 1966), the assumption is close at hand to consider set (b) of collaterals as being derived from the Ib primary afferents. This is also supported by the facts that (i) the number of Clarke neurons contacted by the (b) collaterals can hardly be more than one third of the total cell population, which agrees well with the 36% given by LUNDBERG and OSCARSSON (1956); (ii) the first collaterals given from any seg-

ment during their ascent are type (b) collaterals (Fig. 6) which again are in accord with the observations of OSCARSSON (1957).

The fact that secondary neurons of the DSCT belonging to the muscle spindle afferents and those belonging to the Golgi tendon organ pathway have separate territories is in itself of considerable interest and may give some clue to the design of further physiological experiments. Of even more significance, however, is the observation of the additional connections of Ib primary afferent collaterals in medial parts of the intermediate grey matter (lamina VI, VII) and in lamina VIII of the ventral horn. As it will be shown in a forthcoming paper, the far majority of nerve cells giving rise to crossed ascending ventrolateral funiculus fibres is localized in this very region of the grey matter. It is, therefore, interesting to speculate whether these Ib collaterals are contacting cells originating from the ventral spinocerebellar tract or those from the spinothalamic tract or possibly both. This kind of reasoning should not, however, be carried too far for the present as the main dorsal funiculus fibres of both Ia and Ib afferents can have — and obviously have — other collaterals in other levels to entirely different regions of the grey matter. The fact, therefore, that any collateral giving rise to terminal branches ending around different cells tells us something in the positive sense, but collaterals directed towards a single group of cells does not give any useful information in the negative sense.

REFERENCES

1. ANDERSEN, P., ECCLES, J. C., SEARS, T. A.: (1964) The Ventro-basal Complex of the Thalamus: Types of Cells: their Responses and their Functional Organization. J. Physiol. (Lond.) **174,** 370—399. — 2. CURTIS, D. R., ECCLES, J. C., LUNDBERG, A.: (1958) Intracellular Recording from Cells in Clarke's Column. Acta physiol. scand. **43,** 303—314. — 3. ECCLES, J. C., FATT, P., KOKETSU, K.: (1954) Cholinergic and Inhibitory Synapses in a Pathway from Motor-axon Collaterals to Motoneurones. J. Physiol. (Lond.) **126,** 524—562. — 4. ECCLES, J. C., ITO, M., SZENTÁGOTHAI, J.: (1967) The Cerebellum as a Neuronal Machine. Springer, Berlin. — 5. GRUNDFEST, H., CAMPBELL, B.: (1942) Origin, Conduction and Termination of Impulses in Dorsal Spinocerebellar Tracts of Cats. J. Neurophysiol. **5,** 275—294. — 6. HOLMQUIST, B., LUNDBERG, A., OSCARSSON, O.: (1956) Functional Organization of the Dorsal Spinocerebellar Tract in the Cat. V. Further Experiments on Convergence of Excitatory and Inhibitory Actions. Acta physiol. scand. **38,** 76—90. — 7. JANSEN, J. K. S., NICOLAYSEN, K., RUDJORD, T.: (1966) Discharge Pattern of Neurons of the Dorsal Spinocerebellar Tract Activated by Static Extension of Primary Endings of Muscle Spindles. J. Neurophysiol. 29, 1061—1086. — 8. LAPORTE, Y., LUNDBERG, A., OSCARSSON, O.: (1956) Functional Organization of the Dorsal Spino-Cerebellar Tract in the Cat. II. Single Fibre Recording in Flechsig's Fasciculus on Electrical Stimulation of Various Peripheral Nerves. Acta physiol. scand. **36,** 188—203. — 9. LAPORTE, Y., LUNDBERG, A.: (1956) Functional Organization of the Dorsal Spino-cerebellar Tract in the Cat. III. Single Fibre Recording in Flechsig's Fasciculus on Adequate Stimulation of Primary Afferent Neurons. Acta physiol. scand. **36,** 204—218. — 10. LENHOSSÉK, M. v.: (1895) Der feinere Bau des Nervensystems im Lichte neuester Forschungen. Fischer, Berlin. — 11. LLOYD, D. P. C., MCINTYRE, A. K.: (1950) Dorsal Column Conduction of Group I Muscle Afferent Impulses and their Relay through Clarke's Column. J. Neurophysiol. **13,** 39—54. — 12. LUNDBERG, A., OSCARSSON, O.: (1956) Functional Organization of the Dorsal Spinocerebellar Tract in the Cat. IV. Synaptic Connections of Afferents from Golgi Tendon Organs and Muscle Spindles. Acta physiol. scand. **38,** 53—75. — 13. LUNDBERG, A., OSCARSSON, O.: (1960) Functional Organiza-

tion of the Dorsal Spinocerebellar Tract in the Cat. VII. Identification of Units by Antidromic Activation from the Cerebellar Cortex with Recognition of Five Functional Subdivisions. Acta physiol. scand. **50,** 356—374. — 14. LUNDBERG, A., WINSBURY, G.: (1960) Functional Organization of the Dorsal Spinocerebellar Tract in the Cat. VI. Further Experiments on Excitation from Tendon Organ and Muscle Spindle Afferents. Acta physiol. scand. **49,** 165—170. — 15. OSCARSSON, O.: (1957) Primary Afferent Collaterals and Spinal Relays of the Dorsal and Ventral Spinocerebellar Tracts. Acta physiol. scand. **40,** 222—231. — 16. RAMÓN Y CAJAL, S.: (1909) Histologie du système nerveux de l'homme et des vertébrés. Vol. 1, Maloine, Paris. — 17. REXED, B.: (1954) A Cytoarchitectonic Atlas of the Spinal Cord in the Cat. J. comp. Neurol. **100,** 297—379. — 18. SZENTÁGOTHAI, J.: (1961a) Anatomical Aspects of Inhibitory Pathways and Synapses. In: Nervous Inhibitions, Ed. F. Florey. Pergamon Press, Oxford. Pp. 32—46. — 19. SZENTÁGOTHAI, J.: (1961b) Somatotopic Arrangement of Synapses of Primary Sensory Neurons in Clarke's Column. Acta morph. Acad. Sci. hung. **10,** 307—311. — 20. SZENTÁGOTHAI, J.: (1966) Pathways and Subcortical Relay Mechanisms of Visceral Afferents. Acta neuroveg. (Wien) **28,** 103—120. — 21. SZENTÁGOTHAI, J.: (1967a) Synaptic Architecture of the Spinal Motoneuron Pool. In: Recent Advances in Clinical Neurophysiology. Ed. L. Widén. Electroenceph. clin. Neurophysiol. Suppl. **25,** 4—19. — 22. SZENTÁGOTHAI, J.: (1967b) Models of Specific Neuron Arrays in Thalamic Relay Nuclei. Acta morph. Acad. Sci. hung. **15,** 113—124. — 23. SZENTÁGOTHAI, J., ALBERT, A.: (1955) The Synaptology of Clarke's Column. Acta morph. Acad. Sci. hung. **2,** 43—51. — 24. SZENTÁGOTHAI, J., HÁMORI, J., TÖMBÖL, T.: (1966)Degeneration and Electron Microscope Analysis of the Synaptic Glomeruli in the Lateral Geniculate Body. Exp. Brain Res. **2,** 283—301. — 25. TÖMBÖL, T.: (1966/1967) Short Neurons and their Synaptic Relations in the Specific Thalamic Nuclei. Brain Res. **3,** 307—326.

GOLGI-ARCHITEKTUR DER CLARKESCHEN SÄULE

Die Untersuchung der Clarkeschen Säule von Katzen mit dem Golgischen Imprägnationsverfahren bestätigte das Vorhandensein von zwei Neuronentypen, u.zw.
1. der *Hauptzellen*, aus denen die Fasern des Tractus spinocerebellaris dorsalis ihren Ausgang nehmen, und der *Randzellen*, kleiner Interneuronen mit kurzen Axonen, die den Neuronen vom Typ Golgi II ähnlich sind. Es konnten drei Typen der dorsalen afferenten Stränge identifiziert werden: a) afferente kollaterale Äste (Muskelspindeln, Typ Ia), b) Kollateralen (Golgisches Sehnenorgan, Typ Ib) und c) der Typ c, die vermutlich aus der Haut stammt. Während die Gruppen a) und c) im größeren dorsomedialen Sektor der Säule endigen, endigt die Gruppe b) ausschließlich im kleineren ventrolateralen Sektor. Es wird angenommen, daß sich die Clarkeschen Neuronen, die die Information an die Muskelspindel übermitteln, von den jenen Clarkeschen Neuronen unterscheiden, welche die Information an das Golgische Sehnenorgan weiterleiten und auch verschieden lokalisiert sind. Die wechselseitigen Beziehungen der Neuronen der Clarkeschen Säule und einige geometrische Muster der Synapsenverteilung werden besprochen.

АРХИТЕКТУРА ГОЛЬДЖИ ДОРЗАЛЬНОГО ЯДРА СПИННОГО МОЗГА

М. РЕТХЕЙИ

Методом окраски Гольджи дорзального ядра спинного мозга автор выявил у кошки наличие двух типов нейронов, а именно 1. *главных клеток*, от которых отходят волокна дорзального спинномозгового тракта (ДСМТ) и 2. *краевые клетки*, небольшие промежуточные нейроны, с короткими аксонами и нейроны, напоминающие тип Гольджи II. Удалось идентифицировать 3 типа дорзалных афферентных пучков: *а)* мышечные веретена типа Iа, афферентные коллатеральные ветви, *б)* коллатерали типа Iб, сухожильный орган Гольджи, и *в)* тип *в*, предположительно кожного происхождения. Пучки *а)* и *в)* оканчаются в более крупном, дорзомедиальном секторе дорзального ядра спинного мозга, в то время как группа *в)* кончается искючительно лишь в меньшем, вентролатеральном секторе. Полагается, что нейроны Кларка, передающие информацию мышечному веретену отличаются от тех, которые передают информацию сухожильному органу Гольджи, и что они различно локализованы. Обсуждаются обоюдные синаптические взаимоотношения нейронов дорзального ядра спинного мозга и некоторые геометрические рисунки распределения синапсов.

Dr. Miklós RÉTHELYI; Budapest IX. Tűzoltó u. 58., Hungary

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 331—340, 1968*

Department of Anatomy, University Medical School, Budapest

STRUCTURAL BASES OF BLOOD FLOW REGULATION IN THE SMALL INTESTINE

J. Vajda, T. Raposa and Z. Herpai

(Received November 20, 1967)

A study performed with the aid of various injection techniques (corrosion cast and transparent injection specimens) and silver stains on the small intestine in man and some laboratory animal species (cat and dog) revealed an abundance of arteriovenous (AV) anastomoses. Some of their specific sites of localizations, their various kinds and their rich nerve supply are described. Arterial and venous arborization patterns are thoroughly analyzed and their possible functional significance discussed. Abundance of AV anastomoses and the rich nerve supply either of the anastomoses or their approaches point to their significance in the regulation of blood flow. Venous valves were encountered only in the arcade zone of the mesenteric veins. The common veins of drainage of groups of 10—16 villi and their strange relation to the dense arterial capillary plexus of the submucosa are thought to be of crucial significance in the development of venous stasis in the mucosa.

Introduction

The exceptionally high and additionally very variable metabolic requirements of intestinal absorption over the whole surface of the small intestine — enlarged in gigantic proportions by the villi — have their reflections in the density and complexity of the structural substrates of circulation. Blood flow considerably increases during digestional processes and considerably decreases in starvation. Observations according to which the oral parts of the jejunum are the best supplied whereas blood flow gradually decreases towards the colon are indicative of the close correlation between circulation and absorption.

The mesenteric area, being also a region of blood storage, plays an important role in the control of blood distribution in the whole organism. One of the main instruments in the peripheral regulation of blood flow are arteriovenous (AV) anastomoses. Spanner (1931) and Schumacher (1938) were among the first to describe direct connections between arteries and veins in the mesentery and the intestinal wall, an observation confirmed later by many others. By way of these AV anastomoses the blood supply of the portal system may be increased considerably. Bypassing the capillary bed by a considerable fraction of intestinal blood flow might restrain the usual fall in blood pressure at levels ensuring the sufficient speed of portal circulation.

The aim of the present examinations was to elucidate some of the morphological bases of the local control of blood flow in the intestinal wall and the mesentery.

Material

The studies were performed on the small intestine of the cat, the dog and of man. Human material was obtained from fresh cadavers and from sections of surgically removed intestine. From this we only used parts not showing major pathological changes and to which the mesentery with sufficiently long vessels were attached.

Technical procedures

Preference was given to procedures offering the possibility of direct studies in three dimensions.

1) Arteries and veins were injected separately with filtered Indian ink diluted 1 : 10 with distilled water and kept at body temperature. Both arterial and venous systems were thoroughly cleaned of blood by perfusion with physiological saline to which papaverine had been added, it being impossible to inject arteries or veins of the villi without the previous use of spasmolytic drugs. Filling of the vessels was controlled in both cases under the dissecting microscope and was continued until complete blackening of the villi. The intestines thus injected were examined after clearing according to SPALTEHOLZ (1914).

2) Corrosion specimens were obtained by injection of PVC-powder diluted in equal amounts with acetone and cyclohexanon. After administration of papaverine chloride (0.01 g/kg) and 5% heparin (1 ml/kg), the animals were overnarcotized with ether. After exposure of the abdomen and the thoracic cavity the animals were placed into water of 37° C and the vessels of the head, extremities and of the kidneys were ligated. A cannula was introduced into aorta and vena portae and the whole intact vascular system of the abdominal viscera was perfused under low pressure from the aorta with physiological saline of body temperature. After completion of the perfusion, cyclohexanon was injected from the aorta until it appeared at the lumen of the portal vein. At first about 10—15 ml of completely colourless 2% PVC dilution was used for injection. According to our experience this concentration ensures the filling up of the finest capillaries and extravasates can be avoided by injecting at low pressure. Injection was then continued with plastic solution of the same concentration but stained red by adding powdered dye if the injection was made from the aorta, and blue if the injection was made in the reverse direction, i.e. from the portal vein. Finally an additional 10 ml of a 5% coloured dilution was injected into the larger vessels. The dye could thus diffuse from the more concentrated contents of the larger vessels towards the thinner dilution injected at first. Under stereomicroscopic control of the villi, pressure was slowly raised until their tips had stained red and their basal parts blue. Prior to the polymerization of the resin, a glass or plastic tube of adequate thickness was introduced into the lumen of the intestine in order to maintain its cylindrical shape. Corrosion of the tissue was then performed by placing parts of the intestine and their mesentery into diluted and then gradually more concentrated hydrochloric acid. After thorough washing, the remaining coloured casts of the vessels were subjected to examination under a dissecting microscope.

3) In each case pieces of the mesentery and as a control also sections of the intestine were examined with Gross-Schultze's silver impregnation method.

Results

Numerous AV anastomoses are present between primary side branches of the main radial arteries and veins of the mesentery. Their number increases especially in the region of the arches and immediately centred to the arches.

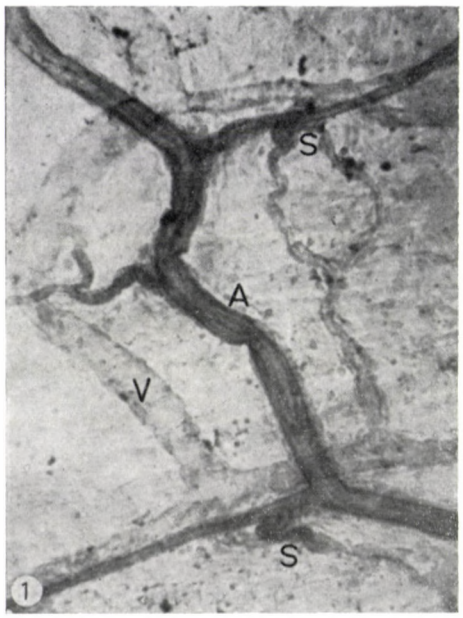


Fig. 1. AV anastomoses in the mesentery of the cat. Silver nitrate impregnation. A = artery, V = vein, S = connecting branch spirally twisted at its origin

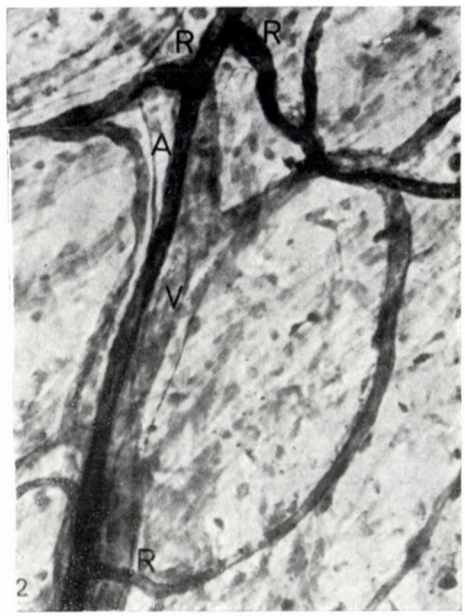


Fig. 2. AV anastomoses with ring-shaped thickenings at their origin in the mesentery of the cat. Silver nitrate impregnation. A = artery, V = vein, R = connecting branch emerging with a ring-shaped thickening

AV anastomoses may be divided into two groups, those that start with spiral loops (Fig. 1) and others having ring-shaped thickenings (Fig. 2). In the former kind the arterial limb turns into the venous limb without any appreciable change in width. In the connections having ring-shaped thickenings the arterial limb narrows down before turning into a vein. The ring-shaped thickening on the initial portion of the AV anastomosis is caused by an increase in the number of smooth-muscle elements. In the immediate vicinity of thickenings, and even among the muscular elements, numerous unmyelinated fibres may be discerned.

From the rich nerve plexus accompanying the mesenterial arteries abundant branches take off at the sites of the AV anastomoses, to attach themselves closely to the vessels connecting the artery and the vein (Fig. 3). The smaller arteries of the mesentery exhibit characteristic coils, which are never seen in the accompanying veins (Fig. 4). No change of the lumen occurs in these coils. The terminal mesenterial vessels arising from the arcades separate into three distinct groups, two immediately below the two surfaces of the mesentery continue subperitoneally to both sides of the intestine, whereas the central group enters the wall of the intestine directly and supplies the mesenterial

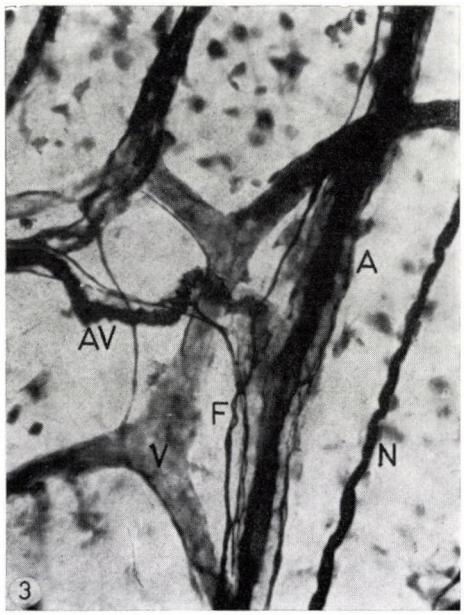


Fig. 3. Nerve fibres in the mesentery of the cat accompanying AV anastomoses which emerge with ring-shaped thickening. Impregnation after silver nitrate injection of the arteries. A = artery, V = vein, N = nerve accompanying the vascular bundle, F = nerve fibres adjoining the artery. AV = anastomosis. Arrangement of the nerve fibres that join the connecting branch is seen, as well as the thickening of the initial part of the anastomoses. Owing to the silver nitrate injection the lumen is dark in colour

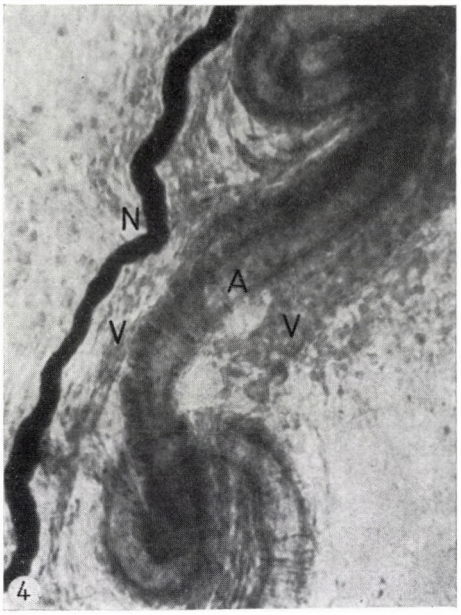


Fig. 4. Loops at the mesenteric artery of the cat. Silver nitrate impregnation. A = twisted artery, V = vein taking a straight course not following the loops of the artery, N = nerve joining the vascular bundle

one third of the total circumference of the intestinal wall (Fig. 5). Numerous interarterial connections are found in the arcade zone mainly between the arteries. They either simply join (Fig. 6) or are connected by smaller branches which thus form islands (Fig. 7). Many AV anastomoses occur in this dense vascular plexus. Two smaller branches arising from arteries often join into a short common trunk before entering the vein (Fig. 8). These AV anastomoses are regularly present between the vessels of the central group, in both plastic casts and Indian ink injection specimens (Fig. 9). Their lumen measures 0.3—0.5 mm in diameter.

The vessels running along the intestinal wall i.e. the lateral group vessels, after a short initial course proceed in the subserosa. Above them a fine capillar network is formed, which issues postcapillary veins radially (Fig. 11). Reaching the middle zone of the lateral surface the arteries divide into two branches, the larger of which divides into the muscle layer, whereas a thinner one con-

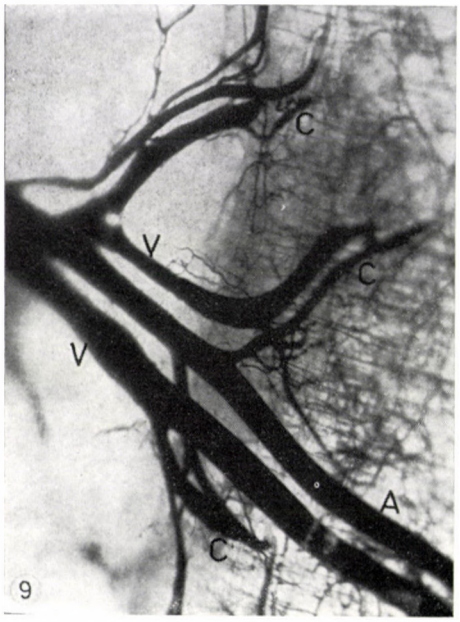


Fig. 9. AV anastomoses arranged in groups on the mesenteric border of the intestine of the cat. Preparation cleared after the arterial system had been injected with India ink. A = artery, V = vein, C = connections. The India ink has flown through the connections into the veins and has filled them up

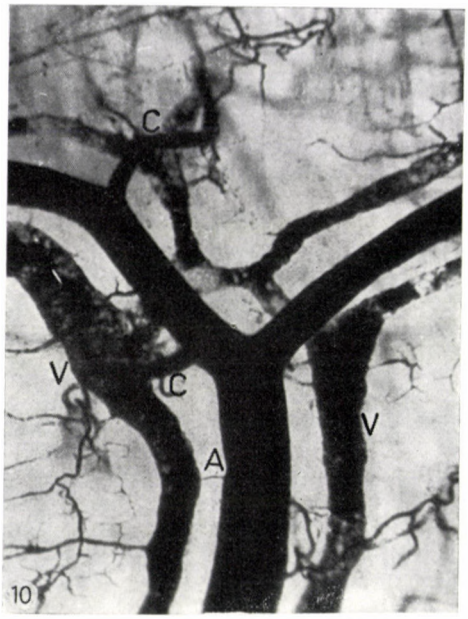


Fig. 10. AV anastomoses in wall of human intestine. The arterial system was injected with diluted India ink — as described in Fig. 9 — then the specimen was cleared. A = artery, V = vein, C = connections. The venous system defectively filling up through the connections is seen, as well as the triad artery — connecting branches — conveying veins — which form a ring

tinues below the surface towards the antimesenteric border. On the very place of division AV anastomoses (Figs 10, 12) are characteristically established between almost each larger arterial branch and its accompanying veins. These AV anastomoses are wider than those in the arcuate zone. The smaller arterial branches proceeding towards the antimesenteric border are often connected by delicate coiled transversal branches. In the antimesenteric region, the arteries from the two sides are having abundant mutual interconnections (Fig. 13).

While penetrating through the muscle layer the arteries are flattened corresponding to the directions of the muscle cells and literally squeeze themselves through the muscle bundles (Fig. 14). The capillary network formed in the muscular layer is sparse, whereas the plexus of the submucosa is of extreme richness (Fig. 15). The skeleton of the submucosal plexus is formed by arteries of about 0.2 mm thickness, the interarterial space being occupied by thin capillaries. Only few larger veins traverse this purely arterial plexus.

The larger vessels of the submucosal plexus break through the lamina muscularis mucosae where on its interior surface they are arranged into a

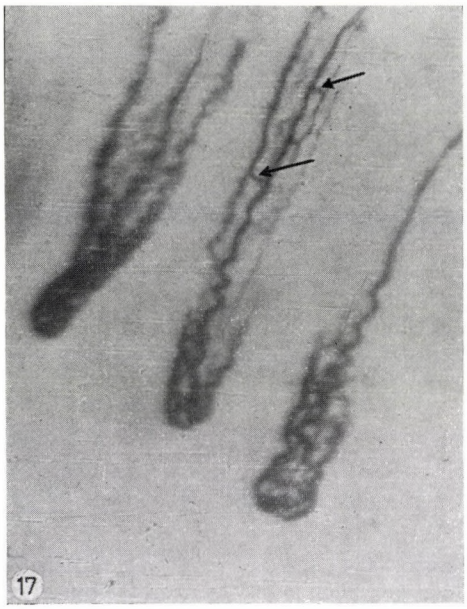


Fig. 17. Arterial capillaries in the intestine of the cat. Specimen cleared after the arterial system has been injected with India ink under the dissecting microscope, taking care that the India ink should not pass into the venous plexus. The central twisted arteriole of the villus is seen, as well as its mantle-like division on the surface of the villus. AV anastomoses on the basic part of the villus are indicated by an arrow

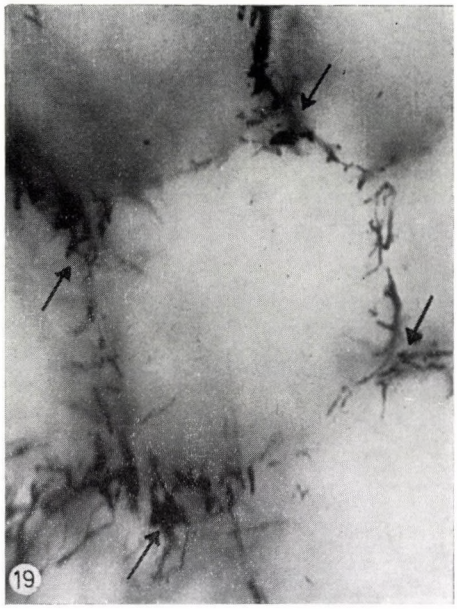


Fig. 19. Cross-section of intestinal villi in the cat. The almost hexagonal form of the cross section and the cross sections of the capillaries arranged on the surface of the villi is seen. The emerging conveying veins are indicated by an arrow

dense tangential arterial meshwork rich in anastomoses. The meshes of the larger branches are interconnected by a dense capillary network. The central arterioles of the villi take their origin from the anastomosing branches (Fig. 16) of this plexus. They arise from its main branches in tufts supplying 10—15 villi and proceed directly towards their tips. The central arterioles of the villi have side-branches — well discernible also in human material — that emerge near the base of the villi. In larger villi occasionally two or even more central vessels are present. The arteriole runs, having but few secondary side branches, up to the peak where it branches in characteristic fountain shaped fashion forming a descending mantle-like plexus below the surface of the villus (Fig. 17). The capillary network of the whole villus is an arterial one in the apical third, becoming venous in the basal two-thirds (Fig. 18). In purely venous corrosion casts the apical part of the villi remains unfilled giving a characteristic pattern of open tubes (Fig. 23). At the base the side branches of the central arteriole form AV connections within the villus itself. In venous Indian ink preparations the superficial venous plexus of the villi is well discernible,

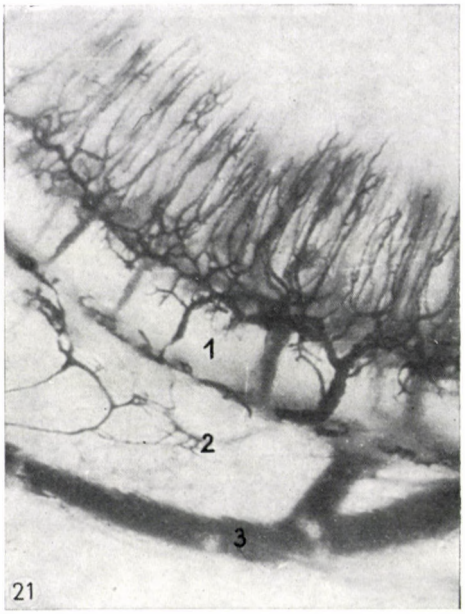


Fig. 21. Venous system of the intestinal wall of the cat in cross-section. Preparation cleared after the veins have been injected with diluted India ink. The arrangement of the venous plexuses is observable. L = submucosa, in which the crossing venous stems are seen: 2 = muscular layer, with conveying veins running between muscle bundles; 3 = larger conveying venous stem proceeding towards the surface

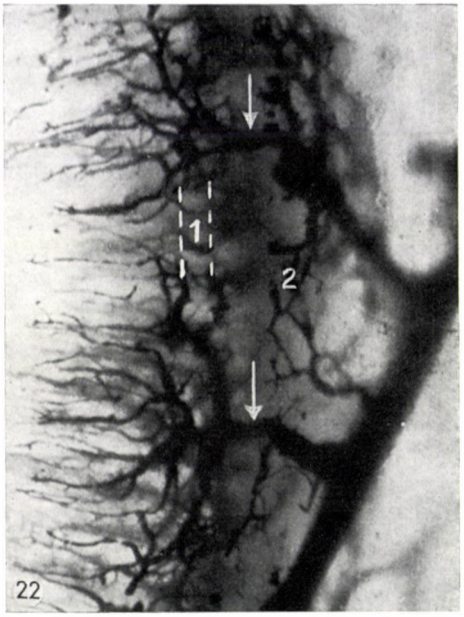


Fig. 22. Magnification of Fig. 21. Number 1 shows the situational plane of the lamina muscularis mucosae. The conveying vein crossing over the submucosa (indicated by an arrow) and collecting the venous branches emerging from 10—16 villi. Number 2 indicates the venous plexus between the submucosa and the muscular layer

which arriving at the base of the villi becomes progressively a hexagonal lattice (Fig. 19) with the main draining veins arising from the angles. There is a simtlar venous network in the layer of the Lieberkühn crypts (Fig. 20) with the difference that here the central arterioles are lacking. The venous plexus of 10—16 villi are collected at the level of the lamina muscularis mucosae into larger stems of drainage. These veins which depending on the density of the villi collect blood from a smaller or greater area of the mucosa (Figs 21, 22) are crossing the profuse arterial capillary network of the submucosa radially. In the muscular layer they accompany the flat ribbon-like coiled arteries and finally the veins pass under the serosa where they take up the blood from the stellate branches of the subserous network and then join the arteries in pairs.

Venous valves occur only in the veins of the arcade zone (Fig. 24), while none are found in the veins of the intestinal wall proper. Additional valves are encountered in mesenterial veins in close neighbourhood of lymph nodes.

Discussion

In spite of all their limitations, corrosion and transparent injection specimens if combined are especially well suited for the examination of the vascular system. The first method yields clear information about the three-dimensional architecture, while the latter shows the relations of the vessels to their surroundings.

According to the present observations, the vascular bed of the small intestine is a highly complex system that owing to its manifold internal connections may be particularly adapted to fundamental changes in direction as well as to those in various functional parameters of blood flow according to actual requirements. Particular significance can be attributed to the various kinds of anastomoses. They have been divided by SCHUMACHER (1938) and TISCHENDORF (1948) into two large groups. This subdivision is in agreement with the types found in the present study, while other authors distinguished a larger number of different kinds of anastomosis.

The third type, the one described by STAUBESAND (1950), as resembling those of the carotid glomus, was not met with in this study.

The significance of AV anastomoses lies primarily in the possibility to increase the venous reflux and consequently accelerate local blood circulation. Additionally, as stressed by PATZELT (1942), AV anastomoses might serve as a device to secure appropriate distribution of blood in various parts of the intestinal wall. Decrease of arterial pressure might be the main purpose of the anastomoses of the lateral subperitoneal arteries. These anastomoses are arranged at the sites immediately before penetrating into the muscular layer. Contraction of the circular muscle layer obviously — as can be surmised also from their flattened shape — blocks blood flow towards the depth and diverts the blood towards the veins situated in the subserosal area. The reflux may even increase in the mesenterial veins during the peristaltic wave due to increased flow through the AV anastomoses.

An important fact, especially in the case of diseases involving local changes of the mucosa, is that the common veins of drainage from the mucosa perforate the profuse capillary network of the submucosa. All pressure changes within the submucosal arterial plexus have their repercussion on blood flow of the perforating veins, including a complete suppression of circulation. In this latter case stasis may occur in the collecting area of the veins.

There is no difference of any significance in vascular architecture between the animal species examined and man.

REFERENCES

1. Barclay, A. E.: (1947) Microarteriography. Brit. J. Radiol., **20,** 394. — 2. Barclay, A. E., Bentley, F. H.: (1949) The Vascularization of the Human Stomach. Brit. J. Radiol., **22,** 62. — 3. Barlow, T. E.: (1951) Arterio-venous Anastomoses in the Human Stomach. J. Anat., **85,** 1. — 4. Boulter, P. S., Parks, A. G.: (1960) Submucosal Vascular Patterns of the Alimentary Tract and Their Significance. Brit. J. Surg., **47,** 546. — 5. Braun, H.: (1944) Die arteriovenösen Anastomosen. Med. Klin. **40,** 138. — 6. Busscher, G.: (1948) Étude morphologique et considérations physiologiques sur la vascularisation de l'estomac. Acta gastro-enterol. belg., **7,** 333. — 7. Chambers, R., Zweifach, B. W.: (1944) Topography and Function of the Mesenteric Capillary Circulation. Amer. J. Anat., **75,** 173. — 8. Chambers, R.: (1948), Blood Capillary Circulation under Normal Conditions and in Traumatic Shock. Nature (Lond.), **162,** 835. — 9. Clara, M.: (1956) Die arterio-venösen Anastomosen. Springer, Wien. — 10. Conti, G.: (1953) Über das Vorkommen von Sperreinrichtungen in Arterien mit spezieller Berücksichtigung der "gestielten Polster". Acta anat. (Basel), **18,** 234. — 11. Doby, T.: (1952) Method for the Quantitative Estimation of Arterio-venous Anastomoses in Organs. Acta med. Acad. Sci. hung., **3,** 201. — 12. Franklin, K. J.: (1951) Aspects of the Circulations Economy. Brit. med. J., **1,** 1343. — 13. Jacobson, L. F., Noer, R. J.: (1952) The Vascular Pattern of the Intestinal Villi in Various Laboratory Animals and Man. Anat. Rec., **114,** 85. — 14. Noer, R. J.: (1943) The Blood Vessels of the Jejunum and Ileum: A Comparative Study of Man and Certain Laboratory Animals. Amer. J. Anat., **73,** 293. — 15. Noer, R. J., Derr, J. W., Johnston, Ch. G.: (1949) The Circulation of the Small Intestine: An Evaluation of its Revascularizing Potential. Ann. Surg., **130,** 608. — 16. Patzelt, V.: (1942) Über das Blutkreislauforgan und die arterio-venösen Anastomosen. Wien. klin. Wschr., **55,** 748. — 17. Petry, G.: (1954) Bau und Vorkommen der arteriovenösen Anastomosen. Klin. Wschr., **32,** 943. — 18. Röhrl, W.: (1951) Die radiographische Darstellung von arterio-venösen Anastomosen. Klin. Wschr., **29,** 307. — 19. Schoedel, W.: (1954) Kurzschlußdurchblutung. Klin. Wschr., **32,** 943. — 20. Schumacher, S. v.: (1938) Über die Bedeutung der arterio-venösen Anastomosen und der epitheloiden Muskelzellen (Quellzellen). Z. mikr. anat. Forsch., **43,** 107. — 21. Sherman, J. L., jr., Newman, St.: (1954) Functioning Arterio-venous Anastomoses in the Stomach and Duodenum. Amer. J. Physiol., **179,** 279. — 22. Spalteholz, W.: (1914) Über das Durchsichtigmachen von menschlichen und tierischen Präparaten. Hirzel, Leipzig. — 23. Spanner, R.: (1931) Die arterio-venösen Anastomosen in Darm. Anat. Anz., **71,** (Erg. Bd.) 24. — 24. Spanner, R.: (1932) Neue Befunde über die Blutwege der Darmwand und ihre funktionelle Bedeutung. Morph. Jb., **69,** 394. — 25. Spanner, R.: (1939) Bau und Funktion der venovenösen Anastomosen. Anat. Anz., **87,** (Erg. Bd.) 320. — 26. Spanner, R.: (1952) Zur Anatomie der arteriovenösen Anastomosen. Verh. dtsch. Ges. Kreisl. Forsch., **18,** 257. — 27. Staubesand, J.: (1950) Über verschiedene Typen arteriovenöser Anastomosen und Glomus Organe in Hahnenkamm. Z. Zellforsch., **35,** 265. — 28. Stein, E.: (1954) Funktionelle Bedeutung der arterio-venösen Anastomosen. Klin. Wschr., **32,** 943. — 29. Varró, V.: (1966) A felszivódás és a vérkeringés kapcsolata a vékonybélben. Orv. Hetil., **107,** 481. — 30. Watzka, M.: (1942) Über Gefäßsperren und arteriovenösen Anastomosen des Menschen. Klin. Wschr., **21,** 263. — 31. Wilbrandt, W., Lautenburg, H.: (1949) Zur Frage des Gewebsdruckes und der physiologischen Bedeutung der arteriovenösen Anastomosen. Pflügers Arch. ges. Physiol., **251,** 225. — 32. Zweifach, B. W.: (1937) The Structure and Reactions of the Small Blood Vessels in Amphibia. Amer. J. Anat., **60,** 473. — 33. Zweifach, B. W.: (1939) The Character and Distribution of the Blood Capillaries. Anat. Rec., **74,** 475.

STRUKTURELLE GRUNDLAGEN DER BLUTSTROMREGELUNG IM DÜNNDARM

J. VAJDA, R. RAPOSA und Z. HERPAI

Im Dünndarm von Menschen, Katzen und Hunde wurden mit Hilfe von verschiedenen Injektionsmethoden (Korrosions- und transparente Injektionspräparate) sowie Silberimprägnation zahlreiche arterio-venöse Anastomosen beobachtet. Die spezifischen Lokalisationsstellen, verschiedenen Arten und die reiche Innervation dieser AV-Anastomosen werden beschrieben. Ihre Anzahl, reiche Nervenversorgung sowie auch die ihrer Umgebung weisen auf eine Bedeutung in der Steuerung des Blutkreislaufs hin. Venenklappen fanden sich nur in der Arkadenzone der Mesenterialvenen. Die gemeinsamen Venen der aus 10—16 Zotten bestehenden Gruppen sowie ihre eigentümlichen Beziehungen zu den dichten arteriellen Kapillargeflecht in der Submucosa haben vermutlich eine entscheidende Bedeutung in der Entstehung der Venenstauung in der Mucosa.

СТРУКТУРНЫЕ ОСНОВЫ РЕГУЛЯЦИИ КРОВОТОКА В ТОНКОЙ КИШКЕ

Й. ВАЙДА, Т. РАПОША и З. ХЕРПАИ

Исследование тонкой кишки человека и подопытных животных (кошки и собаки) с помощью разных инъекционных методов (коррозионные препараты и прозрачные инъекционные препараты), а также красителей серебра, выявило многочисленные артериовенозные анастомозы. Дается описание специфических мест локализации, различных видов артерио-венозных анастомозов, а также их богатой иннервации. Подробно анализируются артериальные и венозные арборизации и обсуждается их предположительная функциональная роль. Значительное число и богатая иннервация артерио-венозных анастомозов и их окружения указывают на значение последних при регуляции кровотока. Венные клапаны обнаружены лишь в зоне дуги мезентериальных вен. Состоящие из групп 10—16 ворсинок общие вены и их специфические связи с густым артериальным капиллярным сплетением в подслизистой предположительно имеют решающее значение при возникновении застоя вен в слизистой.

Dr. János VAJDA
Dr. Tibor RAPOSA
Dr. Zsolt HERPAI
Budapest IX., Tűzoltó u. 58., Hungary

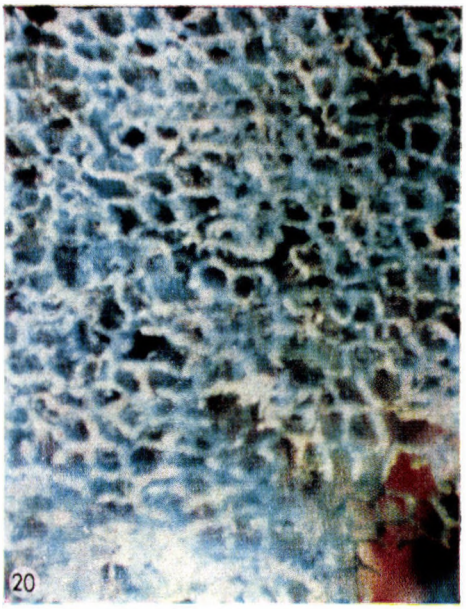


Fig. 20. Venous plexus in the layer of the Lieberkühn crypts of the villus. Corresponding to the basic part of the crypts and the villi, the honey-comb-like design of the venous plexus is well discernible

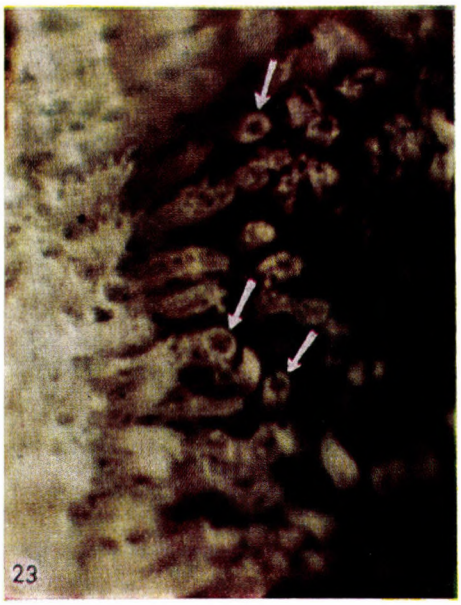


Fig. 23. Intestinal villi of the cat. Corrosion preparation after injection of the veins with PVC. At the sites indicated by arrows the peak of the villus, supplied with arrival capillaries, has not filled up

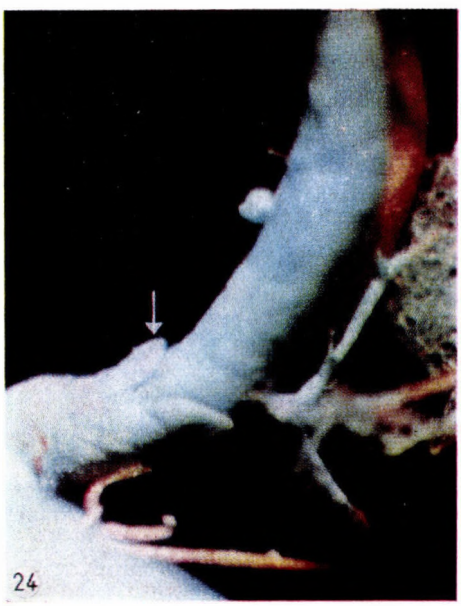


Fig. 24. Cast of venous valve found in intestine of cat. Corrosion preparation of the arterial and venous systems filled up with red and blue PVC. The venous conveying stem, as well as at the sites indicated by arrows, the negative cast of the venous valve, are seen

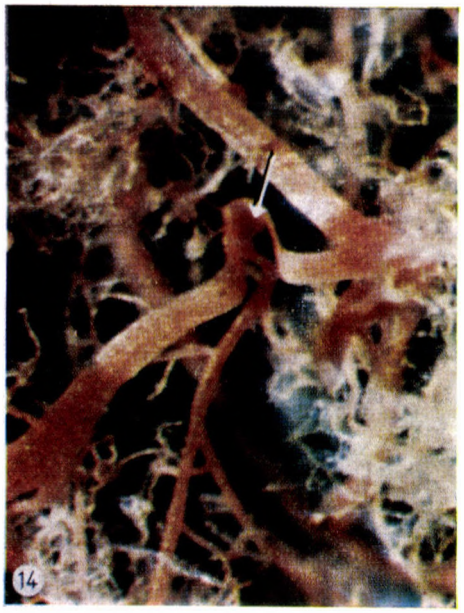


Fig. 14. Corrosion preparation of the arteries perforating the muscular layer in the intestine of the cat. The arterial system has been injected with red PVC. At the site part indicated by an arrow the twisted course of the artery corresponding to the muscle fibres may be observed and also the flattened state of the latter

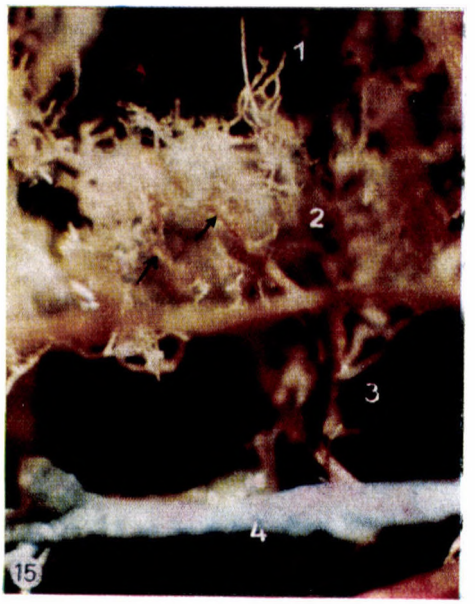


Fig. 15. Cross section of a corrosion preparation of the intestine of the cat. 1 = = central arterioles of the villus, 2 = capillary plexus of the mucosa and the submucosa. The arteries forming the skeleton of the submucosal plexus are indicated by an arrow. 3 = flattened, twisted arteries crossing the muscular layer, 4 = superficial conveying vein

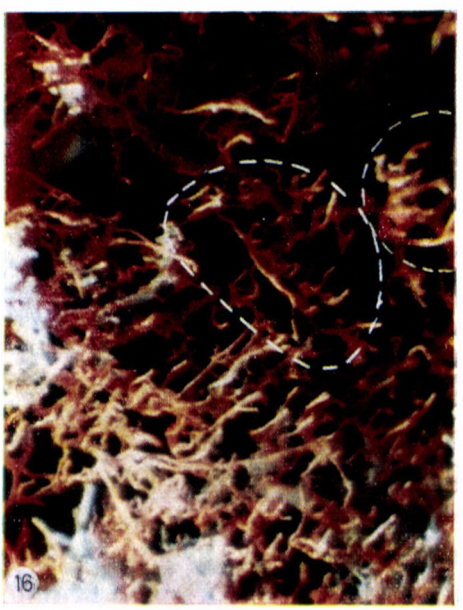


Fig. 16. Central arterioles of the villi in the intestine of the cat. Corrosion preparation after injection with stained PVC. In the area bordered by the dotted line the central arterioles of about 10—16 villi emerge from the same common stem

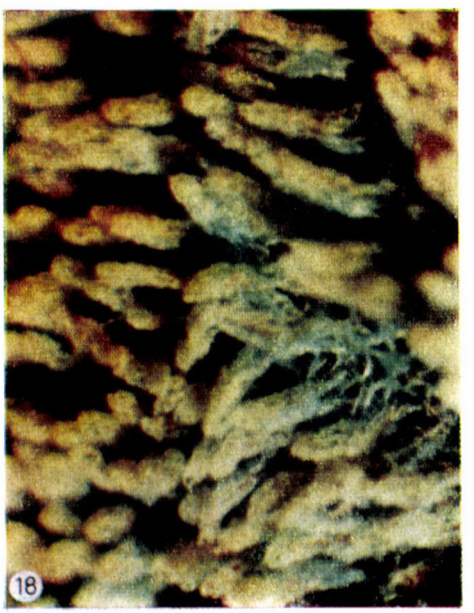


Fig. 18. Corrosion preparation of villi in the intestine of the cat, after the simultaneous injection of the arterial and venous systems with red and blue stained PVC at identical pressure. The peak of the villi stained red, the basic two-thirds blue, corresponding to the arterial and venous systems, respectively

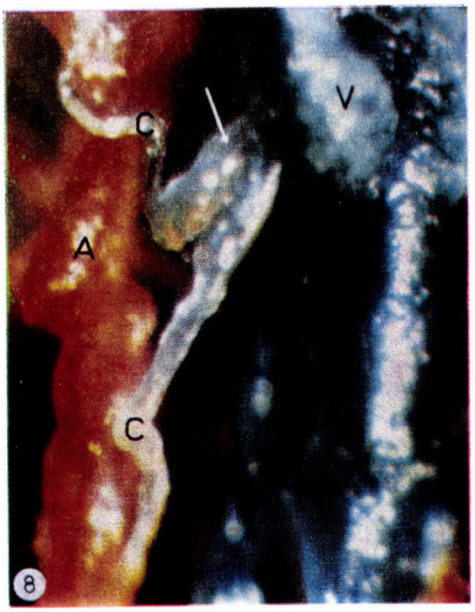


Fig. 8. AV anastomoses emerging with two thin arterial stalke on the mesenteric border of the intestine of the cat. Corrosion preparation after the simultaneous injection of the arteries with red, and of the veins with blue stained PVC. A = artery, V = vein, C = connecting branches. The arrow points to an ampullar dilatation before the flowing into the vein

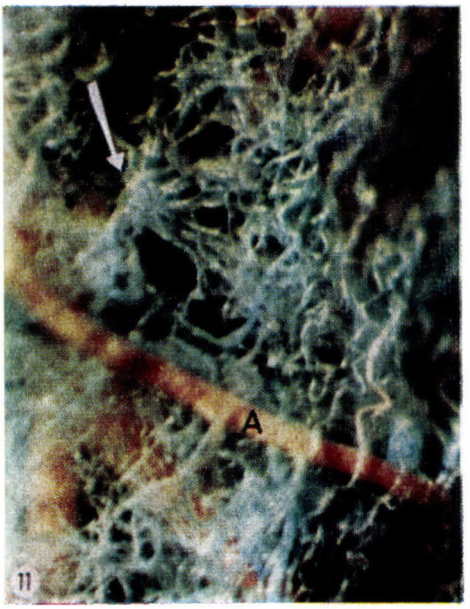


Fig. 11. Subserous vascular plexus in the intestine of the cat. Corrosion preparation after injection of the vascular system. A = arteries running under the subserous plexus. At the site indicated by an arrow a fine capillary network is formed which continues radially into postcapillary veins

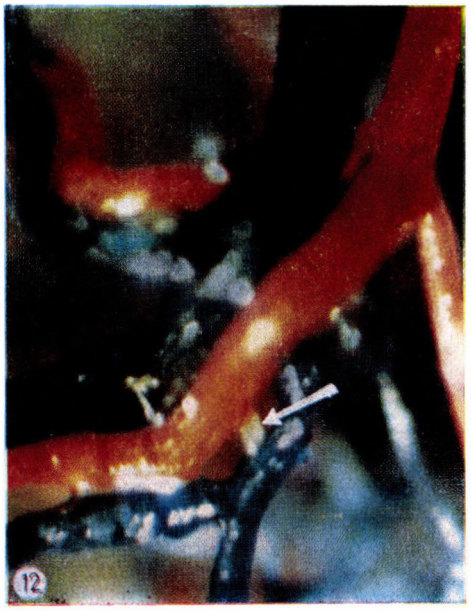


Fig. 12. AV anastomoses in the intestinal wall of the cat. Corrosion preparation of the arterial and venous system simultaneously injected with red and blue PVC. Red = artery, blue = vein. The connecting branch, indicated by an arrow, is transparently thin

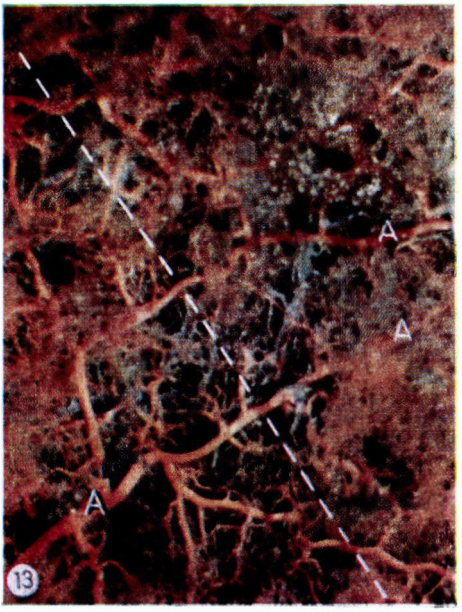


Fig. 13. Interarterial connections of the antimesenteric border of the intestine of the cat. Corrosion preparation of the vascular system after injection with PVC. The antimesenteric border is indicated by a dotted line. A = arteries. It is seen that — without any change of width — the arteries pass into another with an almost straight course

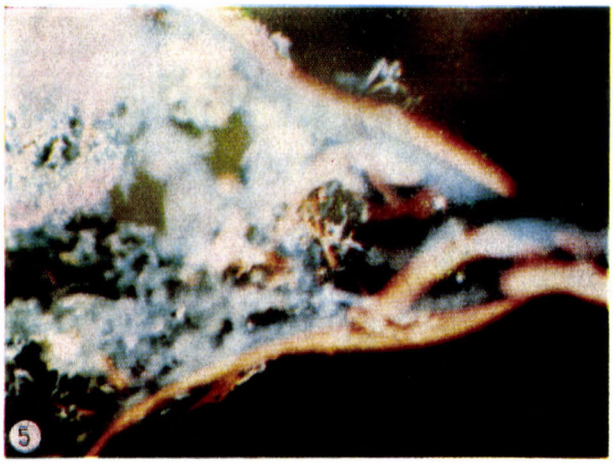


Fig. 5. Arterial and venous plexus in the mesenteric border of the intestine of the cat. Corrosion preparation of the mesenteric and intestinal vessels after injection with PVC. Red = arteries running to the intestine, blue = veins

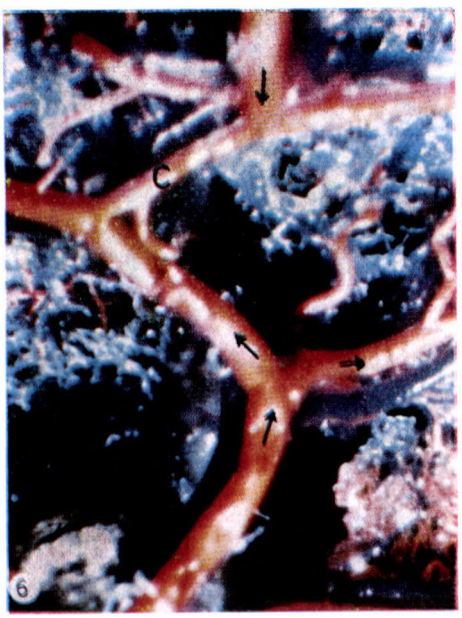


Fig. 6. Interarterial connections on the mesenteric border of the intestine of the dog. Red = arteries in which arrows indicate the direction of the blood flow, C = interarterial connection in which — depending on changes of pressure prevailing in the two arteries — direction of the blood flow might turn

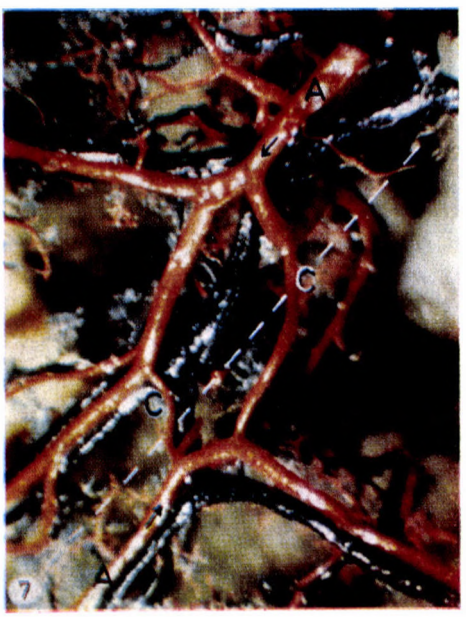


Fig. 7. Islands formed by the interarterial connection on the mesenteric border of the intestine of the cat. A = arteries with the direction of the blood flow indicated, C = = interarterial connections acting as pressure-equalizers. The mesenteric border is indicated by a dotted line

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 341—352, 1968*

Department of Forensic Medicine (Head: Prof. Dr. I. Gy. FAZEKAS), University Medical School, Szeged

EFFECT OF ALCOHOL ON THE CORTICOSTEROID FRACTIONS IN RAT TISSUE EXTRACTS*

I. GY. FAZEKAS and A. T. FAZEKAS

The authors investigated the effect of subcutaneously injected alcohol on the corticosteroid fractions of rats' various tissue extracts. The steroid fractions were revealed by chromatography developed in alkalic solution of tetrazolium blue and they were estimated quantitatively, too. As compared with the values of control animals the total corticosteroid content of all tissues decreased in the first hour after alcohol administration; this was followed by an increase after 2 and 3 hours and by an expressed decrease again after 5 and 6 hours. The number of corticosteroid fractions varied in the organs, having been the highest in the liver [9] and skeletal muscle [10]. The amount of steroids related to the unit weight of tissues was found to be highest in the adrenals, in the heart and in the kidneys. The changes in the total amount of corticosteroids after alcohol administration are described to changes in adrenal activity. The authors discussed the possible explanations of different steroid content of various organs after the use of alcohol and the ways of alcohol effects on steroid metabolism.

It has been shown that tissue extracts derived from any of the organs of rabbits or dogs contain glucocorticoid and mineralocorticoid activity [4—35] which, in a series of paper chromatographic analyses of rat [53, 54] and guinea pig [68, 69] tissues proved identical with various corticosteroid fractions. These observations provided ample evidence that, under physiological circumstances, the various organs and tissues are able to store corticosteroids (peripheral corticosteroid hormone depot). Experiments in this laboratory have shown that administration of alcohol to intact [55, 56] and adrenalectomized rats [51, 52, 57—60], and to humans [66, 67], causes an initial rise in the corticosteroid (corticosterone, hydrocortisone) content of peripheral blood, which is followed by a substantial diminution. The adrenal corticosteroid fractions of the rat were demonstrated to decrease in the first hour, then markedly to increase during the second to third hour, returning successively to control values or somewhat below them in the course of the following 8 to 10 hours [64, 65].

It is a well established fact that alcohol enhances the secretory activity of the adrenals [48, 49, 51, 52, 59, 60], while corticosteroids have been shown to increase the activity of alcohol dehydrogenase in the liver [43—47] and catalase activity in peripheral blood [70—73], thus, the adrenal gland and

* This work is dedicated to Professor Joseph BALÓ on the occasion of having completed five decades in the service of science.

corticosteroids seem to play an important part in the oxidative destruction of alcohol. In view of these results it appeared interesting to investigate the effect of alcohol on the corticosteroid fractions of various tissue extracts. The question seemed to bear some importance not only from the point of view of alcohol and corticosteroid metabolism, but also from the toxicological, endocrinological, and pathological aspects.

The present paper deals with results obtained in the rat.

Materials

Experiments were performed on 260 male Wistar rats weighing 150 to 200 g, kept on a standard diet. The animals were treated in separate groups of 20 rats each. Five groups, i.e. 100 rats, served as controls. Rats of the control groups were killed by decapitation every morning at 9.00 a.m., without any previous treatment; the organs of these rats were immediately removed and stored for 2 hours at —10° C. The experimental animals received at 9.00 a.m. 0.8 g per 100 g body weight of alcohol in a 20% aqueous solution, subcutaneously. One, 2, 3, 4, 5, 6, 8, and 10 hours after the injection of alcohol, the animals were killed by decapitation, one whole group on each subsequent occasion. The organs were removed and the identical ones pooled, stored at —10° C for 2 hours, and then homogenized. The various organs and tissues were extracted, and their corticosteroid content estimated by a semiquantitative paper chromatographic method. The weight of the tissue samples was the same in the control and experimental groups, and attained the following extremes: adrenal, 0.5 to 0.9 g; brain, 25 to 32 g; lungs, 20 to 37 g; heart, 10 to 15 g; spleen, 10 to 20 g; liver, 70 to 130 g; kidneys, 22 to 35 g; skeletal muscle, 200 to 280 g.

Methods

Tissue extracts were prepared by the methods of BUSH and FERGUSON [1, 2] and ENDRŐCZI [3a], originally described for blood and adrenal tissue, respectively, and modified by us in several respects. In order to remove chromogens, purification chromatography was undertaken in the BUSH 5 system, using Whatman's No. 3 paper; the material was then rechromatographed in the BUSH 5 system, allowing cortisol, cortisone, corticosterone, and 11-deoxycorticosterone standards (1, 2, 2.5, 5, and 10 μg) to run parallel with the extracts. Chromatography in the latter system took 6 and a half hours, after which the chromatograms were allowed to dry at room temperature. The starting line was indicated by a mark in ultraviolet light, and the chromogen impurities which gave a white fluorescence were also noted. The material thus prepared was developed in a 9 : 1 mixture of 2 *N* sodium hydroxide and 0.1% aqueous tetrazolium blue. Within a few minutes, the steroid compounds possessing a 17-alpha-keto-side chain appeared in the form of blue formazan spots of varying intensity. By comparing the colour intensity of the spots derived from the tissue extracts with that of the standard preparations it was possible to determine the Rf values. Quantitative estimation was undertaken by measuring the extension and colour intensity of the formazan spots. The chromatogram was then dried under an infrared lamp for 45 to 60 min. This procedure converted the whole chromatogram into dark violet, rendering the formazan spots no more distinguishable from other areas of the paper. On drying at 70 to 80° C the alkaline fluorescent reaction appeared, which is given only by steroids with a 3-oxo-delta-4 side chain.

The dried chromatogram was then exposed to ultraviolet light, using an analytical quartz lamp and a UG 5 filter. The spots showing yellow fluorescence were roughly compared with the spots given by the reference standards. On basis of the Rf values and the results obtained by comparing the two kinds of reaction, the corticosteroid content of the spots was calculated and expressed in μg per 100 g tissue weight. The spots which gave the tetrazolium blue and the alkaline fluorescent reactions, and to which no reference standard was available, were designated as compounds X_1, X_2, X_3, X_4, X_5, X_6, and X_7; these coupounds, on basis of their Rf values, could be identified with a certain risk of uncertainty; in the Tables a question mark serves to indicate this circumstance.

Table I

Corticosteroid fractions in μg per 100 g in the tissues of control rats without alcohol treatment. Rf values, tetrazolium blue reaction, NaOH reaction (fluorescence) and the corresponding standards

Organs	Tetrazolium blue r: + NaOH r.: +					Tetrazolium blue r: + NaOH r.: +					Tetrazolium blue r: + NaOH r.: —					Tetrazolium blue r: + NaOH r.: +					Tetrazolium blue r.: + NaOH r.: +					Tetrazolium blue r.: + NaOH r.: +				
	Rf.: 0.10 = X_1 hexahydrocortisone?					Rf.: 0.13 = X_2 tetrahydrocortisone?					Rf.: 0.18 = X_3 tetrahydrocortisone?					Rf.: 0.29 = Cortisol: +					Rf.: 0.42 = X_4 aldosterone					Rf.: 0.47 = cortisone				
	Group of animals					Group of animals					Group of animals					Group of animals					Group of animals					Group of animals				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Adrenals	—	—	—	—	—	271.4	270	262	272	282	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Brain	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lungs	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.3	0.4	0.6	0.3	0.5	—	—	—	—	—	—	—	—	—	—
Heart	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	1.2	0.8	1.5	0.6	—	—	—	—	—	—	—	—	—	—
Spleen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.8	—	1.0	1.5	0.8	—	—	—	—	—	—	—	—	—	—
Liver	0.2	0.5	1.0	2.2	3.8	0.2	0.4	0.5	0.2	0.3	—	—	—	—	—	1.4	2.3	1.8	3.2	3.8	2.4	2.0	2.4	2.0	2.4	0.6	0.9	0.8	0.6	0.8
Kidneys	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.4	1.2	0.3	0.3	0.3	—	—	—	—	—	3.8	4.8	6.1	4.9	5.2
Skeletal muscle	1.0	2.0	0.5	0.5	1.0	0.2	0.2	0.2	0.2	0.2	0.5	0.4	0.6	0.5	0.5	0.3	0.3	0.2	0.4	0.3	0.3	0.2	0.2	0.3	0.2	1.8	1.0	1.2	1.0	0.2

	Tetrazolium blue r.: + NaOH r.: +					Tetrazolium blue r.: + NaOH r.: blue fluorescence					Tetrazolium blue r.: + NaOH r.: +					Tetrazolium blue r.: + NaOH r.: +					Tetrazolium blue r.: + NaOH r.: +				
	Rf.: 0.58 = X_5 tetrahydrocorticosterone?					Rf.: 0.69 = X_6 tetrahydro-17-hydroxy-11-desoxycorticosterone?					Rf.: 0.72 = X_7 dihydrocortisone?					Rf.: 0.80 = corticosterone					Rf.: 0.87 = 11-desoxy-17-hydroxycorticosterone?				
	Group of animals					Group of animals					Group of animals					Group of animals					Group of animals				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Adrenals	452.8	230	87.7	160	200	452.8	155	235	80	600	543.2	577.8	735	620	240	2717.3	1552	1315.7	1123	1000	815.2	540	380	675	200
Brain	4.9	3.3	3.3	2.9	3.4	2.0	3.7	7.2	1.9	10.0	—	—	—	—	—	8.9	7.9	3.7	3.8	17.8	1.6	3.6	4.8	7.6	7.1
Lungs	4.7	4.3	3.8	0.5	0.3	9.4	6.3	0.4	1.5	5.0	—	—	—	—	—	10.9	5.0	4.0	3.0	3.3	6.3	2.0	3.4	4.2	5.0
Heart	9.5	3.5	4.5	6.2	7.3	19.0	12.4	6.5	9.0	7.9	—	—	—	—	—	19.0	10.0	4.2	18.1	3.4	14.3	8.2	6.4	2.7	0.8
Spleen	7.6	8.7	4.2	6.6	6.5	1.9	10.2	6.4	5.2	10.0	—	—	—	—	—	15.4	4.1	3.0	4.4	5.0	7.7	3.9	5.3	8.8	2.5
Liver	1.4	2.0	1.6	0.9	0.2	1.0	0.8	1.2	0.5	0.4	—	—	—	—	—	2.9	4.2	2.5	3.0	1.2	2.9	3.4	3.5	2.0	3.8
Kidneys	3.2	2.8	2.4	1.7	5.0	9.4	4.6	7.4	5.4	10.0	—	—	—	—	—	16.9	8.0	6.2	7.3	8.3	11.3	5.8	4.8	3.6	3.4
Skeletal muscle	1.3	1.2	0.9	0.7	0.5	1.5	1.4	0.9	0.6	0.2	—	—	—	—	—	14.0	2.7	3.1	2.5	1.5	2.0	3.0	2.7	2.0	3.0

Table III

Corticosteroid fractions in μg/per 100 g in the tissues of rats 1—10 hours after injection of 0.8 g/100 g of aethyl alcohol

	Tetrazolium blue r.: + NaOH r. = yellow fluorescence								Tetrazolium blue r.: + NaOH r. = —								Tetrazolium blue r.: + NaOH r.: yellow fluorescence								Tetrazolium blue r.: + NaOH r.: yellow fluoresceence							
	Rf.: 0.13 = X_2 = tetrahydrocortisol?								Rf.: 0.18 = X_3 = tetrahydrocortisone?								Rf.: 0.29 = cortisol								Rf.: 0.42 = X_4 = aldosterone?							
	after hours alcohol								after hours alcohol								after hours alcohol								after hours alcohol							
	1	2	3	4	5	6	8	10	1	2	3	4	5	6	8	10	1	2	3	4	5	6	8	10	1	2	3	4	5	6	8	10
Adrenals	—	—	216.6	—	—	—	—	—	472.5	—	—	—	—	—	—	—	189.9	—	—	—	—	—	—	—	202.7	—	—	—	56.8	—	71.8	97.4
Brain	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.8	—	—	5.3	—	—	—	—	—	—	—	—	—	—	—	—
Lungs	—	—	—	—	—	—	—	—	0.3	—	—	—	—	—	—	—	2.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Heart	—	—	—	—	—	—	—	—	0.4	—	—	—	—	—	—	—	3.4	—	6.0	—	—	—	—	—	—	—	—	—	—	—	—	—
Spleen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Liver	—	—	—	—	—	—	—	—	—	—	—	1.8	—	—	—	—	2.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kidneys	—	—	—	—	—	—	—	—	0.5	—	—	4.2	—	—	—	—	0.6	—	—	9.1	—	—	—	—	—	—	—	—	—	—	—	1.6
Skeletal muscle	—	—	—	—	0.9	—	—	—	—	—	—	—	—	—	—	—	2.8	0.9	—	1.0	—	—	—	—	—	—	—	—	—	—	—	1.7

	Tetrazolium blue r.: + NaOH r. = yellow fluorescence								Tetrazolium blue r.: + NaOH r. = yellow fluorescence								Tetrazolium blue r.: + NaOH r. = blue fluorescence								Tetrazolium blue r.: + NaOH r.: = yellow fluorescence							
	Rf.: 0.47 = cortisone								Rf.: 0.58 = X_5 = tetrahydrocorticosterone								Rf.: 0.69 = X_6 = tetrahydro-17-hydroxy-11-desoxycorticosterone?								Rf.: 0.72 = X_7 = dihydrocortisone?							
	after hours alcohol								after hours alcohol								after hours alcohol								after hours alcohol							
	1	2	3	4	5	6	8	10	1	2	3	4	5	6	8	10	1	2	3	4	5	6	8	10	1	2	3	4	5	6	8	10
Adrenals	—	—	—	—	—	—	—	—	202.7	206.8	144.4	714.2	56.8	23	1197.6	649.3	540.4	1034.4	72.2	285.7	340	—	119.8	161.3	—	—	772.8	—	—	—	—	—
Brain	—	—	—	—	—	—	—	—	—	—	5.8	4.9	3.1	—	2.7	2.4	7.4	—	3.5	3.1	—	—	1.4	5.2	—	—	3.8	—	—	—	—	—
Lungs	—	—	—	—	—	—	—	0.7	5.5	—	6.9	—	5.1	—	3.5	3.6	6.1	4.0	5.1	—	—	—	1.2	3.6	—	—	—	—	—	—	—	—
Heart	—	—	—	—	—	—	—	—	—	13.8	12.8	—	8.1	—	9.7	4.7	6.4	10.3	12.8	—	—	—	1.6	—	—	6.9	—	—	—	—	—
Spleen	—	—	—	—	—	—	—	—	8.1	10.3	11.5	10.1	—	—	—	10.7	8.1	10.0	11.5	6.0	—	—	—	4.2	—	—	—	—	—	—	—	—
Liver	1.0	—	—	—	—	—	—	—	2.1	0.9	0.1	—	0.9	—	—	—	1.5	1.4	3.9	1.3	0.3	—	—	1.4	—	—	0.5	—	—	—	—	—
Kidneys	—	—	—	—	—	—	—	2.0	3.5	3.5	11.1	6.6	6.5	—	6.8	2.7	1.5	1.7	3.2	5.3	2.6	—	4.1	3.4	—	2.3	—	—	—	—	—	—
Skeletal muscle	0.5	—	—	—	—	—	—	—	1.3	3.0	1.2	1.1	1.9	—	—	3.0	2.7	3.0	0.6	0.5	—	—	2.2	1.0	—	—	1,2	—	—	—	—	—

	Tetrazolium blue r.: + NaOH r.: yellow fluorescence								Tetrazolium blue r.: + NaOH r.: yellow fluorescence							
	Rf.: 0.80 = corticosterone								Rf.: 0.87 = 11-desoxy-17-hydroxycorticosterone?							
	after hours alcohol								after hours alcohol							
	1	2	3	4	5	6	8	10	1	2	3	4	5	6	8	10
Adrenals	608.2	3448.3	4173.1	257.1	56.8	459.7	1796.4	2164.5	270.2	517.2	293.6	642.8	—	—	179.6	108.2
Brain	1.9	8.5	7.7	6.3	0.9	4.7	8.5	8.7	1.8	—	0.4	—	—	—	2.7	0.7
Lungs	7.7	8.0	3.4	0.3	19.2	9.3	11.8	12.7	3.7	6.7	1.7	—	—	—	2.7	2.9
Heart	4.3	17.2	17.1	8.6	4.5	8.0	12.2	7.8	6.4	5.5	8.5	—	—	—	3.2	—
Spleen	10.8	12.5	23.0	12.1	2.5	8.6	6.4	2.5	6.5	10.0	3.8	—	—	—	—	2.1
Liver	1.5	2.8	2.0	—	1.2	1.1	2.7	1.5	1.5	—	1.2	—	—	—	1.3	1.6
Kidneys	5.5	5.8	12.7	6.7	2.2	6.0	8.5	33.8	2.9	1.4	6.3	—	—	—	5.1	5.0
Skeletal muscle	2.1	7.0	3.0	1.8	4.7	9.0	5.7	8.1	1.1	2.3	1.5	—	—	—	1.8	0.3

The method has a range of error of about ± 10 to 15%. The method used for extraction in this study proved to have an efficiency of about 60 to 70% thus, approximately 30 to 40% of the total corticosteroids present in the tissues had escaped detection. Accordingly, the extracts analysed by the above chromatographic method, contained only about 60 to 70% of the corticosteroids present in the tissues and organs.

Results

Table I summarizes the corticosteroid fractions present in the tissue extracts of control rats not receiving alcohol; all values were computed for 100 g of wet tissue weight. The results were expressed in micrograms, determined on basis of the tetrazolium blue and NaOH reactions. In all the 5 control groups the adrenals contained 5 fractions of corticosteroids: tetrahydro-

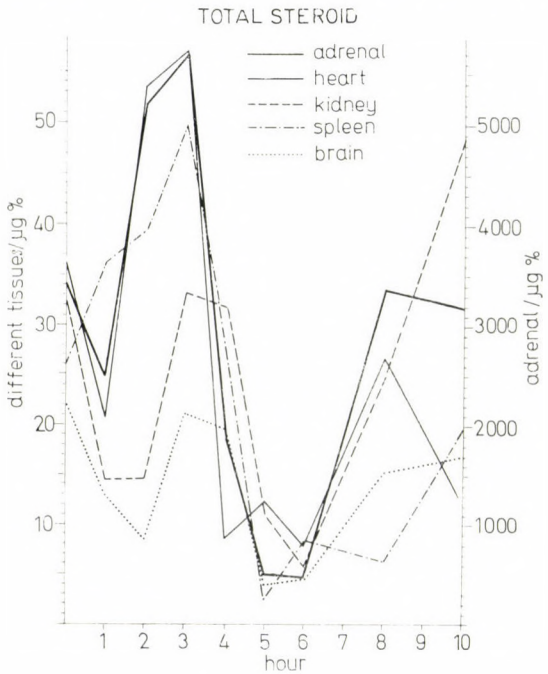


Fig. 1

corticosterone (Rf = 0.58), tetrahydro-17-hydroxy-11-desoxycorticosterone? (Rf = 0.69), dihydrocortisone? (Rf = 0.72), corticosterone (Rf = 0.80), and 11-deoxy-17-hydroxy-corticosterone? (Rf = 0.87). Tetrahydrocortisol (Rf = 0.13) could be detected in a single. It is remarkable that compounds having Rf values of 0.10, 0.18, 0.29, 0.42, and 0.47 should not have been detected in the adrenal tissue extracts, although these compounds were present in some of the other organs analysed in this study. It should be stressed that the compounds with Rf values of 0.58, 0.69, 0.80 and 0.87, were invariably present in

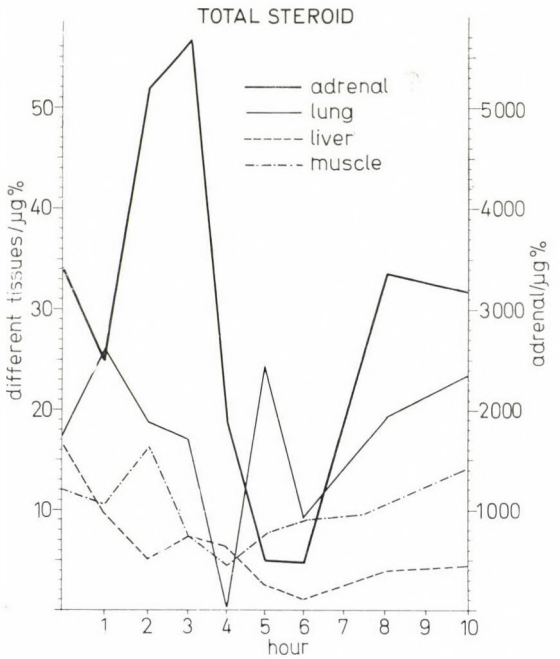


Fig. 2

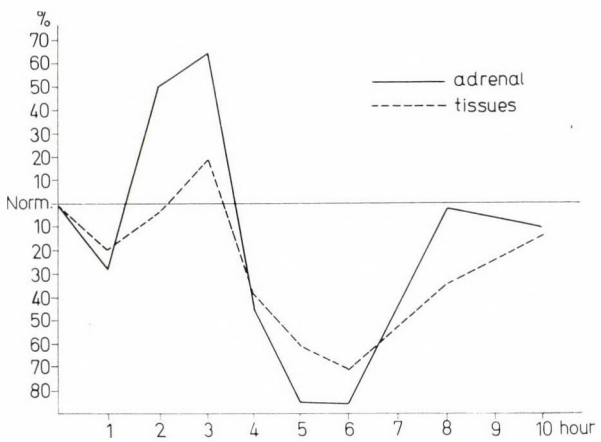


Fig. 3

the adrenals and all other organs and tissues, while those with an Rf of 0.72, only in the adrenals. The values obtained for the corticosteroid content of different organs and tissues of the control rats were in agreement with the data obtained by us in previous studies [53, 54].

Table II shows the corticosteroid fractions of the tissues and organs of the control rats, arranged according to the hormone content of the various

Table II

Corticosteroid fractions in the order of Rf values and total steroid content of control male rats

Rf	Compound	Total corticosteroid content							
		Adrenal	Heart	Kidney	Spleen	Brain	Lung	Liver	Skeletal muscle
0.10	X_1 = hexahydrocortisone?	—	—	—	—	—	—	1.5±1.4	1.0±0.6
0.13	X_2 = tetrahydrocortisol?	271.4±7.1	—	—	—	—	—	0.3±0.1	0.2±0.0
0.18	X_3 = tetrahydrocortisone?	—	—	—	—	—	—	—	0.5±0.1
0.29	cortisol	—	1.0±0.2	0.3±0.1	1.0±0.2	—	0.4±0.1	2.5±0.9	0.3±0.1
0.42	X_4 aldosterone?	—	—	—	—	—	—	2.2±0.2	0.2±0.1
0.47	cortisone	—	—	4.9±0.8	—	—	—	0.8±0.2	1.0±0.1
0.58	X_5 = tetrahydrocorticosterone	226.1±137.4	6.7±2.3	3.0±1.2	6.7±3.5	3.9±0.7	2.7±2.2	2.4±0.7	0.9±0.3
0.69	X_6 = tetrahydroxy-17-hydroxy 11-desoxycorticosterone	304.5±214.9	10.9±5	9.3±2.3	6.7±4.4	4.9±3.8	4.5±3.6	0.8±0.3	0.9±0.5
0.72	X_7 = dihydrocortisone?	543.2±91.3	—	—	—	—	—	—	—
0.80	corticosterone	1541.6±307.7	10.9±7.4	9.3±4.2	6.3±5	8.6±5	5.2±3.2	2.8±1	4.7±5.1
0.87	11-desoxy-17-hydroxycorticosterone	522.2±265.3	6.5±5.2	5.8±3.2	5.6±1.3	4.9±2.5	4.2±1.4	3.1±0.7	2.3±0.5
	Total steroid content	3409.0	36.0	32.6	26.3	22.3	17.0	16.4	12.0

Table IV

Total corticosteroid content in μg per 100 g in the tissues of control rats and rats 1—10 hours

Organ	Total steroid content of control rats μg g per 100 g	Total steroid content in μg per 100 g in alcohol			
		1h	2h	3h	4h
Adrenals	3409.0	2486.6	5206.7	5671.7	1898.8
Heart	36.0	20.9	53.7	57.2	8.6
Liver	32.6	14.5	14.7	33.3	31.9
Spleen	26.3	36.2	39.3	49.8	28.2
Brain	22.3	12.9	8.5	21.2	19.6
Lungs	17.4	26.0	18.7	17.1	0.3
Liver	16.4	9.6	5.1	7.3	6.4
Skeletal muscle	12.0	10.5	16.2	7.5	4.4
Total steroid content of organs without adrenals	163.0	130.6	156.2	193.4	99.4
in per cent	100	80.1	95.8	118.6	60.9
Change in μg per 100 g	0	−19.9	−4.2	+18.6	−39.1
Total steroid content of organs including the adrenals μg per 100 g	3572.0	2548.9	5362.9	5865.1	1998.2
in per cent	100	71.3	150.1	164.1	55.9
Change in μg per 100 g	0	−28.7	+50.1	+64.1	−44.1

organs. The total corticosteroid content of the different organs of the rat is also shown.

Table III summarizes the changes observed in response to 0.8 g/100 g body weight of 20% alcohol in the corticosteroid content of the various tissues and organs, as checked at different points of time between 1 and 10 hours after alcohol administration.

Table IV shows the total corticosteroid content of the different tissues and organs of the rats receiving 0.8 g/100 g of alcohol subcutaneously, as compared to parallel values obtained in the control groups. The time course of the changes occurring in response to alcohol was as follows. Total corticosteroid content of all the organs and tissues attained 163.0 μg/100 g in the control animals, which in the first hour after alcohol administration was reduced to an average of 130.6 μg. This was followed by an increase to 156.2 μg and 193.4 μg after 2 and 3 hours, respectively, thus, the maximum rise was obtained 3 hours after alcohol treatment, and the increment attained 30.0 μg/100 g, 18.4% of the initial value. Four hours after the administration of alcohol, however, total corticosteroid content diminished to an average of 99.4 μg/100 g, then to 64.7 μg and 46.7 μg after 5 and 6 hours, respectively. The greatest reduction was, thus, observed 6 hours after the injection of alcohol, and it attained 116.3 μg, i.e. 71.3% of the control value. Eight hours after alcohol administration an average of 105.9 μg/100 g was recorded, while after 10 hours, 136.9 μg/100 g. This latter value was still less by 23.4 μg/100 g (14.3%) than the initial value.

after the injection of 0.8 g/100 g aethylalcohol

treated rats. Hours after the giving of alcohol				Maximal change in total steroid content			
				Increase		Decrease	
5h	6h	8h	10h	μg per 100 g	per cent	μg per 100 g	per cent
510.4	482.7	3365.4	3181.7	2262.7	+66.3	−2927.0	−85.8
12.6	8.0	26.7	12.5	+21.2	+58.8	−28.0	−77.7
11.3	6.0	24.5	48.5	+15.9	+48.7	−26.6	−81.5
2.5	8.6	6.4	19.5	+23.5	+89.3	−23.8	−90.4
4.0	4.7	15.3	17.0	—	—	−18.3	−82.0
24.3	9.3	19.3	23.5	+8.6	+49.3	−17.1	−98.2
2.5	1.1	4.0	4.5	—	—	−15.3	−92.0
7.5	9.0	9.7	14.1	+4.2	+35.0	−7.6	−63.3
64.7	46.7	105.9	139.6	+30.0	+18.4	−116.3	−71.13
39.6	28.6	64.9	85.6				
−60.4	−71.4	−35.1	−14.4				
575.1	529.4	3471.3	3321.3	+2293.1	+64.1	−3042.6	−85.1
16.1	14.8	97.1	92.9				
−23.9	−85.2	−2.9	−7.1				

Discussion

As shown in Table II, in the untreated control rats the adrenals invariably contained 5 corticosteroid fractions, while 1 fraction was found only occasionally. The skeletal muscle of these animals contained 10 fractions, while the liver 9 fractions, the kidneys 6 fractions, the heart, spleen, and lungs 5 fractions each, and the brain 4 fractions. Thus, skeletal muscle and the liver contain the greatest number of corticosteroid fractions.

The total amount of steroids as computed for unit of tissue weight was highest in the adrenals, then in the heart (36.0 μg/100 g), the kidneys (32.6 μg/100 g), while smaller quantities were found in the spleen (26.3 μg/100 g), brain (22.3 μg/100 g), lungs (17.0 μg/100 g), liver (16.4 μg/100 g), and the least in the skeletal muscle (12.0 μg/100 g).

The high corticosteroid content of cardiac muscle might in some way be related to the high activity of the heart, while the high steroid level found in the kidneys to the excretory function of this organ. The low steroid content of the liver is in all probability a consequence of the high rate of steroid destruction in this organ, and this might be responsible for the presence of these corticosteroid fractions. Skeletal muscle contained 10 corticosteroid fractions but the total steroid content per unit of tissue weight was the lowest (12.0 μg/100 g), a finding difficult to explain. Considering, however, that the total steroid concentration in skeletal muscle amounted to one third of that in cardiac muscle, the lower steroid content of skeletal muscle may be related

to its lower activity level as compared to heart muscle. Further investigations are required to check this assumption.

The fact that the liver and the skeletal muscle contained 5 corticosteroid fractions, the kidneys 2, and the heart, spleen, and lungs 1, which were not present in adrenal tissue extracts can be ascribed to the conversion of adrenal steroids in these organs, while the presence here of other corticosteroid compounds (cortisol, cortisone, aldosterone?) indicates that steroid hormones are elaborated in organs other than the adrenals, too. This assumption needs further confirmation.

The data in Table II are in accord with results obtained in previous studies [53, 54].

Table IV indicates that 1 hour after the administration of alcohol the total corticosteroid level was reduced in the adrenals, heart, kidneys, brain, liver, and skeletal muscle, and elevated in the spleen and lungs. In view of our earlier finding according to which alcohol causes a rise of the plasma corticosteroid level, it seems reasonable to assume that the observed reduction of the total steroid content of the adrenals and other organs in response to alcohol was due to a release of these compounds into the blood, leading to a depletion of the tissue stores. Two and 3 hours after the administration of alcohol, the total steroid content of the various tissue extracts exhibited a substantial rise which could be accounted for by the increased adrenocortical activity. This is in agreement with the observation that blood steroid levels undergo a further increase during this period [55, 56], allowing an augmented accumulation of these compounds in the various tissues. The total corticosteroid content of most of the organs exhibited a substantial reduction 4, 5, and 6 hours after the injection of alcohol; this might have been the result of diminished adrenal activity, as indicated by a previous study where corticosteroids had been detected in blood plasma and adrenal tissue [55, 56, 63, 64, 65].

It is evident, that the diminution of adrenocortical activity 4 to 6 hours after the administration of alcohol and the consequent reduction of the total corticosteroid content of the various tissues are not without effect on the function of these organs and the whole organism. Thus, for example, the cardiac muscle contained only 77.7% of the corticosteroids found in the normal rats 6 hours after the administration of alcohol; as already mentioned, after the adrenals the heart muscle contained the largest amount of corticosteroids per unit of tissue weight. This observation may bear some practical importance. It has been shown previously [36, 37] that 121 of a total of 137 patients (88%), who had died of acute cardiac failure, had some alcohol in their blood. This observation calls for a study in this direction of the mechanism of acute cardiac death.

The total corticosteroid content of the brain after the administration of alcohol showed parallel changes with that of the adrenal glands; the values

were always below the control level. The diminution in response to alcohol might be related to the diminished functional capacity of the central nervous system and the loss of consciousness during alcohol intoxication. The reduction in total corticosteroid content was substantial: 1 hour after the administration of alcohol it was reduced by 42.2% (from 23.3 to 12.9 μg per 100 g), after 2 hours by 61.8% (to 8.5 μg per 100 g). Although there was a subsequent rise in the course of the 3rd and 4th hours, despite the considerable increase in adrenocortical activity during this period, the values still remained far below normal values, and at 5 to 6 hours a further diminution to 4.0 and 4.7 μg/100 g occurred, i.e. an 82% decrease as compared to the control values, and 8 to 10 hours after the administration of alcohol, the values were still depressed.

The progressive diminution of the total steroid content of the liver, which amounted to 92% at 6 hours, and the reduced values observed during the 2nd and 3rd hours when adrenocortical activity was transiently elevated, were probably due to the augmented metabolism of steroids in response to alcohol. The reduction of total steroid content after alcohol seems to be inconsistent with the observations of KANAI [74] and earlier results from this laboratory [49, 50], according to which the administration of alcohol, similarly to glucocorticoids [75], was increasing the glycogen stores of the liver. The reducing effect of alcohol on the total hepatic steroid content and the augmenting effect of alcohol and corticosteroids on its total glycogen, point to an eventual correlation between the enhanced destruction of corticosteroids and the formation of glycogen. On basis of the present as well as previous results it seems reasonable to assume that this holds true also for the skeletal muscle, on alcohol administration a similar diminution of the total corticosteroid level was associated with an increase in glycogen content, with the difference that in the 2nd and 10th hours there was a 35 and 12.5% increase of total steroid concentration, respectively, all remaining values being considerably depressed; the maximum reduction, observed at 4 hours, amounted to 63.3%.

A satisfactory explanation for the increased corticosteroid content of splenic extracts 1, 2, 3, and 4 hours after the administration of alcohol (with a maximum increase of 89.3% in the 3rd hour) could not be obtained in this study. A considerable reduction, similar to that observed in other organs, followed this initial rise which was maximal (90.4%) in the 5th hour, but even 10 hours after the injection of alcohol the values were 25.8% below the control level.

The lungs contained significantly more corticosteroid at 1, 5 and 10 hours than the controls did, although both in the 1st and 5th hour the adrenal hormone content was substantially depressed, indicating a reduced activity of the gland. In contrast, 4 hours after the administration of alcohol, a reduction was observed in the total corticosteroid level of the lungs which was the greatest of all the organs tested, amounting to 98.2%. During the 2nd and 3rd

hours when adrenal activity was maximal, the lungs contained corticosteroids in amounts which were not significantly different from the control values. A satisfactory explanation for this observation is lacking. The mechanisms underlying the above changes and their eventual consequences are beyond the scope of this paper, and the data obtained are insufficient for such conclusions to be drawn. It should first be established whether the observed changes in the corticosteroid fractions are specific to alcohol, or other stressor agents are likewise capable of eliciting alterations in the steroid content of various organs, eventually alterations of different character. It would be important to determine whether the repeated administration of alcohol, or prolonged stress effects would cause a change in the corticosteroid fractions of the tissues and organs, particularly the adrenals, and whether there is any difference in the responses given to the various stressor impacts. These and other questions can be answered, of course, only on the basis of further investigations.

It has been established by STEVENS et al. [78] and COHN et al. [3] that delta-4,3-ketosteroids are conjugated with glucuronic acid on the A ring in the liver of mice. They also showed that liver and kidney extracts are capable of binding corticosterone in increased amounts as compared to other organs. As the rat adrenal produces corticosterone in the highest amount of all the steroid hormones, this compound may be bound in the various organs as a glucuronide ester, is accumulated in the cells in this form, and liberated from this bond in response to stressor stimuli such as alcohol. Compatible with this assumption is our previous observation [61, 62] that both intact and adrenalectomized rats exhibit increased serum beta-glucuronidase activity after the administration of alcohol. It is possible that the organism, by increasing plasma beta glucuronidase activity enhances the liberation by specific hydrolysis of glucuronic acid-bound steroid stores. The corticosteroids thus liberated enter the blood stream together with free glucuronic acid. This latter forms with alcohol a glucuronide, thereby reducing its toxicity, while the free steroids enhance the metabolization of alcohol partly by increasing the activity of alcohol dehydrogenase in the liver [43—52, 55, 57, 58, 65] and augmenting catalase activity in blood [70—73].

REFERENCES

1. BUSH, I. E.: (1952) Biochem. J. **50,** 370. — 2. BUSH, I. E., FERGUSON, K. H.: (1953) J. Endocr. **10,** 1.—3. COHN, G., HUME, L. M., BONDY, P. K.: (1960) 1st Int. Congr. Endocrinology, Copenhagen. — 3a. ENDRŐCZI, E.: cit. 76. — 4. FAZEKAS, I. GY.: (1954) Endokrinologie **31,** 365. — 5. FAZEKAS, I. GY.: (1955) Kísérl. Orvostud. **7,** 196. — 6. FAZEKAS, I. GY.: (1955) Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **255,** 279. — 7. FAZEKAS, I. GY.: (1955) Kísérl. Orvostud. **7,** 258. — 8. FAZEKAS, I. GY.: (1955) Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **226,** 236. — 9. FAZEKAS, I. GY.: (1955) Kísérl. Orvostud. **7,** 536. — 10. FAZEKAS, I. GY.: (1955) Endokrinologie **32,** 315. — 11. FAZEKAS, I. GY.: (1955) Kísérl. Orvostud. **7,** 267. — 12. FAZEKAS, I. GY.: (1956) Endokrinologie **33,** 151. — 13. FAZEKAS, I. GY.: (1956) Pflügers Arch. ges. Physiol. **262**, 431. — 14. FAZEKAS, I. GY.: (1956) Naunyn-Schmiedebergs Arch.

exp. Path. Pharmak. **229,** 152. — 15. FAZEKAS, I. GY.: (1956) Kísérl. Orvostud. **8,** 518. — 16. FAZEKAS, I. GY.: (1957) Kísérl. Orvostud. **9,** 15. — 17. FAZEKAS, I. GY.: (1957) Kísérl. Orvostud. **9,** 295. — 18. FAZEKAS, I. GY.: (1957) J. Endocr. **15,** 440. — 19. FAZEKAS, I. GY.: (1957) Endokrinologie **35,** 96. — 20. FAZEKAS, I. GY.: (1957) Endokrinologie **35,** 108. — 21. FAZEKAS, I. GY.: (1957) Ann. Endocr. (Paris) **18,** 706. — 22. FAZEKAS, I. GY.: (1958) Folia endocr. (Roma) Vol. XI. **3,** 316. — 23. FAZEKAS, I. GY.: (1958) Kísérl. Orvostud. **10,** 186. — 24. FAZEKAS, I. GY.: (1958) Kísérl. Orvostud. **10,** 221. — 25. FAZEKAS, I. GY.: (1959) Kísérl. Orvostud. **11,** 561. — 26. FAZEKAS, I. GY.: (1961) Acta physiol. Acad. Sci. hung. **20,** 1. — 27. FAZEKAS, I. GY.: (1960) Vitamin-, Hormon-u. Fermentforsch. **11,** 29. — 28. FAZEKAS, I. GY.: (1960) Ann. Endocr. (Paris) **21,** 42. — 29. FAZEKAS, I. GY.: (1959) Kísérl. Orvostud. **11,** 573. — 30. FAZEKAS, I. GY.: (1960) Endokrinologie **40,** 60. — 31. FAZEKAS, I. GY.: (1961) Hautarzt **12,** 20. — 32. FAZEKAS, I. GY.: (1960) Kísérl. Orvostud. **12,** 430. — 33. FAZEKAS, I. GY.: (1961) Kísérl. Orvostud. **13,** 19. — 34. FAZEKAS, I. GY.: (1961) Acta biol. med. germ. **6,** 297. — 35. FAZEKAS, I. GY.: (1961) Folia endocr. (Roma) **14,** 402. — 36. FAZEKAS, I. GY.: (1955) Zacchia **18,** 242. — 37. FAZEKAS, I. GY.: (1956) Kísérl. Orvostud, **8,** 92. — 38. FAZEKAS, I. GY.: (1960) Arch. Toxikol. **18,** 205. — 39. FAZEKAS, I. GY.: (1960) Zacchia **23,** 165. — 40. FAZEKAS, I. GY.: (1964) Acta Med. leg. soc. (Liège) **17,** 77. — 41. FAZEKAS, I. GY.: (1961) Kísérl. Orvostud. **13,** 361. — 42. FAZEKAS, I. GY.: (1961) Arch. Toxikol. **19,** 205. — 43. FAZEKAS, I. GY., RENGEI, B.: (1961) Kísérl. Orvostud. **13,** 434. — 44. FAZEKAS, I. GY., RENGEI, B., FAZEKAS, Á. T.: (1961). Arch. Toxikol. **19,** 229. — 45. FAZEKAS, I. GY.: (1962) Arch. Toxikol. **19,** 388. — 46. FAZEKAS, I. GY.: (1962) Kísérl. Orvostud. **14,** 497. — 47. FAZEKAS, I. GY.: (1963) Kísérl. Orvostud. **15,** 94. — 48. FAZEKAS, I. GY.: (1963) Kísérl. Orvostud. **15,** 200. — 49. FAZEKAS, I. GY.: (1963) Kísérl. Orvostud. **15,** 176. — 50. FAZEKAS, I. GY.: (1963) Kísérl. Orvostud. **15,** 381. — 51. FAZEKAS, I. GY.: (1963) Folia endocr. (Roma) **16,** 600. — 52. FAZEKAS, I. GY.: (1963) Kísérl. Orvostud. **15,** 611. — 53. FAZEKAS, I. GY., FAZEKAS, Á. T.: (1964) Endocrinologie **46,** 247. — 54. FAZEKAS, I. GY., FAZEKAS, A. T.: (1964) Kísérl. Orvostud. **16,** 623. — 55. FAZEKAS, I. GY.: (1964) Kísérl. Orvostud. **16,** 50. — 56. FAZEKAS, I. GY.: (1964) Folia endocr. (Roma) **XVII,** 143. — 57. FAZEKAS, I. GY.: (1964) Kísérl. Orvostud. **16,** 55. — 58. FAZEKAS, I. GY.: (1964) Folia endocr. (Roma) **XVII.** 256. — 59. FAZEKAS, I. GY.: (1964) Dtsch. Z. ges. gerichtl. Med. **55,** 81. — 60. FAZEKAS, I. GY.: (1964) Kísérl. Orvostud. **16,** 506. — 61. FAZEKAS, I. GY. RENGEI, B.: (1964) Enzymologia **27,** 345. — 62. FAZEKAS, I. GY.: (1964) Enzymologia **27,** 353. — 63. FAZEKAS, I. GY.: (1964) Endokrinologie **46,** 133. — 64. FAZEKAS, I. GY., FAZEKAS, A. T.: (1965) Endokrinologie **47,** 136. — 65. FAZEKAS, I. GY., FAZEKAS, A. T.: (1965) Kísérl. Orvostud. **17,** 65. — 66. FAZEKAS, I. GY.: (1965) Kísérl. Orvostud. **17,** 366. — 67. FAZEKAS, I. GY.: (1966) Quart. J. Stud. Alcohol **27,** 439. — 68. FAZEKAS, I. GY., FAZEKAS, A. T.: (1965) Folia endocr. (Roma) **18,** 94. — 69. FAZEKAS, I. GY., FAZEKAS, A. T.: (1966) Kisérl. Orvostud. **18,** 113. — 70. FAZEKAS, I. GY., POSGAY, K., FARKAS, I.: (1965) Kísérl. Orvostud. **17,** 481. — 71. FAZEKAS, I. GY., POSGAY, K., FARKAS, I.: (1966) Enzymologia **30,** 116. — 72. FAZEKAS, I. GY.: (1965) Kísérl. Orvostud. **17,** 533. — 73. FAZEKAS, I. GY.: (In press) Dtsch. Z. ges. gerichtl. Med. — 74. KANAI, J.: (1933) Biochem. Z. **262,** 41. — 75. KERPOLA, W.: (1952) Endocrinology **51,** 192. — 76. KOVÁCH, A.: (1962) A kísérletes orvostudomány vizsgáló módszerei. Vol. VI. Akadémiai Kiadó, Budapest. — 77. MASSZI, J., MECHER, T.: (1963) Kísérl. Orvostud. **15,** 567. — 78. STEVENS, W., BERLINER, D. L., DOUGHERTY, T. F.: (1961) Endocrinology **68,** 875.

WIRKUNG DES ALKOHOLS AUF DIE CORTICOSTEROIDFRAKTIONEN IN RATTENGEWEBE-EXTRAKTEN

I. GY. FAZEKAS und A. T. FAZEKAS

Die Wirkung von subkutan injiziertem Alkohol auf die Steroidfraktionen wurde in verschiedenen Rattengewebe-Extrakten untersucht. Die Steroidfraktionen wurden nach chromatographischer Trennung in alkalischer Tetrasoliumblau-Lösung entwickelt und quantitativ bestimmt. Der Gesamtcorticoidgehalt nahm in der ersten Stunde nach der Alkoholverabreichung in allen Geweben ab, 2 bis 3 Stunden danach erfolgte ein Anstieg und 5 bis 6 Stunden später eine ausgeprägte Abnahme. In den einzelnen Organen war die Zahl der Steroidfraktionen unterschiedlich, in der Leber wurden 9 und in den Skelettmuskeln 10 Fraktionen gefunden. Bezogen auf das Einheitsgewicht der Gewebe war der Steroidgehabt in den Nebennieren, im Herzen und in den Nieren am höchsten. Die nach Alkoholverabreichung beobachteten Veränderungen des Gesamtsteroidgehalts werden der veränderten Nebennierenaktivität zugeschrieben. Die möglichen Ursachen des unterschiedlichen Steroidgehalts in den verschiedenen Organen nach Alkoholgaben und die Mechanismen der Alkoholwirkung auf den Steroidmetabolismus werden besprochen.

ДЕЙСТВИЕ АЛКОГОЛЯ НА ФРАКЦИИ КОРТИКОСТЕРОИДОВ В ВЫТЯЖКАХ ТКАНЕЙ КРЫСЫ

И. Д. ФАЗЕКАШ и А. Т. ФАЗЕКАШ

Авторы исследовали действие подкожно введенного алкоголя на фракции кортикостероидов в вытяжках различных тканей крысы. Проводилось также качественное определение отдельных фракций. По сравнению с величинами контролей в первый час после введения алкоголя во всех тканях наблюдалось понижение общего содержания кортикостероидов. По истечении 2 и 3 часов следовало повышение и через дальнейшие 5 и 6 часов — опять выраженное понижение. Число кортикостероидных фракций в отдельных органах было различным, наивысшее число было выявлено в печени (9) и в скелетной мышце (10). В переводе на единицу веса тканей количество стероидов оказалось выше всего в надпочечниках, в сердце и в почках. Изменения общего количества кортикостероидов, наблюдаемые после введения алкоголя, приписываются изменениям активности надпочечников.

Dr. I. Gyula Fazekas
Dr. A. Tibor Fazekas
Szeged, Kossuth Lajos sugárút 40, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 353—362, 1968*

Second Department of Medicine (Director: P. GÖMÖRI); First Institute of Pathological Anatomy and Cancer Research (Director: J. BALÓ); Institute of Anatomy (Director: J. SZENTÁGOTHAI), University Medical School, Budapest

RENAL VASCULAR CHANGES IN CHRONIC RENAL INSUFFICIENCY

P. GÖMÖRI, I. MUNKÁCSI, E. SZALAY, I. VARGA and B. ZOLNAI

(Received October 5, 1967)

The kidney of 38 patients who had died of chronic uraemia has been injected with polyvinyl chloride dissolved in a mixture of cyclohexanol and acetone, and corroded in hydrochloric acid. In the preparations spindle formation on the vessels, microaneurysms in the arterioles, strictures, mostly at the origin of the arteries, complete arterial obliteration, appearance of spiral and tortuous vessels were observed. There was extensive glomerular destruction, while the afferent and efferent arterioles became recanalized at the vascular pole of the disintegrating glomeruli, a process that led to the formation of aglomerular vessels. The latter were able to ensure tubular blood supply and to maintain tubular activity after the destruction of glomeruli. In addition to these phenomena, recapillarization was observed. It is suggested that grave vascular changes accompanying chronic renal failure are due to hypertension; therefore, drug treatment of renal hypertension is recommended. A correlation was revealed between the gravity of renal vascular changes and the gravity of changes in the eyeground.

Examinations of corrosion preparations of the kidney of seven patients as also the results of related animal experiments were described in previous communications [2, 3]. The kidneys of five patients who had died of chronic glomerulonephritis were found to have undergone characteristic changes such as extensive destruction of the glomeruli followed by recanalization of the afferent and the efferent arterioles with the formation of aglomerular vessels at the site of the degenerating glomeruli, which ensured tubular blood supply after glomerular destruction. No such changes were observed in two patients who had succumbed to subacute glomerulonephritic uraemia.

Corrosion preparations made *post mortem* from the kidneys of 47 patients have been examined in the present study.

Material and method

38 of the 47 patients died of chronic uraemia, while 9 had been suffering from essential hypertension or a nephropathy which did not result in uraemia so that they died of some other disease such as heart failure or apoplexy.

Corrosion preparations were made according to the method of MUNKÁCSI [6], with ZOLNAI's modification [10] in some cases. NPN of patients who had died of uraemia usually exceeded 100 to 120 mg per 100 ml, while creatinine clearance amounted to, or was less than, 10 to 12 ml/min. All patients were isosthenuric, and the clinical manifestations were those of chronic uraemia. Both kidneys were removed after death; one for corrosion, the other for histological study. The present paper deals only with cases in which the clinical diagnosis agreed with the histological diagnosis. Diagnosis of chronic pyelonephritis was based on the histolog-

ical criteria described by KIMMELSTIEL [4]. Polyvinyl chloride dissolved in a mixture of cyclohexanol and acetone was injected into the renal artery according to Munkácsi's technique [6]; the kidney was corroded in concentrated hydrochloric acid, and the preparation examined under a stereomicroscope. The following diseases were diagnozed in 38 patients who had died of uraemia: 14 cases of chronic glomerulonephritis, 5 cases of subacute glomerulonephritis, 7 cases of chronic pyelonephritis, 2 cases of Kimmelstiel-Wilson's syndrome, 2 cases of renal scleroderma, 3 cases of malignant hypertension, 4 cases of amyloidosis, one case of essential hypertension combined with secondary renal failure. In addition, 9 patients were examined who had no chronic renal failure: five had essential hypertension or arteriosclerosis, two had renal scleroderma and two had lupus erythematodes. The cause of death in these cases was apoplexy or cardiac failure.

Results

Corrosion preparations showed the following differences between normal (Figs 1, 8) and diseased kidneys.

(1) Spindle-like vessels. The lumen of major vessels, e.g. the interlobar and interlobular arteries, the arciform arteries, showed spindle-like dilatations and strictures. The latter were no local myogenic constrictions but consisted in an initial dilatation and gradual narrowing of the vessels so that the lumen showed an uneven surface (Fig. 2).

(2) Microaneurysms. Encountered chiefly in the arterioles (interlobular and afferent), these presumably represented a later stage of the spindle-like change. In these microaneurysms the lumen was uneven and displayed superposed, irregularly arranged dilatations and constrictions. The vessels had ceased to have a uniform lumen as also the tendency to narrow towards the periphery; they usually ended with an abrupt obliteration ("stop") or continued suddenly as thin structures of spiral or tortuous course (Fig. 3).

(3) Strictures, obliterations, "stops". Usually in combination with spindle-like or microaneurysmal vessels, a constriction was visible at the origin of the arteries, although it sometimes occurred at a greater distance as well. Obliterations of this kind completely occluded some minor vessels, suddenly forming "stops". Their end portion was always rounded (Figs 4, 5).

(4) Spiral and tortuous vessels. These occurred in cortex and medulla alike. They dominated the picture in cases of renal cirrhosis whatever its origin. The development of such vessels is presumably due to parenchymal contraction, a phenomenon well known from histological preparations. It may be produced experimentally by ligation of the renal artery and is known to occur under physiological conditions in the renal pelvis, the ureter and the uterus, while it has not been observed in the renal parenchyma under normal conditions. The vasa recta, too, took a spiral form (Fig. 6).

(5) Glomerular degeneration and aglomerular vascularization. The delicate intraglomerular loops were destroyed, while the part around the vascular pole persisted. The afferent vessel formed a microaneurysm at the pole so that the vestigial glomerulus showed a chicken-leg ramification. With advancing

Fig. 1. Vascular cast from normal kidney showing interlobular artery and glomerali. PVC corrosion preparation. 100 ×

Fig. 2. Spindle-form interlobular artery and degenerated glomeruli. PVC corrosion preparation. 120 × . a = spindle-form artery

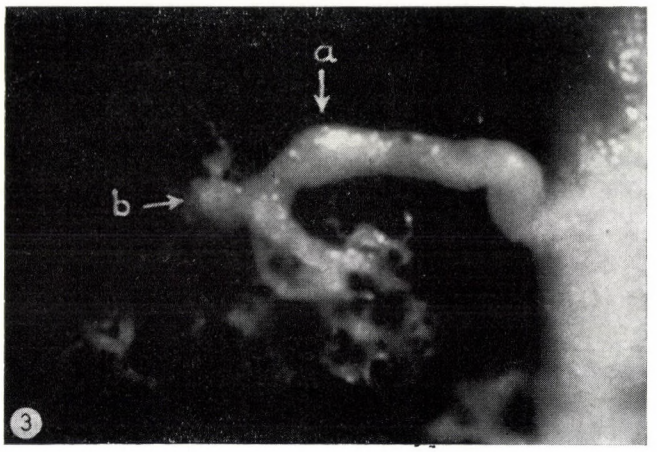


Fig. 3. Afferent arteriole showing microaneurysms, and degenerated glomerulus. PVC corrosion preparation. 300 ×

Fig. 4. Narrowing and vascular "stop". PVC corrosion preparation. 300 ×
a = narrowing at the origin of vas rectum
b = incomplete filling of afferent arterioles

degeneration, the afferent vessel became increasingly narrow, then disappeared, so that the glomerulus ceased to fill and the vessel ended in a "stop". If vascular degeneration was restricted to the glomerular capillaries, the afferent vessel communicated with the efferent one at the vascular pole which led to the formation of aglomerular vessels. These developed at an early stage, during capillary destruction. In a previous paper we have described aglomerular vessels which had developed in rabbits with chronic Masugi nephritis, whereas no such vessels were found in the case of acute Masugi nephritis (Figs 7, 8, 9a, 9b).

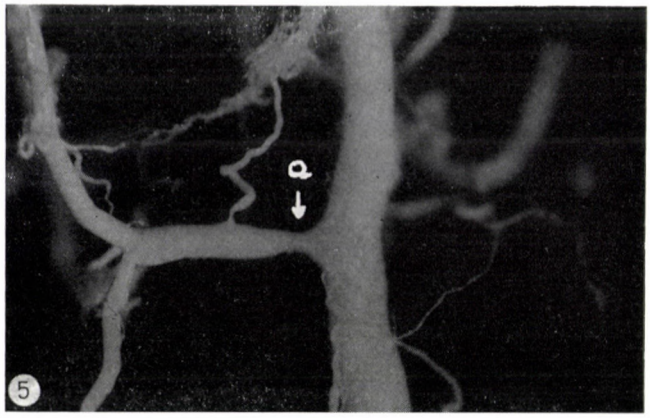


Fig. 5. Narrowing at the origin of interlobular artery. PVC corrosion preparation. 120 ×
a = narrowing

Fig. 6. Spiral interlobular artery, with degenerated and hypertrophied glomeruli. PVC corrosion preparation. 300 ×

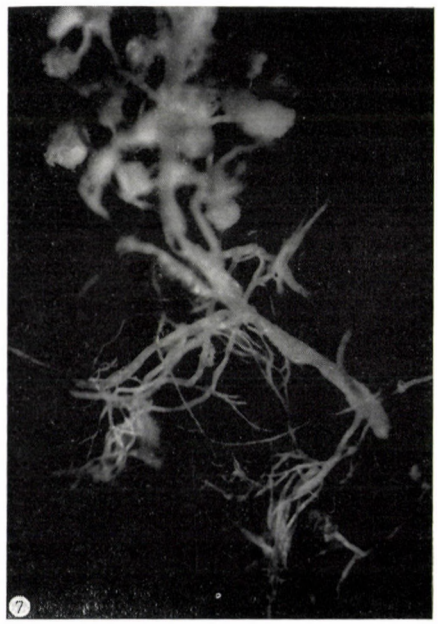


Fig. 7. Aglomerular arteries at the basal part of an interlobular artery; note glomeruli in its peripheral portion. PVC corrosion preparation. 120 ×

(6) Glomeruli of various sizes. Hypertrophic and small degenerated glomeruli were found side by side right from the initial phase of glomerular degeneration.

(7) Capillarization. New capillaries may form in chronic renal failure. While the glomerular capillaries are degenerating, the afferent arteriole may

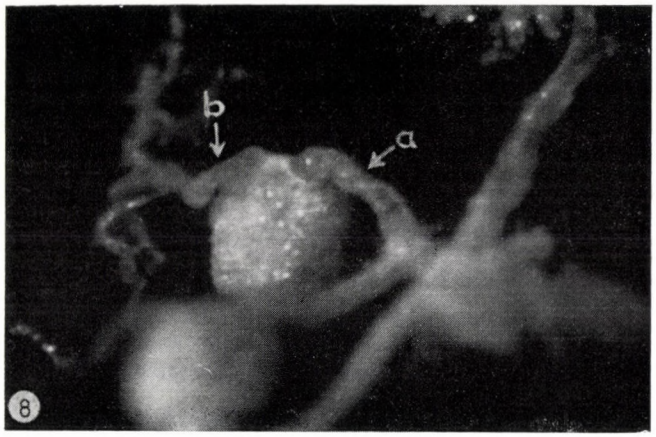


Fig. 8. Normal vascular pole of glomerulus. PVC corrosion preparation. 220 ×. *a* = afferent arteriole, *b* = efferent arteriole

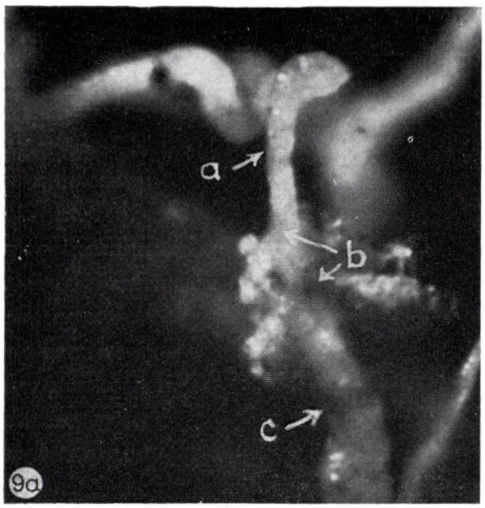


Fig. 9/a. Afferent–efferent communication at vascular pole of degenerated glomerulus. PVC corrosion preparation. 200 ×. *a* = vas afferens. *b* = afferent–efferent communication, *c* = vas efferens

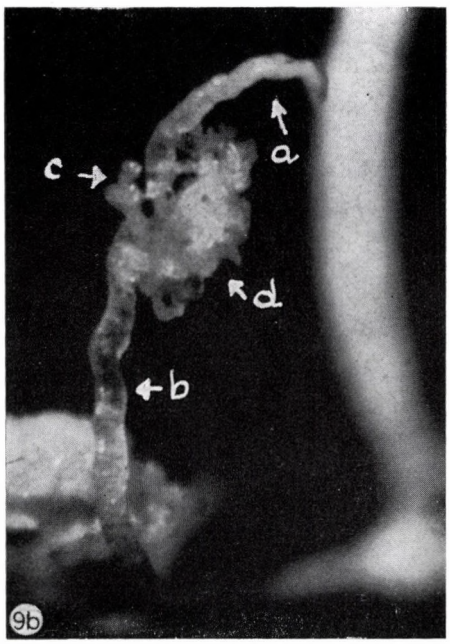


Fig. 9/b. Afferent–efferent communication at vascular pole of degenerated glomerulus. PVC corrosion preparation. 200 ×, *a* = vas afferens, *b* = vas efferens, *c* = afferent–efferent communication, *d* = degenerated glomerulus

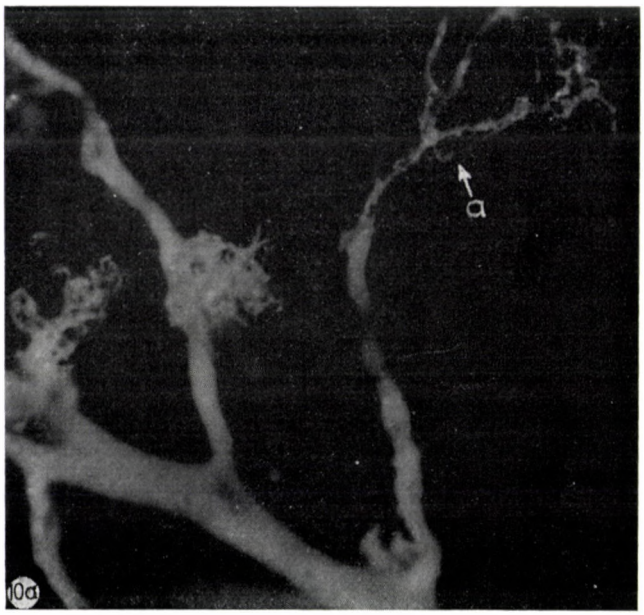


Fig. 10/a. Capillarization of afferent arteriole. PVC corrosion preparation. 120 ×.
a = capillaries of afferent arteriole

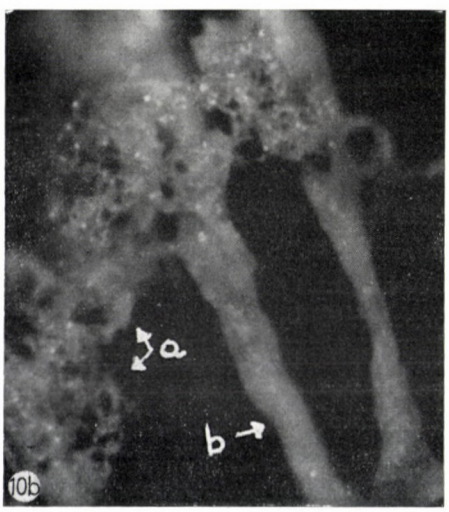


Fig. 10/b. Capillarization of interlobular arteries. PVC corrosion preparation. 220 ×.
a = capillaries, *b* = interlobular artery

emit at the vascular pole or at the side of the interlobular vessels new capillaries which anastomose extraglomerularly with the postglomerular capillaries (Figs 10a, 10b).

Discussion

The joint occurrence of the above changes was found almost exclusively in patients who had died of chronic uraemia due to chronic glomerulonephritis (14 cases). Although patients suffering from subacute glomerulonephritis had died likewise of uraemia, it was only in a single such case that the described changes were observed; this patient survived, however, a whole year after the onset of the disease. The said anomalies are to be found also in cases of chronic pyelonephritis but not as regularly as in chronic glomerulonephritis, a phenomenon possibly due to the fact that it is in the interstices that the pathological process takes place. LJUNGQUIST [5] observed numerous aglomerular vessels in cases of pyelonephritis. As regards the material of this study, one of the hypertensive patients who had had secondary renal failure and three patients with malignant hypertension who had died of chronic uraemia, likewise displayed all the changes described above. Aglomerular vascularization has moreover been described also in hypertension without renal insufficiency [8]. It occurs also in the kidney of aged persons likewise without renal insufficiency. While no aglomerular vascularization was observed in patients with Kimmelstiel–Wilson's syndrome, their kidney contained a great number of disintegrating juxtamedullary glomeruli which would probably have disappeared owing to recanalization. A case of renal scleroderma has already been reported from this department by URAI et al. [9]. Another patient with scleroderma who was admitted subsequently and who died of chronic uraemia, exhibited the same changes as those accompanying chronic glomerulonephritis. We had two patients who were suffering from scleroderma, whereas their urine showed no pathological signs, their renal function was normal, and it was some other disease to which they succumbed. Corrosion preparations of their kidney showed numerous aglomerular vessels and a recanalization of the afferent and the efferent vessels, although neither of them had renal failure. These two cases were similar to those of hypertensive or senile patients in whom the number of aglomerular vessels is increased without chronic renal failure. The cause of this phenomenon is obscure, but it shows that not only essential hypertension or old age but also scleroderma is able to increase the number of aglomerular vessels without renal failure. We found numerous destroyed glomeruli and recanalized aglomerular vessels in two patients with lupus erythematodes who had died of uraemia, although changes of this kind are considerably less frequent in such cases than in chronic glomerulonephritis. Four patients suffering from amyloidosis exhibited likewise extensive glomerular destruction

and aglomerular vascularization; as in chronic glomerulonephritis, the uneven filling of vessels, their constriction and deformation were observed in these cases as well. No difference between primary and secondary amyloidosis was revealed. Aglomerular vascularization in essential hypertension without renal failure, as described by TRUETA et al. [8], occurred also in the present material.

The present experiments have shown that renal vessels suffer significant structural changes in chronic renal failure. Aglomerular vascularization, observable in aged and hypertensive persons even without renal insufficiency, is not without pathological significance. Formation of aglomerular vessels, recanalization of the afferent and efferent arterioles following glomerular destruction evidently ensure tubular blood supply. This is probably the reason why, in contrast to acute renal failure, there is no tubular necrosis in chronic renal insufficiency, and it is presumably for this reason that, after the impairment of glomerular activity, the tubules are able to reabsorb and to excrete certain substances such as carbamide, creatinine or phosphate [1]. Another observation of clinical interest was that with recapillarization the renal vessels underwent grave structural changes such as the formation of spindle microaneurysms, and, still more important obliteration of minor vessels. It is obvious that the destruction of minor vessels leads, in itself, to a further glomerular degeneration independently of the primary disease. Hypertension, a concomitant of glomerulonephritis, is the presumable cause of these structural changes. In the present material no correlation was found between the duration of hypertension and the changes in the renal vessels, and it was the more surprising that a marked positive correlation occurred between the gravity of vascular lesions and the gravity of the eyeground finding.

Considering that, in general, pathological changes in the fundus indicate a grave prognosis of hypertension, the present results may be construed as a warning that blood pressure should be controlled in renal hypertension in the same manner as it is done in essential hypertension, and this the more so as lesions of the renal vessels appeared to be extremely grave in all cases where haemorrhage, retinitis, or oedema were found in the eyeground. It is possible that, as in the eyeground, hypotensive treatment may retard the process in the renal vessels as well.

REFERENCES

1. FISCHER, A., LÁNG, E., SZÉKELY, J.: (1959) Die Tubulusfunction bei chronischer Niereninsuffizienz. Acta med. Acad. Sci. hung. **14,** 379. — 2. GÖMÖRI, P., MUNKÁCSI, I., SZALAY, E., TU SÜJ-HAJ, ZOLNAI, B.: (1962) Intrarenal Blood Circulation in Chronic Renal Failure. I. Human Material. Acta med. Acad. Sci. hung. **18,** 441. — 3. GÖMÖRI, P., SZALAY, E., TU SÜJ-HAJ, ZOLNAI, B.: (1962) Intrarenal Blood Circulation in Chronic Renal Failure. II. Experimental Masugi nephritis. Acta med. Acad. Sci. hung. **18,** 451. — 4. KIMMELSTIEL, P., ON JA KIM, BERES, I., WELLMANN, K.: (1961) Chronic Pyelonephritis. Amer. J. Med. **30,** 589. — 5. LJUNG-

QUIST, A.: (1963) The Intrarenal Arterial Pattern in the Normal and Diseased Human Kidney. Acta med. scand. **174,** Suppl. — 6. MUNKÁCSI, I.: (1954) Über die Verwendung der Kunststoffe Polyvinylchlorid (PVC) und Piacryl in der Korrosionstechnik. Z. wiss. Mikr. **63,** 352. — 7. SZALAY, E., VARGA, I.: (1967) Renal Amyloidosis. Acta morph. Acad. Sci. hung. **15,** 81. — 8. TRUETA, I., BARCLAY, A. E., DANIEL, P., FRANKLIN, K. J., PRICHARD, M. M.: (1947) Studies on Renal Circulation. Blackwell, Oxford. — 9. URAI, L., MUNKÁCSI, I., SZINAI, G.: (1961) New Data on the Pathology of "True Scleroderma Kidney". Brit. med. J. **1,** 713. — 10. ZOLNAI, B.: (1962) Bemerkungen zur anatomischen Injektionskorrosionsmethode. Anat. Anz. **111,** 383.

VERÄNDERUNGEN DER NIERENGEFÄSSE BEI CHRONISCHER NIERENINSUFFIZIENZ

P. GÖMÖRI, I. MUNKÁCSI, E. SZALAY, I. VARGA und B. ZOLNAI

An den Nieren von 40 an chronischer Urämie verstorbenen Kranken wurden mittels in Aceton und Cyclohexan gelöstem Polyvinylchlorid Korrosionsuntersuchungen vorgenommen. An den Korrosionspräparaten wurden folgende Veränderungen nachgewiesen: spindelförmige Gefäßentartung, Entstehung von Mikroaneurismen an den Arteriolen, Verengerungen, zumeist an der Ursprungsstelle der Arterien, völlige Obliteration der Arterien, spirale und gewundene Gefäße, und eine bedeutende Destruktion der Glomeruli. An den Gefäßpolen der zerstörten Glomeruli ließ sich jedoch die Rekanalisation der Vas afferens und efferens beobachten, das zur Bildung von aglomerulären Gefäßen geführt hat. Diese aglomerulären Gefäße hatten die Blutversorgung der Kanälchen auch nach der Zerstörung der Glomeruli und damit auch die Tubulärfunktion gesichert. Neben den obigen Veränderungen war eine Neubildung der Kapillargefäße zu sehen. Die Ansicht wird vertreten, daß für die bei chronischer Niereninsuffizienz auftretenden schweren Gefäßveränderungen die Hypertonie verantwortlich ist, und daß es unerläßlich sei, die renale Hypertonie medikamentös zu behandeln. Die Schwere der Veränderungen der Nierengefäße steht mit dem Ausmaß der Veränderungen des Augengrundes in Korrelation.

ИЗМЕНЕНИЯ СОСУДОВ ПОЧЕК ПРИ ХРОНИЧЕСКОЙ ПОЧЕЧНОЙ НЕДОСТАТОЧНОСТИ

П. ГЁМЁРИ, И МУНКАЧИ, Е. САЛАИ, И ВАРГА и Б. ЗОЛНАИ

На почках 40 больных, умерших вследствие хронической уремии, были проведены коррозионные исследования с помощью поливинилхлорида, растворенного в смеси ацетона и циклогексанона. На коррозионных препаратах были обнаружены следующие изменения: веретенообразное перерождение сосудов, на артериолах развитие микроаневризма, сужения, в большинстве случаев в месте отхода артерий, полная облитерация артерий, появление спиралевидных и извилистых сосудов. Коррозионные препараты показали значительное разрушение клубочков, однако, в месте сосуда истого полюса разрушенных клубочков наблюдается реканализация афферентных и эфферентных сосудов, что приводит к образованию агломерулярных сосудов. Эти агломерулярные сосуды и после разрушения клубочка обеспечивают кровоснабжение канальцев и их функцию. Кроме вышеуказанных изменений наблюдалось также новообразование капиллярных сосудов. По мнению авторов ответственной за тяжелые изменения сосудов, наблюдаемые при хронической недостаточности почек, по всей вероятности следует считать гипертонию, и поэтому непременно нужно проводить медикаментозное лечение ренальной гипертонии. Степень тяжести изменений, обнаруживаемых на почечных сосудах, находится в корреляции с выраженностью изменения глазного дна.

Dr. Pál GÖMÖRI
Dr. István MUNKÁCSI
Dr. Elemér SZALAY
Dr. István VARGA
Dr. Balázs ZOLNAI

Budapest VIII., Szentkirályi u. 46, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(3), pp. 363—372, 1968*

Section of Pathology (Head: A. KÁLLÓ), János Hospital, Budapest

BRONCHIOLAR EMPHYSEMA AND RELATED DISEASES*

A. KÁLLÓ

Author reviewed the literature on bronchiolar emphysema. On the basis of these data and his own experiences he stressed the significance of both dysplasia and inflammation in the pathogenesis of such diseases as muscular cirrhosis, leiomyosis, fibrocystosis, bronchiolectasis and multiple vascular lesions of the lung.

The first report to deal with the condition termed bronchiolar emphysema was that of SIEBERT and FISHER from 1957 [15], although the term itself was coined by LOESCHKE [13]. Comprehensive surveys of the pertinent studies have been presented in several papers [1, 2, 4, 7, 9, 10, 14].

Although bronchiolar emphysema is mostly found in aged persons, it has also been observed in children, and even congenital and foetal cases have been mentioned [3, 16].

Morphology

The postmortem findings are most characteristic provided bronchiolar emphysema is not associated with some other disease.

The lungs are heavy and enlarged; their nodular surface resembles that of the cirrhotic liver (Fig. 1). The nodules are normally hobnailsized and soft as if they would contain air or some fluid. The lungs are rubber-like and small nodes can be palpated in them.

The cut surface of the lungs shows cysts of variable extension, density and size which make it appear cystic if the pores are large, and honeycomb-like if they are small (Fig. 2). The pores may be so minute as to be perceptible only if inspected under the microscope.

Connective tissue proliferation can be seen around the holes (pericystic fibrosis); this is why the condition is sometimes termed fibrocystic lung. Interstitial haemorrhages may complicate the picture. Inflammatory signs

* This work is dedicated to Professor Joseph BALÓ on the occasion of having completed five decades in the service of science.

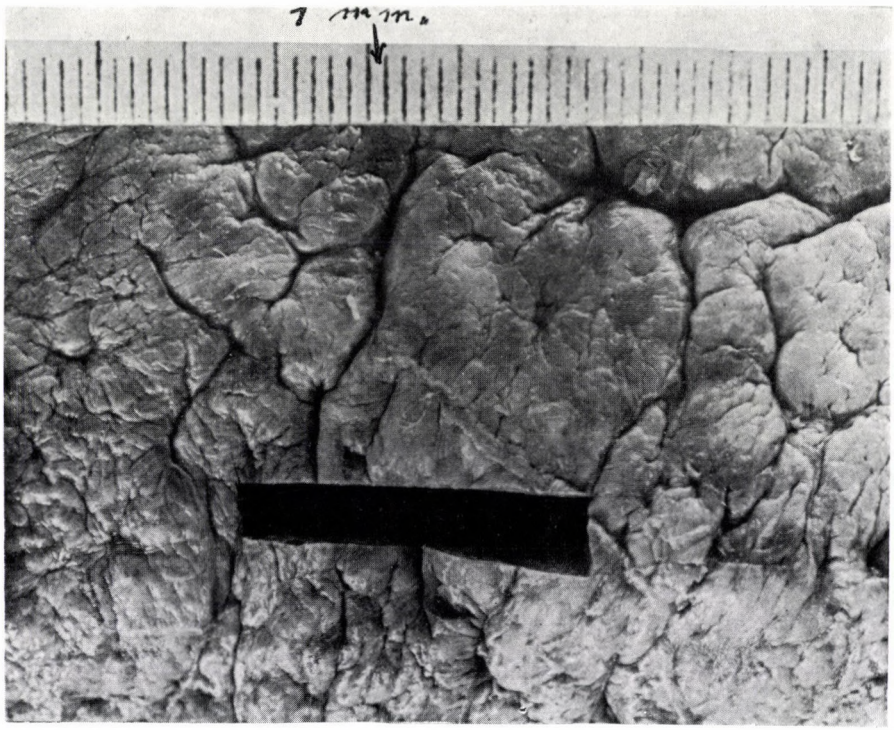


Fig. 1. Nodular surface of cirrhotic lung

may be observable; it depends on whether the changes outlined in the foregoing are accompanied by secondary inflammation in the unaffected portions of the lung or in the dilated portions themselves, and if so, on the extent of the inflammatory process.

Most authors emphasize the bilateral and diffuse character of the disease, whereas our own observations have not proved either of these phenomena to be necessary concomitants of the condition.

Right heart hypertrophy has been registered in all grave forms; it is so characteristic a concomitant of the pulmonary changes at issue that they should always be taken into account in cases of cor pulmonale.

The microscopic picture, too, is characterized by the presence of cysts of different shapes, sizes and origins. In the cases reported under the term bronchiolar emphysema, the bronchi were distended in the first place. According to our observations, the dilatations occur in both the lower and the upper respiratory tract. Bronchiolar emphysema is frequently accompanied by alveolar ectasis characteristic of vesicular emphysema. There were cases in which dilatation of the proximal respiratory tract was accompanied by distensions in the lower respiratory segments (bronchiectasis + bronchiolecta-

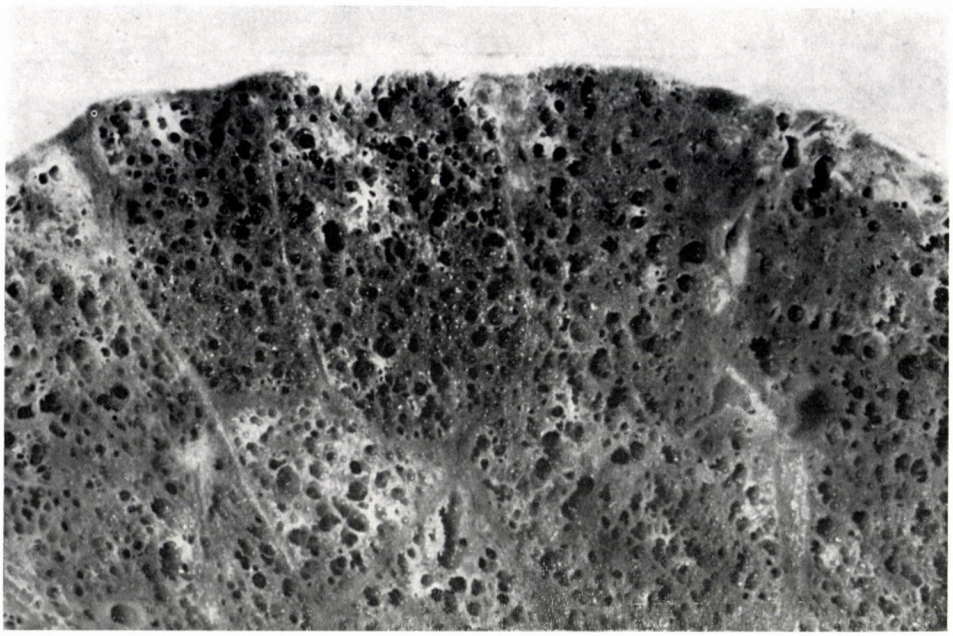


Fig. 2. Cut surface of microcystic lung

sis, = panbronchiectasis). These are borderline cases, transitions from common upper multiple bronchiectasis to vesicular and bronchiolar emphysema.

The inner surface of the cysts is coated by ciliated or unciliated, cylindrical or squamous epithelium. The character of the epithelial lining may be radically modified by repeated inflammations. The dilatations are either empty or may contain mucous matter, clotted protein-rich serum, cellular elements with eosinophils and sometimes giant cells, further detritus.

Authors are unanimous in stating that the wall of the dilatations contains no cartilage. We sometimes encountered vestigial lamellae of cartilage in dilated segments, and sometimes the chondral portion was absent from the wall of dilatations which would otherwise have been classified as parts of the upper respiratory tract. It is generally difficult to determine the exact segment in which the dilatation has been formed.

Though to a less extent, the foregoing applies to the mucous glands of the respiratory path and the goblet cells of the epithelium as well.

The faulty construction of myoelastic component is the most conspicuous.

The elastic system of the air trays and lungs showed changes in all cases. The most frequent phenomenon was a decrease in the amount or a fragmentation of elastic fibres in the wall of the dilated segments. The opposite, fibro-

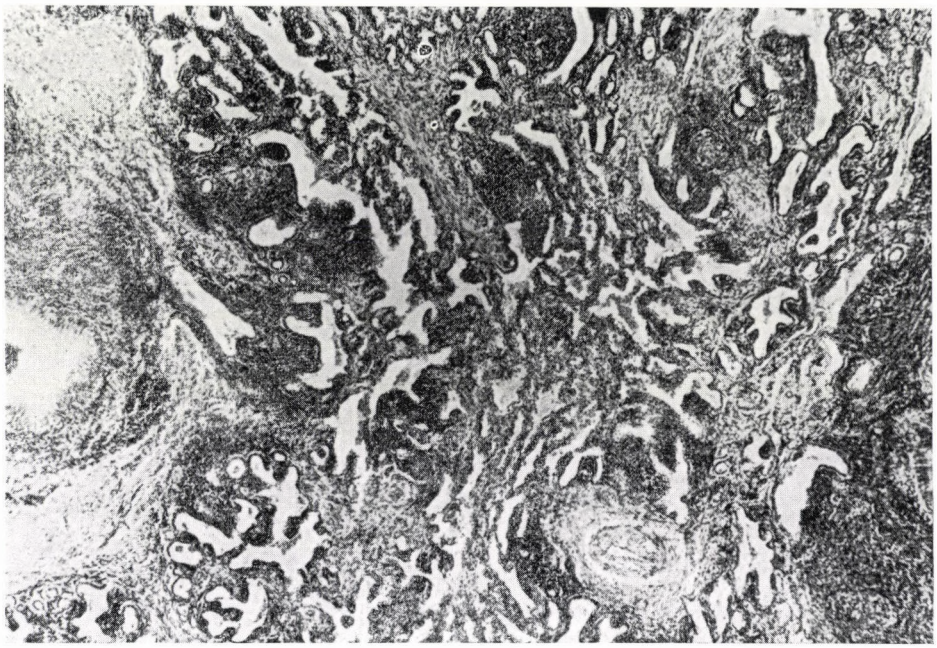


Fig. 3. Periectatic lymphoid cell infiltration and adenomatoid structure originating from the bronchiolization of ducts, atria and alveoli. (Low power)

Fig. 4. Leiomyotic dystopy in infiltrated pulmonary ground substance (Low power)

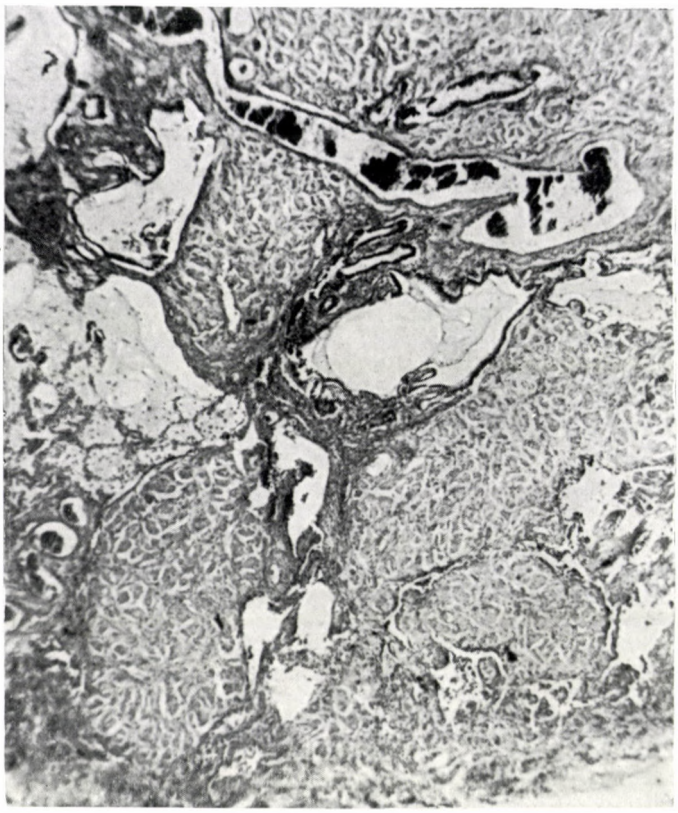


Fig. 5. Bundles of muscle tissue, dilated bronchioli and vessels in the lung, without inflammatory signs

elastosis, may, however, also occur. Remnants of desintegrated elastic fibres are situated around the dilatations in the form of irregular bundles and clusters. Changes in the elastic elements may occur also in the interstitial space or around the alveoli.

Changes in the smooth muscles are even more characteristic and more frequent (Fig. 4). Such changes may even dominate the picture, as expressed by the terms, myomatosis cystica pulmonum or muscular cirrhosis of the lung.

Smooth muscle anomalies may appear as hypoplasia, hyperplasia, or with an irregular arrangement of the fibres. They are most frequent in the wall of dilatations. The muscular layer may be defective, irregular or missing. The missing elements are sometimes still near the dilatation but no longer in the wall of the distended segment: they are scattered over the interstitial space in a haphazard fashion and possibly also in the fibrotic tissues. Less frequent is a scattered or diffuse proliferation of smooth muscles (Fig. 5). Although such cases, too, belong to the category of cystic changes, it is better

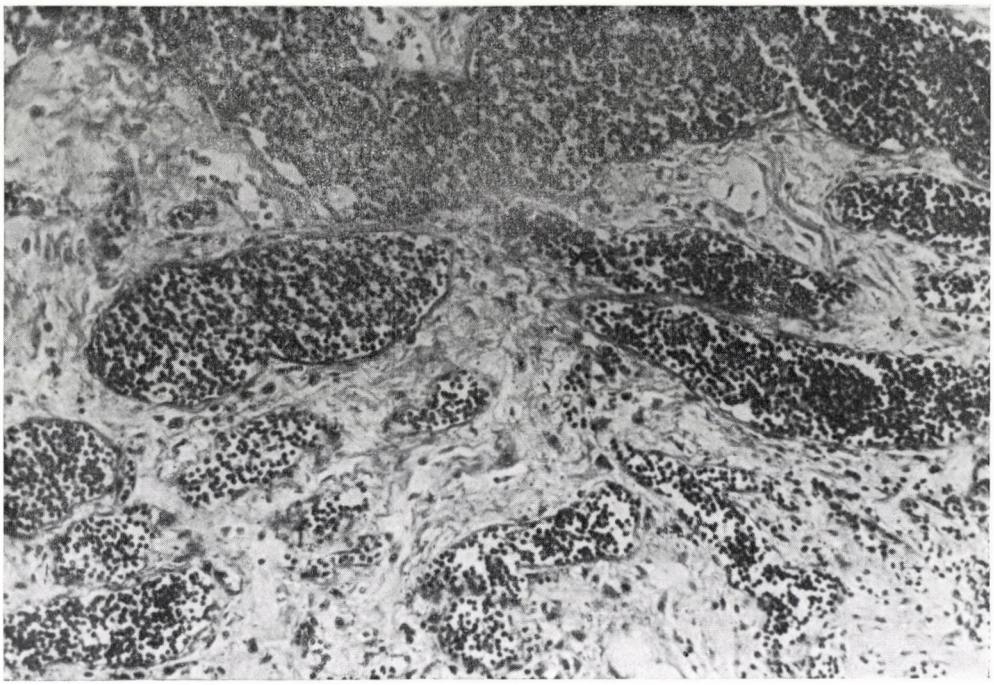


Fig. 6. Cavernous angiomatosis in fibrotic ground substance

to distinguish them by some special term, such as myomatosis or pulmonary leiomyosis. Extreme forms of this kind [12] may constitute a transition to Bourneville's syndrome; they belong to the category of tumorous proliferations (hamartosis, hamartoma) rather than to those characterized by hypertrophy.

In addition to the changes of myoelastic elements, inflammation and consequential fibrosis may develop around the dilated portions of the airways.

Haemorrhages are frequently seen in the unaffected pulmonary parenchyma, while there are many siderophorous and coniphagous cells in the alveoli. The disposition to haemorrhage is due to the peculiar lesions which affect both the major and the minor vessels. Manifestations of this nature form a transition to multiple pulmonary aneurysms and arteriovenous fistulae which, too, may be accompanied by bronchiectasis.

Only two authors ascribed significance to these vascular phenomena. FANCONI [6] described a case in which capillary proliferation was so intensive as to justify its qualification as angioreticulosis. The phenomenon was classified by UEHLINGER as a congenital developmental disturbance of the capillaries. A similar case was described by GOLDEN and BRONK [8]. In one of our cases the change resembled cavernous hepatic angiomatosis (Fig. 6). Consid-

ering that vascular phenomena of such extent and aspect have not yet been reported, it seemed justified to describe this angiocavernous form of the honeycomb lung.

Pathogenesis

According to certain authors the changes in question are of inflammatory origin, others attribute them to dysplasia, and still others accept both factors as pathogenic. We share the latter view. Reports on the occurrence of the described phenomena in foetuses, neonates and in whole families argue in favour of the theory of dysplasia [3, 5, 16]. To this the obvious objection is that, if dysplasia is a decisive pathogenic factor, why does the disease so frequently arise at advanced age.

While acknowledging the significance of dysplasia, the inflammatory factor must also be considered, especially from the point of view of the pro-

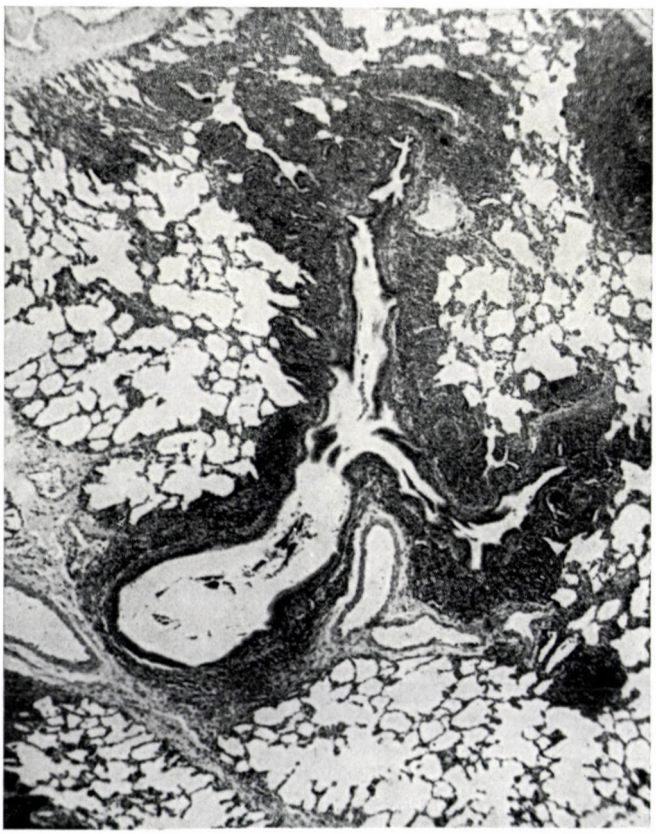


Fig. 7. Follicular (lymphoid cell) infiltration around dilated bronchiole. The surrounding alveolar parenchyma is comparatively intact (Initial inflammatory phase)

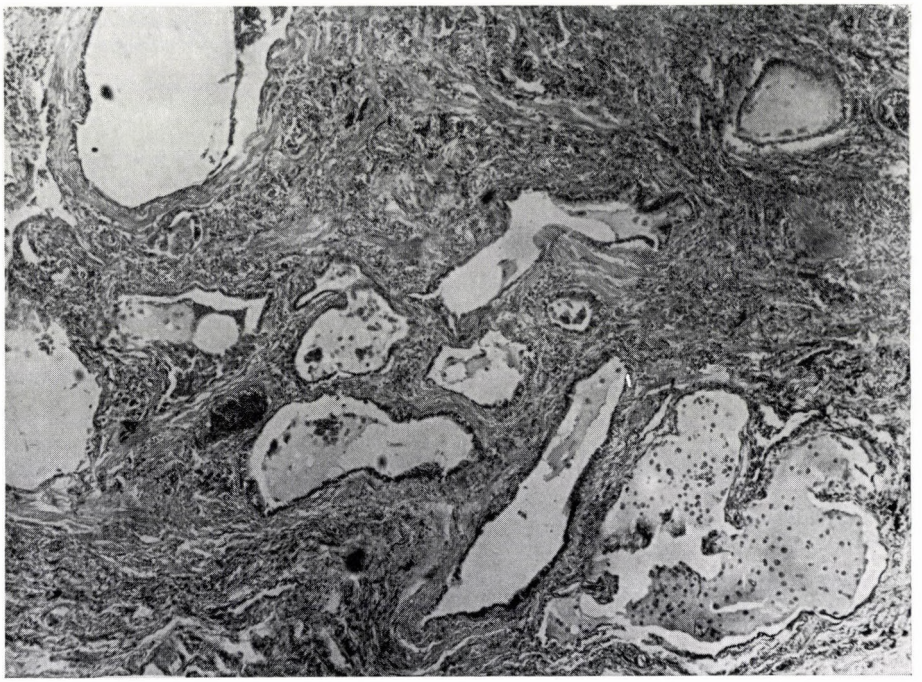


Fig. 8. Fibrocystic lung under medium power. Note slight inflammatory infiltration and dystopic leiomyosis in the fibrotic ground substance, without alveolar parenchyma

gression of the disease. Inflammation was practically present in every published cases in each of those observed in our material.

It is especially with an infiltration of lymphoid elements around the dilated parts that inflammation starts in the initial phase (Figs. 3 and 7). Certain authors ascribe such phenomena to viral infections and regard the bronchiolectasis rather than the infiltrative inflammation as the secondary manifestation. The more extensive, more serious and more frequently recurring the inflammation, the more rapid and extensive the development of fibrosis. While dysplasia is the basis and condition of the disease, inflammation and fibrosis are the chief factors in its progression. By expansion and subsequent shrinkage the fibroplasia gradually constricts and then destroys the alveolar parenchyma (Fig. 8). With advancing fibrosis, the respiratory surface becomes more and more restricted, and the clinical symptoms of malignant dyspnoea, of chronic obstructive ventilatory disturbance will be dominant.

Discussion

The morphological picture of emphysema in the classic sense of the term is often at variance with the clinical syndrome of chronic diffuse obstructive ventilatory disturbance. In general, disorders of this kind are characterized by

structural irregularity and cystic transformation of pulmonary tissue. Most authors emphasize the development of vesicles (cystic and honeycomb lungs), others point to smooth muscle anomalies (leiomyosis, muscular cirrhosis). In recent communications the term bronchiolar emphysema is being used and dysplasia and inflammatory changes are considered the pathogenic factors.

Our observations have convinced us that, in spite of the multiplicity of terms and the diversity of morphological changes, these cases represent different varieties of a single disease entity. The tissue anomalies manifest themselves with dilatations at different points of the respiratory tract, and with disorders of the smooth muscles (leiomyosis, elastofibrodysplasia). Diseases referred to as leiomyosis, fibrocystosis, bronchiolectasis as well as certain related forms of bronchiectasis and multiple vascular lesions are, essentially, but varieties of a particular group of dysplasic conditions, manifestations of emphysema in a wider sense of the term, accompanied by chronic diffuse obstructive ventilatory disturbance. If the said varieties originate chiefly from dysplasia, susceptibility to it may play some part in the development of the classical, vesicular form of emphysema.

The syndrome at issue is not as rare as would seem from the available data. Although typically bilateral and diffuse forms are rare, non-diffuse atypical forms are fairly frequent. They easily escape notice at post-mortem examination, especially if morphological signs of classical emphysema are present. Although such mixed forms are more frequent than genuine emphysema textbooks hardly refer to them.

In order to differentiate between classical emphysema or some atypical manifestation, it is necessary to study the material histologically whenever pulmonary disease is associated with progressive dyspnoea ("chronic diffuse obstructive ventilatory disturbance") and whenever cor pulmonale is diagnosed postmortem even if there are gross signs of classical emphysema.

The term emphysema covers, thus, a wider range than the term in its traditional sense. Apart from the alveoli also prealveolar air passages may undergo dilatation and so give rise to atypical forms of emphysema. Looked at from a morphological angle, variants of this kind may be in the background of chronic diffuse obstructive ventilatory disturbance quite as frequently as is the classical form. Such a wider interpretation of emphysema will reduce discrepancy in the interpretation of chronic obstructive ventilatory disturbance and its pathomorphology. Since these morphologically less explored entities are in the background of the problem of the so-called "bronchitic emphysema", a broader definition of the term cannot fail to result in a satisfactory morphological interpretation of bronchiolar emphysema.

REFERENCES

1. BÓNIS, I., MACCONE, V.: (1936) Pulmo polycysticus. 5th Congress of Hungarian Pathological Association. — 2. BOWERMAN, D. L., TOWBIN, M. N.: (1964) Bronchial Emphysema: its Significance in Industrial Medicine. Report of a Case. Dis. Chest **46,** 349. — 3. BRECKENRIDGE, R. L., REHERMANN, R. L., GIBSON, E. T.: (1965) Congenital Cystic Adenomatoid Malformation of the Lung. J. Pediat. **67,** 863. — 4. CSORNAI, M.: (1951) Myomatosis cystica pulmonum. Kísérl. Orvostud. **4.** — 5. DONOHUE, W. L., LASKI, B., UCHIDA, I., MÜNN, J. D.: (1959) Familial Fibrocystic Pulmonary Dysplasia and its Relation to the Hamman-Rich Syndrome. Pediatrics **24,** 786. — 6. FANCONI, G.: (1959) Zur Differentialdiagnose der Lungengerüsterkrankungen im Kindesalter. Dtsch. med. Wschr. **84,** 709. — 7. FISHER, J. H.: (1965) Bronchiolar Emphysema (Diffuse bronchiolectasis). So-called Muscular Cirrhosis of the Lungs. Canad. med. Ass. J. **93,** 681. — 8. GOLDEN, A., BRONK, T.: (1953) Diffuse Interstitial Fibrosis of the Lungs. A Form of Diffuse Interstitial Angiosis and Reticulosis of the Lungs. Arch. intern. Med. **92,** 606. — 9. JELLINEK, H., SZINAY, G.: (1955) Granulomatöse "Wabenlungen". Acta morph. Acad. Sci. hung. **6.** — 10. JERRY, L. M., RITCHIE, A. C.: (1964) Bronchiolar Emphysema. A Report of Necropsied Case of Diffuse Bronchiolectasis and Review of the Literature. Canad. med. Ass. J. **90,** 964. — 11. KÁLLÓ, A., TAKÓ, J., HAMVAS, J., SZIJÁRTÓ, L.: (1965) Über die angiocavernomatöse Form der fibrocystischen Lunge. Zbl. allg. Path. **108,** 215. — 12. KESZLER, P., SZINAI, G., VOITH, L.: (1959) Lungenmyomatose. Thoraxchirurgie **7,** 293. — 13. LOESCHKE, H.: (1926) Emphysema bronchiolectaticum und präterminale Bronchiectasen als Systemerkrankung isolierter Abschnitte des Bronchialbaumes. Verh. dtsch. Ges. Path. **37,** 242. — 14. ROMHÁNYI, G., MACCONE, V.: (1936—37) Zur Pathogenese der polycystischen Lungenveränderungen. Frankf. Z. Path. **50,** 442. — 15. SIEBERT, F. T., FISHER, E. R.: (1957) Bronchiolar Emphysema: So-called Muscular Cirrhosis of the Lungs. Amer. J. Path. **33,** 1137. — 16. VLCEK, A., ULCOVA, I., ČERNIK, V.: (1965) Beitrag zur Problematik der angeborenen Lungencysten bei Säuglingen. Kinderärztl. Praxis **33,** 355.

DAS BRONCHIOLÄREMPHYSEM UND DIE DAMIT VERBUNDENEN KRANKHEITEN

A. KÁLLÓ

Nach einer Übersicht der Literaturangaben über das Bronchioläremphysem wird auf Grund eigener Beobachtungen die Bedeutung der Dysplasie und der Entzündung in der Pathogenese von Krankheiten, wie Muskelcirrhose, Leiomyose, Fibrozystose, Bronchiolektasie und multiple Gefäßschädigungen der Lunge hervorgehoben.

ЭМФИЗЕМА БРОНХИОЛ И СВЯЗАННЫЕ С НЕЙ ЗАБОЛЕВАНИЯ

А. КАЛЛО

После обзора литературных данных об эмфиземе бронхиол выдвигается на основе этих данных и собственных наблюдений значение дисплазии и воспаления в патогенезе заболеваний, как цирроз мышц, мейомиоз, фиброкистоза, бронхиолектазии и множественные поражения сосудов легких.

Dr. Antal KÁLLÓ; Budapest XII., Diósárok 1. János Kórház, Hungary

RECENSIONES

Morphologie von Wirbelsäule und Brustkorb

Primatologia, Handbuch der Primatenkunde. Vol. IV. No. 4

F. Ankel

VIII + 120 pp., 69 figures, 1 table. Price: sFr. /DM 53. — Ed. Karger, Basel—New York, 1967

Examination of the spinal column, the characteristic structure of vertebrates, is an important object of comparative anatomy. Changed locomotion and the development of manipulative activity, consequences of the formation of the vertical spine were decisive factors in the evolution of the human species; they raise new problems concerning the phylogenesis and ontogenesis of the spinal column, problems that can nowadays be approached by qualitative methods.

Dispensing with a discussion extending to the whole widely ramifying subphylum of Vertebrata, Ankel's monograph deals only with the morphology of the vertebral column and the chest of Primates, further with a functional interpretation of the data so obtained.

Relying on investigations concerning half apes (Tupaia, Lemur, Galago, etc.) and apes (Cebidae, Callithricidae, Cercopithecoidea, Hominoidea), further on literary data, Ankel analyses the general structure of the spinal column and the vertebrae, and discusses the special features of the spine of the various simian species.

A separate chapter contains a comparative description of the costae and the sternum.

It is not sufficient to base a functional analysis of the vertebral column and the chest on the osseous structure alone. The skeleton itself is a passive structure which, to become active, needs the cooperation of muscles and ligaments. The latter cause a steady change in the mutual arrangement of the vertebrae so that it is a concerted action of the active and passive factors which determines kinetic phenomena.

The author starts from the principle that the functional adaptation of the bones exerts a morphological effect on the vertebrae, the mechanic units, so that a comparative analysis of the bones admits of functional conclusions.

The manner in which the osseous spinal column is exposed to pressure, traction and bending varies according to the various modes of locomotion. Although the mechanical utilization of the spinal column shows considerable differences among the Primates, the differences do not manifest themselves with corresponding deviations in the structure of the axial skeleton.

Careful analysis of the various simian species allowed to conclude that the axial skeleton of Primates is a structure which may be generalized in the sense that it is able to satisfy multiple functional requirements or, in other words, that, while Primates may be divided according to different types of locomotion, their axial skeleton shows a common structure. It is worthy of especial note that, despite a decisive change in the locomotion of humans, the vertebral column of man is hardly different from that of monkeys. Analysis of the bones constituting the axial skeleton revealed no differences in regard of sex.

Owing to careful morphological and functional analyses, Ankel's monograph is a valuable contribution to primatological research work.

T. Donáth

Humerus, Ulna und Radius der Simiae

Vergleichendmorphologische Untersuchungen mit Berücksichtigung der Funktion

R. Knussmann

Bibliotheca Primatologica Fasc. 5. XII + 399 pp., 228 figures, 46 tables. Price: SFr/DM 79. — Ed. Karger, Basel—New York, 1967.

Inferences from investigations into primatology can always be utilized in human anatomy, especially if — as in the present work — data yielded by the study of Primates are compared with data obtained on human material.

Relying on a systematic study of the humerus, ulna and radius in all simian species, the monograph under review presents a functional interpretation of the morphological data gained by the analysis of the said bones. The monograph is especially valuable insofar as, besides using the classical method of description and conventional drawings, it evaluates the results of measurements by up-to-date biomathematical-statistical methods.

In order to be able to subject his numerous data to a thorough analysis, the author does not extend his investigations in a horizontal direction and dispenses, accordingly, with discussing the functionally inseparable structures as the muscles and ligaments connected with the bones. This notwithstanding, the work — a vertical one since it is restricted to a detailed analysis of the bones themselves — admits of important conclusions on account of the biostatistical evaluation of the material.

The monograph has the purpose to offer an osteological analysis of the anterior extremities which, according to the different manners of locomotion, are differently used by the various Primates. It was further intended to find a correlation between the development of brachiation with that of the human upper extremities.

Studies made on 541 recent monkeys of 24 genera have led to the conclusion that brachiation represents a functional adaptation which, by an elongation of the diaphyses, facilitates swinging from one hold to another.

Corpus and distal epiphysis (olecranon) of the ulnar bone grow in diameter in proportion to the growth of the body and its weight, an evident adaptation to increased pressures, tractions and strains.

The monograph presents detailed comparisons between the osteological data of monkeys and man. A comparison of the osteological measurements of the Neanderthal man with those concerning the upper extremity of the present races reveals a certain degree of regression (olecranon, trochlear incisure, neck of the radius, etc.) in the latter.

Pursuant to the factor analysis applied in the course of the biomathematical evaluations, the author distinguishes eleven factors that belong to three main categories: (1) factors of embryology and taxonomy; (2) factors of functional adaptation; (3) special factors of the bony structure.

With its exemplary arrangement, vivid style, 228 drawings in the appendix to illustrate skeletal details, 46 biostatistical tables, and an exhaustive bibliography, the work cannot fail to please all students of primatology.

T. Donáth

INDEX

Morphologia Normalis et Experimentalis

Pathologia

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Farkas Sándor

A kézirat nyomdába érkezett: 1968. VII. 25. — Terjedelem: 10,75 (A/5) ív, 102 ábra, (25 színes), 4 melléklet

68.66049 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

The Acta Morphologica publish papers on experimental medical subjects in English, German, French and Russian.

The Acta Morphologica appear in parts of varying size, making up volumes.

Manuscripts should be addressed to:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Correspondence with the editors and publishers should be sent to the same address.

The rate of subscription to the Acta Morphologica is 165 forints a volume. Orders may be placed with "Kultúra" Foreign Trade Company for Books and Newspapers (Budapest I., Fő utca 32. Account No. 43-790-057-181) or with representatives abroad.

Les Acta Morphologica paraissent en français, allemand, anglais et russe et publient des travaux du domaine des sciences médicales expérimentales.

Les Acta Morphologica sont publiés sous forme de fascicules qui seront réunis en volumes.

On est prié d'envoyer les manuscrits destinés à l'adresse suivante:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Toute correspondence doit être envoyée à cette même adresse.

Le prix de l'abonnement est de 165 forints par volume.

On peut s'abonner à l'Entreprise du Commerce Extérieur de Livres et Journaux «Kultúra» (Budapest I., Fő utca 32. Compte-courant No. 43-790-057-181) ou à l'étranger chez tous les représentants ou dépositaires.

«Acta Morphologica» публикуют трактаты из области экспериментальных медицинских наук на русском, немецком, английском и французском языках.

«Acta Morphologica» выходят отдельными выпусками разного объема. Несколько выпусков составляют один том.

Предназначенные для публикации авторские рукописи следует направлять по адресу:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

По этому же адресу направлять всякую корреспонденцию для редакции и администрации.

Подписная цена «Acta Morphologica» — 165 форинтов за том. Заказы принимает предприятие по внешней торговле книг и газет «Kultúra» (Budapest I., Fő utca 32. Текущий счет № 43-790-057-181) или его заграничные представительства и уполномоченные.

54,— Ft

Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

ALBANIA
Ndermarja Shtetnore e Botimeve
Tirana

AUSTRALIA
A. Keesing
Box 4886, GPO
Sydney

AUSTRIA
Globus Buchvertrieb
Salzgries 16
Wien I

BELGIUM
Office International de Librairie
30, Avenue Marnix
Bruxelles 5
Du Monde Entier
5, Place St. Jean
Bruxelles

BULGARIA
Raznoiznos
1, Tzar Assen
Sofia

CANADA
Pannonia Books
2, Spadina Road
Toronto 4, Ont.

CHINA
Waiwen Shudian
Peking
P .O. B. 88

CZECHOSLOVAKIA
Artia
Ve Směčkách 30
Praha 2
Poštovná Novinová Služba
Dovoz tisku
Vinohradská 46
Praha 2
Mad'arská Kultura
Václavské nám. 2
Praha I
Poštová Novinová Služba
Dovoz tlače
Leningradská 14
Bratislava

DENMARK
Ejnar Munksgaard
Nörregade 6
Copenhagen

FINLAND
Akateeminen Kirjakauppa
Keskuskatu 2
Helsinki

FRANCE
Office International de Documentation et Librairie
48, rue Gay Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC
Deutscher Buch-Export und Import
Leninstraße 16
Leipzig 701
Zeitungsvertriebsamt
Fruchtstrasse 3—4
1004 Berlin

GERMAN FEDERAL REPUBLIC
Kunst und Wissen
Erich Bieber
Postfach 46
7 Stuttgart S.

GREAT BRITAIN
Collet's Holdings Ltd.
Dennington Estate
London Rd.
Wellingborough. Northants.
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
Oxford

HOLLAND
Swetz and Zeitlinger
Keizersgracht 471—487
Amsterdam C
Martinus Nijhof
Lange Voorhout 9
The Hague

INDIA
Current Technical Literature
Co Private Ltd.
India House OPP
GPO Post Box 1374
Bombay I

ITALY
Santo Vanasia
Via M. Macchi 71
Milano
Libreria Commissionaria Sansoni
Via La Marmora 45
Firenze

JAPAN
Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
Tokyo
Maruzen and Co. Ltd.
P. O. Box 605
Tokyo-Central
Far Eastern Booksellers
Kanda P. O. Box 72
Tokyo

KOREA
Chulpanmul
Phenjan

NORWAY
Johan Grundt Tanum
Karl Johansgatan 43
Oslo

POLAND
RUCH
ul. Wronia 23
Warszawa

ROUMANIA
Cartimex
Str. Aristide Briand 14—18
Bucureşti

SOVIET UNION
Mezhdunarodnaya Kniga
Moscow G—200

SWEDEN
Almquist and Wiksell
Gamla Brogatan 26
Stockholm

USA
Stechert Hafner Inc.
31, East 10th Street
New York, N. Y. 10003
Walter J. Johnson
111, Fifth Avenue
New York, N. Y. 10003

VIETNAM
Xunhasaba
19, Tran Quoc Toan
Hanoi

YUGOSLAVIA
Forum
Vojvode Mišića broj 1
Novi Sad
Jugoslovenska Knjiga
Terazije 27
Beograd

29. XII. 1968 Index: 26.017

Acta Morphologica

Academiae Scientiarum Hungaricae

ADIUVANTIBUS

J. BALÓ, P. ENDES, K. FARKAS, L. HARANGHY, B. KELLNER, I. KROMPECHER, GY. ROMHÁNYI, E. SOMOGYI, J. SZENTÁGOTHAI

REDIGIT

I. TÖRŐ

TOMUS XVI * FASCICULUS 4

Akadémiai Kiadó Budapest

1968

ACTA MORPH. HUNG

ACTA MORPHOLOGICA

A MAGYAR TUDOMÁNYOS AKADÉMIA ORVOSTUDOMÁNYI KÖZLEMÉNYEI

SZERKESZTŐSÉG ÉS KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY U. 21.

Technikai szerkesztő:
Dr. Somogyi Endre

Az Acta Morphologica német, angol, francia és orosz nyelven közöl értekezéseket a kísérletes orvostudomány tárgyköréből.

Az Acta Morphologica változó terjedelmű füzetekben jelenik meg. Több füzet alkot egy kötetet.

A közlésre szánt kéziratok a következő címre küldendők:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Ugyanerre a címre küldendő minden szerkesztőségi és kiadóhivatali levelezés.

Az Acta Morphologica előfizetési ára kötetenként belföldre 120, külföldre 165 Ft. Megrendelhető a belföld számára az Akadémiai Kiadónál (Budapest V., Alkotmány utca 21. Bankszámla 05-915-111-46), a külföld számára pedig a „Kultúra" Könyv- és Hírlap Külkereskedelmi Vállalatnál (Budapest I., Fő utca 32. Bankszámla: 43-790-057-181) vagy annak külföldi képviseleteinél és bizományosainál.

Die Acta Morphologica veröffentlichen Abhandlungen aus dem Bereiche der experimental-medizinischen Wissenschaften in deutscher, englischer, französischer und russischer Sprache.

Die Acta Morphologica erscheinen in Heften wechselnden Umfages. Mehrere Hefte bilden einen Band.

Die zur Veröffentlichung bestimmten Manuskripte sind an die folgende Adresse zu senden:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

An die gleiche Anschrift ist auch jede für die Schriftleitung und den Verlag bestimmte Korrespondenz zu richten.

Abonnementspreis pro Band: 165 Forint. Bestellbar bei dem Buch- und Zeitungs-Außenhandels-Unternehmen »Kultúra« (Budapest I., Fő utca 32. Bankkonto Nr. 43-790-057-181) oder bei seinen Auslandsvertretungen und Kommissionären.

Acta Morphologica Acad. Sci. hung. **16** *(4), pp. 375—390, 1968*

Department of Neurology (Director: Prof. B. HORÁNYI), University Medical School, Budapest

ACETYLCHOLINESTERASE ACTIVITY OF THE HUMAN LOWER BRAIN STEM WITH SPECIAL REGARD TO THE RETICULAR FORMATION

M. PAPP

(Received July 28, 1966)

The acetylcholinesterase (AChE) activity of the lower brain stem with special regard to the reticular formation has been studied in preparations from 10 suddenly died subjects. The nerve cells could be divided into 5 groups on the basis of AChE activity of the perikaryon and in its surroundings.

1. Nerve cells of the first group in the reticular formation exhibited a high AChE activity in the cytoplasm in patterns resembling the Nissl substance or fine granular in distribution. This AChE activity is identical to that observed in the extensively studied cholinergic nerve cells (large motor cells of the anterior horn, preganglionic nerve cells in the lateral column).
2. Nerve cells of the second group exhibited a moderate AChE activity.
3. In the majority of nerve cells AChE activity was low in the entire cell body and in the dendrites.
4. Nerve cells of non-cholinergic character with external AChE activity.
5. AChE-negative nerve cells.

The reticular formation of the human lower brain stem can be divided on the basis of AChE activity into the following cell groups.

1. Uniformly moderate enzyme activity constitutes the column of raphe cells. The AChE activities of the individual cells differ in a minor degree.
2. Uniformly low AChE activity was found in two of the dorsolateral cell columns (n. parvocellularis and n. cuneiformis).
3. A variable, but on the whole moderate AChE activity characterizes the ventromedial cell column.
4. A variable, but on the whole high AChE activity is shown by the dorsolateral cell group (dorsolateral part of the n. pontis centralis oralis, ventral part of the locus coeruleus and the dorsal part of the n. tegmenti pedunculopontinus).
5. The n. papilliformis which is characterized by uniformly medium AChE activity of the nerve cells differs from the above cell group with a significant activity of the neuropil.

The significance of the variability of the AChE activity in the third and fourth cell groups is discussed,

Nerve cells of very low AChH activity represented the overwhelming majority in all cell groups of the lower brain stem reticular formation, even in those of moderate and high activity.

The Koelle method (KOELLE and FRIEDENWALD 1949) may indicate the extent of participation of acetylcholine (ACh) in neurotransmission (KOELLE 1955) and, at the histotopochemical examination of the neuron and its surroundings, allow the recognition of non-cholinergic-cholinoceptive nerve cells (KOELLE 1957, KOELLE and KOELLE 1959, MCISAAC and KOELLE 1959). This has been supported by fluorescence methods used for the demonstration of biogenic amines (HAMBERGER, NORBERG and SJÖQVIST 1963).

The aim of the present study has been to classify the nerve cells and cell groups of the human lower brain stem on the basis of the histochemical morphology of their AChE activity. The examinations were centred on the reticular formation (rf).

Material and methods

The lower brain stem (medulla oblongata, pons, mesencephalon) from 10 subjects 20 to 45 years of age was examined. Each of them had died suddenly without medical attendance for some days preceding death. In four cases, death had been due to cardiac arrest, in one case to a stab wound of the heart, in one case to skull injury and in four cases to suicide by hanging. Examination was made 10—18 hours after death, in some cases earlier. At various levels of the lower brain stem blocks 3—4 mm thick were excised and fixed in 6—8% neutral formaline at +4°C for 18—20 hours. For the demonstration of AChE and pseudocholinesterase (ChE), 40 μ frozen sections were incubated for 120 minutes in acetylthiocholine iodide (AThCh) and butyrylthiocholine iodide (BuThCh), pH 5.0—6.2 (KOELLE and FRIEDENWALD 1949; GEREBTZOFF 1959). In three cases some sections were incubated at pH 5.5 for 20 hours to demonstrate the enzymic activity of the axons. AChE was inhibited with 5×10^{-7}—10^{-6} M ambenonium chloride, ChE with 10^{-6} M diisopropylfluorophosphate (DFP).

Results

I. General histochemical observations

In sections incubated in AThCh, the bulk of enzyme activity was localized in the perikaryon, dendrites and axons (Figs 1—12), while its minor part in the neuropil (Figs 14—18). No difference was demonstrable between sections incubated with AThCh and treated and not treated with ChE inhibitor. In contrast, ambenonium chloride (LANDS et al. 1955) eliminated all enzyme activity except for traces left in the vessel walls or sometimes in the lumina. Thus, in agreement with the findings of FOLDES et al. (1962) the cholinesterase activity of human brain was due primarily to its AChE content, and the end product of the histochemical reaction taking place under the described conditions may be regarded the site of AChE activity.

The pH optimum for demonstrating AChE activity in the reticular formation was in the range from 5.5 to 6.0. In certain so-called specific cell groups (n. tractus solitarii) AChE activity in the nucleoli of nerve cells and in the glia cells was seen at pH 6.0 already. However, in the greater part of the reticular formation, the end product signifying enzyme activity was localized at the

Fig. 1. Nerve cell without staining for AChE (arrows) close to another nerve cell with moderate enzyme activity in the dorsolateral part of the *n. pontis centralis oralis.* pH 5.5; magnification 550×

Fig. 2. Nerve cells of high enzyme activity in the rostral part of *n. dorsalis nervi vagi.* At the sites marked by arrows the enzyme activity exhibits double contours. Granules of marking enzyme activity are seen throughout the total calibre of dendrites. pH 5.5; magnification 830×

Fig. 3. Enzyme activity in a distribution resembling the tigroid pattern. Lateral part of the caudal subnucleus of *n. gigantocellularis.* pH 6.0; magnification 280×

Fig. 4. Nerve cells from the caudal end of the facial nerve. Distribution of enzyme activity resembles the tigroid pattern. pH 6.0; magnification 280×

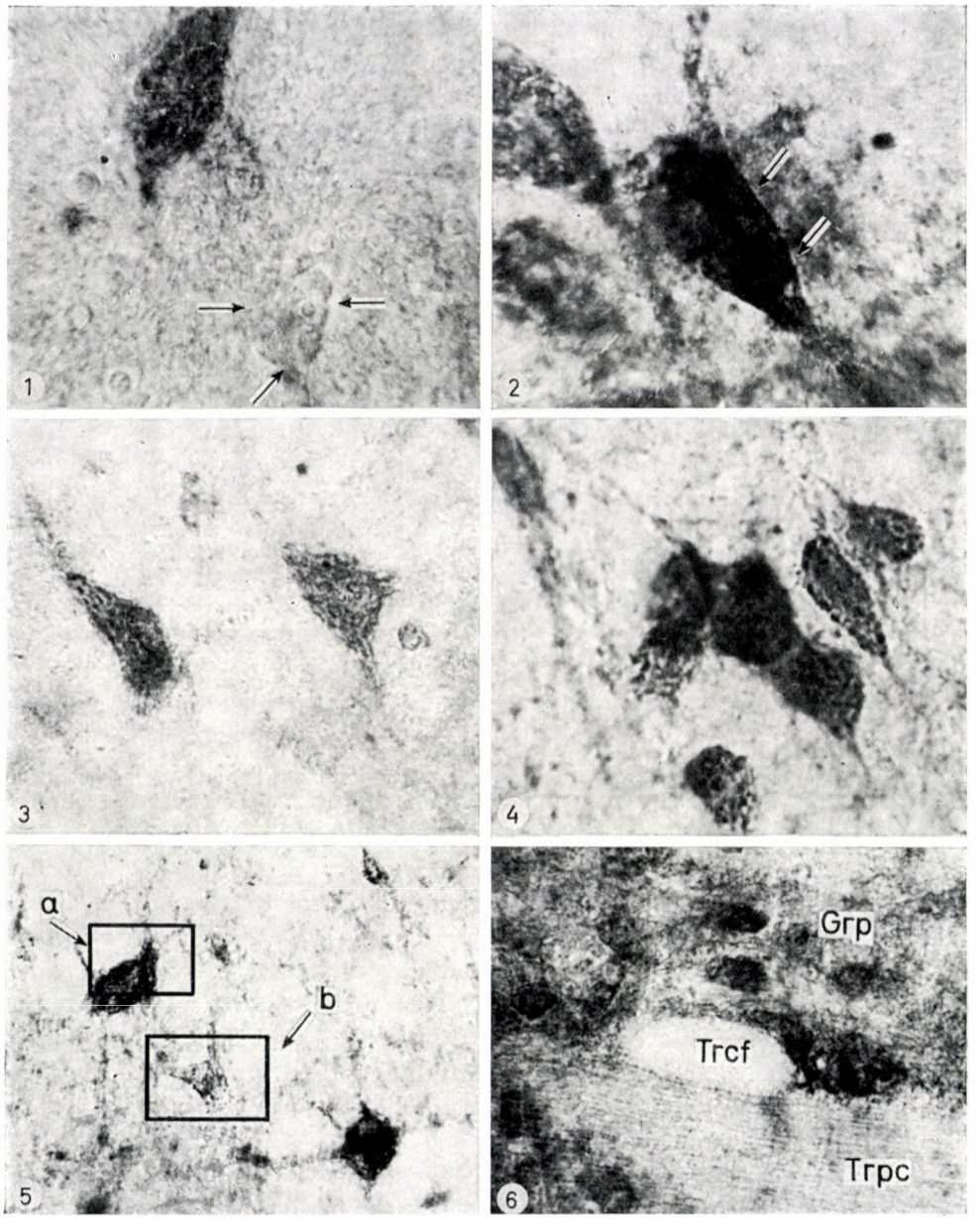


Fig. 5. Part of the subnucleus caudalis of *n.gigantocellularis*. A medium size nerve cell of cholinoceptive enzyme activity is shown between two large multipolar cells of cholinergic type activity. pH 5.5; magnification 110 ×

Fig. 6. Basal part of the pons. AChE active transverse fibres and inactive corticofugal fibre bundles. pH 5.5; magnification 280 ×

same site as at a lower pH, increasing only in intensity. The more distal parts of the dendrites were better seen at a lower pH (Fig. 8).

Following 2 hours incubation with BuThCh the staining was not sufficiently intensive for evaluation. In view of the long time between death and fixation, the amount of biochemically demonstrable pseudocholinesterases (ORD and THOMPSON 1952) might have decreased below the histochemically demonstrable threshold.

II. Classification of nerve cells according to AChE activity

Localization of AChE activity in the nerve cells and around them was of the following types.

1. The entire perikaryon showed a strong enzymic activity; its distribution was either reminiscent of the pattern of Nissl substance (Figs 2, 3, 4, 5a and 7) or was finely granular (Figs 1 and 8) and its localization corresponded to that of the internal or cytoplasmic AChE (KOELLE and STEINER 1956) as demonstrated by FUKUDA and KOELLE (1959). Enzyme activity in the dendrite was seen either diffusely throughout its entire calibre (Fig. 2), or exclusively along the edges (Fig. 7). In the nucleus dorsalis nervi vagi exclusively the former type, whereas in the "effector" part of the reticular formation (BRODAL 1957) both types were apparent.

This type of AChE activity was shown by the general and special visceral efferent cells (Figs 2, 4) as well as by the majority of somatic efferent and pontine cells (Fig. 6). Among the cell groups of the reticular formation, such enzyme activity was found in the n. gigantocellularis subnucleus caudalis (Figs 3, 5, 7), n. paragigantocellularis lateralis (some type "a" and "b" cells, OLSZEWSKI and BAXTER 1954), in the dorsolateral part of the n. pontis centralis oralis (Fig. 8) and n. tegmenti pedunculopontinus, in many large and medium, less often in small nerve cells.

2. The entire nerve cell showed moderate enzyme activity in the same distribution as in the cells of the preceding group. In the reticular formation many cells showing this type of AChE activity were found in the raphe nuclei, n. medullae oblongatae centralis, n. gigantocellularis, n. paragigantocellularis dorsalis and lateralis, nn. pontis centralis oralis and caudalis (Fig. 1) and n. tegmenti pedunculopontinus.

3. Very low, uniformly distributed enzyme activity was seen in the perikaryon and dendrites. Nearly exclusively this type of AChE activity was present in the somatic afferent cranial nerve nuclei, the n. cuneatus and gracilis, the parvocellular part of the n. ruber (Fig. 17), n. parvocellularis of the reticular formation (Fig. 19), the n. cuneiformis and subcuneiformis.

Fig. 7. Part "a" of Fig. 5. Large multipolar cell with enzyme activity resembling the tigroid pattern, in the caudal subnucleus of *n. gigantocellularis*. The enzyme activity of dendrites is limited next to the surface of the nerve cell. Magnification 830 ×

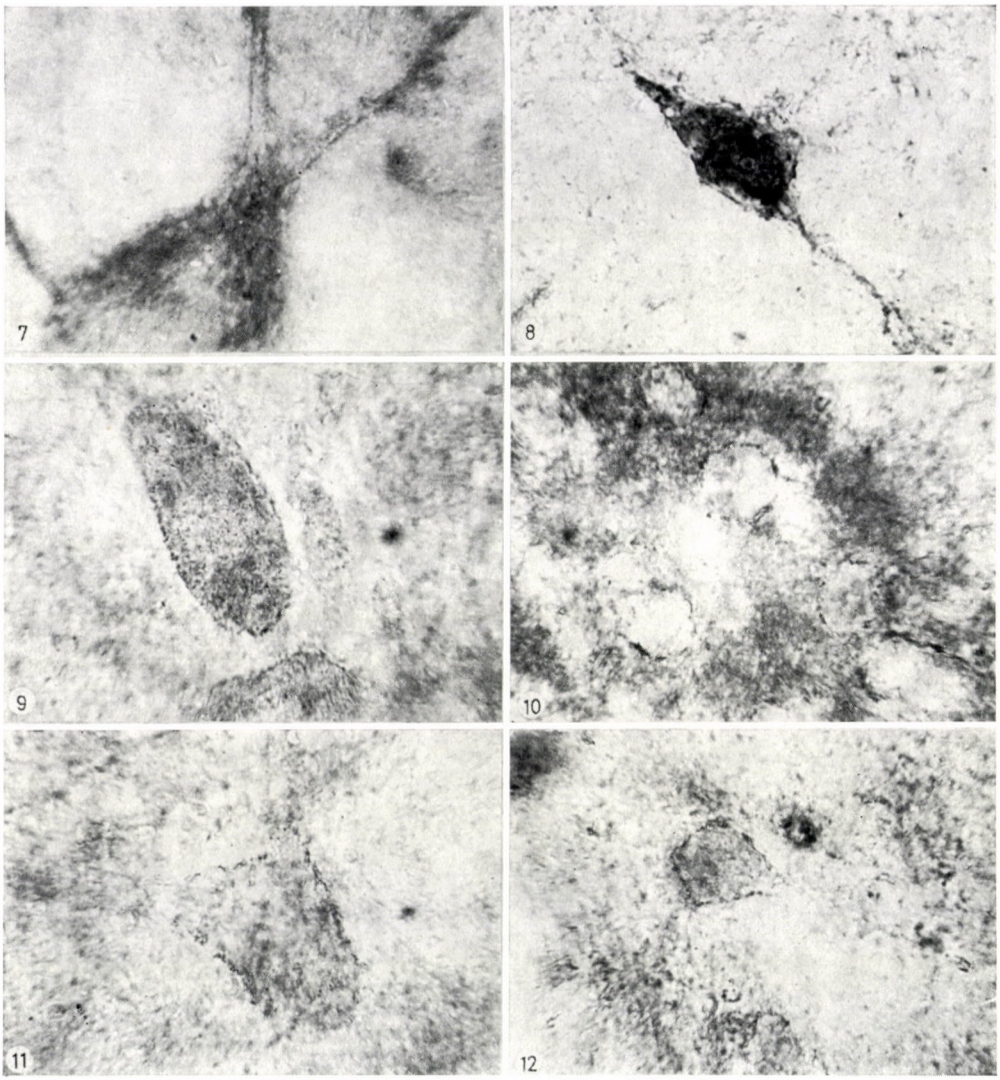


Fig. 8. Cholinergic type enzyme activity in a nerve cell of medium size from the dorsolateral part of the *n. pontis centralis oralis*. The enzyme activity of the dendrite is traceable down to the distal branches. pH 5.0; magnification 550×

Fig. 9. Medium-size nerve cell with enzyme activity next to the cell surface and low activity in the perikaryon. Caudal subnucleus of *n. gigantocellularis*. pH 6.0; magnification 550×

Fig. 10. Non-cholinergic-cholinoceptive type enzyme activity in the oral part of *n. tractus solitarii*. Intensive staining for AChE next to the cell surface and in the neuropil. pH 5.5; magnification 550×

Fig. 11. Part "b" of Fig. 5. Multipolar nerve cell of medium size exhibiting non-cholinergic-cholinoceptive type enzyme activity. Caudal subnucleus of *n. gigantocellularis*. Magnification 550×

Fig. 12. A medium-size nerve cell with external AChE activity from the dorsolateral part of *n. pontis centralis oralis*. Slightly to right and upward a transversally cut dendrite is seen. pH 6.0; magnification 550×

4. In the rostral part of the nucleus tractus solitarii some nerve cells displayed a very low or no AChE activity in the cytoplasm, while a significant activity around the perikaryon (Fig. 10). This type of activity was, among the cell groups of the reticular formation, found mostly in the dorsolateral part of the n. pontis centralis oralis (Fig. 12) and in the ventral part of the locus coeruleus; less often also in the caudal subnucleus of the n. gigantocellularis (Figs 9, 11).

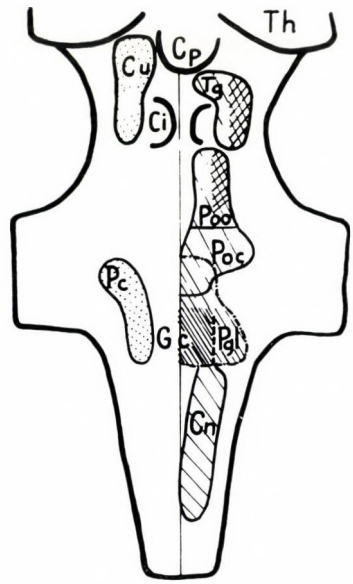


Fig. 13. Schematic presentation of AChE activity of the majority of cell groups in the human lower brain stem reticular formation (Olszewski, 1956). Dotted area: uniformly low AChE activity in the dorsolateral cell groups. Hatched area: variable, on the whole medium activity in the medial cell column. The shade of the area refers to the intensity of activity. Cross-hatched area: variable, on the average high AChE activity in one of the dorsolateral matrix

5. In some cells AChE activity was lacking in both the perikaryon and around them even at higher pH. Such AChE-negative cells were found among nerve cells of strong, moderate and low AChE activity in the dorsolateral half of the n. pontis centralis oralis (Fig. 1).

III. Distribution of AChE activity in various cell groups of the reticular formation

On the basis of AChE activity the human lower brain stem reticular formation could be divided into the next five cell groups.

1. Cells of uniformly moderate enzyme activity constitute the column of raphe nuclei except for the n. raphe pallidus. Under this category come the n. raphe obscurus (Fig. 14), n. centralis superior (Fig. 18) and the n. supratrochlearis. Enzyme activity in the various cells differs only in a minor degree.

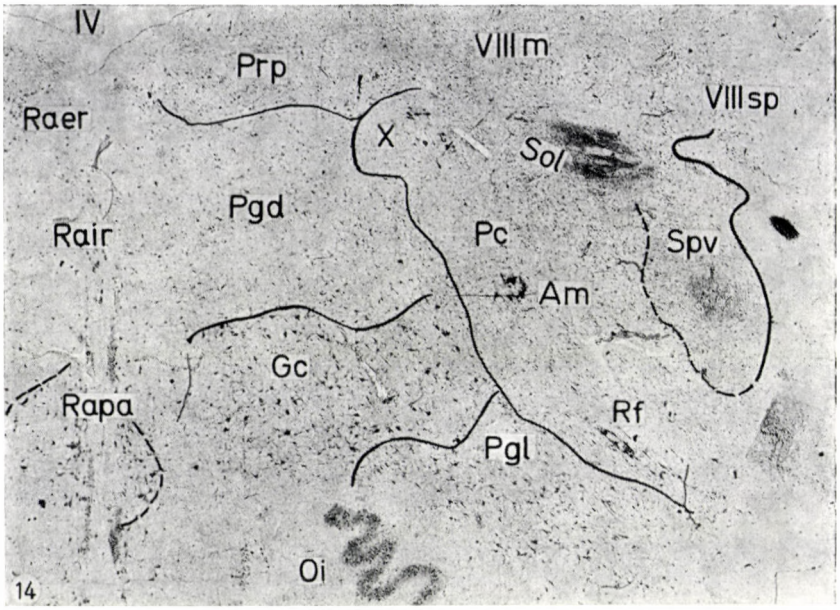


Fig. 14. Low-power view of the human lower brain stem at the level of the caudal subnucleus of *n. gigantocellularis.* pH 6.0; magnification 14×

2. AChE activity was uniformly low in two of the dorsolateral cell columns, caudally the n. parvocellularis (Figs 13, 14, 19) and rostrally the n. cuneiformis. These two columns are made up predominantly of "c" type cells (Olszewski and Baxter 1954). The few medium size cells display also a low activity.

3. AChE activity in the ventromedial cell column is on the average moderate, but cells of low, moderate and very high activity are likewise found. The structures belonging here are the n. medullae oblongatae centralis, n. gigantocellularis, nn. paragigantocellularis dorsalis and lateralis, and the ventromedial parts of n. pontis centralis caudalis and n. pontis centralis oralis as well as the ventral part of the n. tegmenti pedunculopontinus (Fig. 13). The variations in activity are particularly pronounced in the n. gigantocellularis subnucleus caudalis and paragigantocellularis lateralis (Figs 3, 5, 7, 9, 11). A somewhat weaker activity is shown by the nerve cells of the n. paragigantocellularis dorsalis (Fig. 14). The intensity of staining seemed to weaken in the caudal and rostral direction, beginning from the caudal subnucleus of the n. gigantocellularis (Figs 13, 15, 16).

In frontal sections cut at the level of the caudal peak of the n. nervi facialis, counting of nerve cells according to size and intensity of AChE activity was made in two subjects. The ventral border of the area was the white matter surrounding the oliva inferior, its dorsolateral border the n. parvocellularis, its

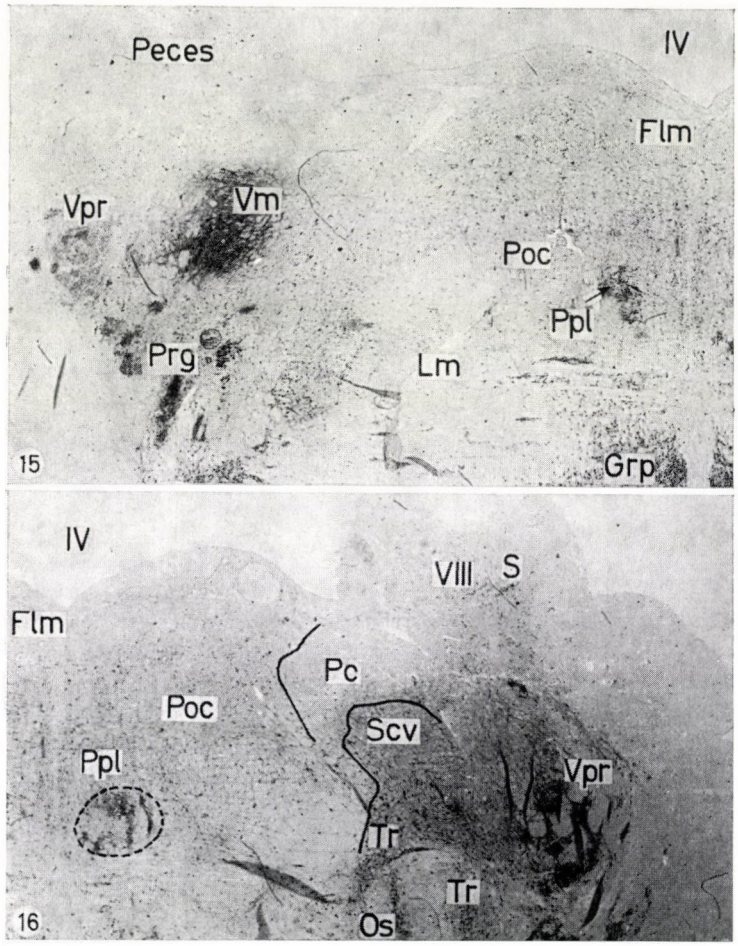


Fig. 15—16. The human lower brain stem at the level of the superior oliva (Fig. 16) and the motor nucleus of the trigeminal nerve. (Fig. 15) The medial two thirds of *n. pontis centralis caudalis* exhibit more intensive activity than its lateral part or the *n. parvocellularis*, though the difference is considerably less pronounced than at the level of the caudal subnucleus of *n. gigantocellularis*. At these levels highly AChE active medium-size cells are present in the *n. subcoeruleus ventralis*. pH 6.0; magnification 7×

medial border the raphe and its dorsal border a perpendicular line drawn from the raphe to the centre of the nucleus of the spinal tract of the trigeminal nerve. Thus this area comprised the caudal subnucleus of the n. gigantocellularis, the n. paragigantocellularis lateralis and part of the n. raphe pallidus. In this area 65% of the cells showed low AChE activity and were mostly of small size ("c" type), 25% moderate activity and were mostly of medium size, and 8—10% showed high activity and were predominantly large or medium less often small in size.

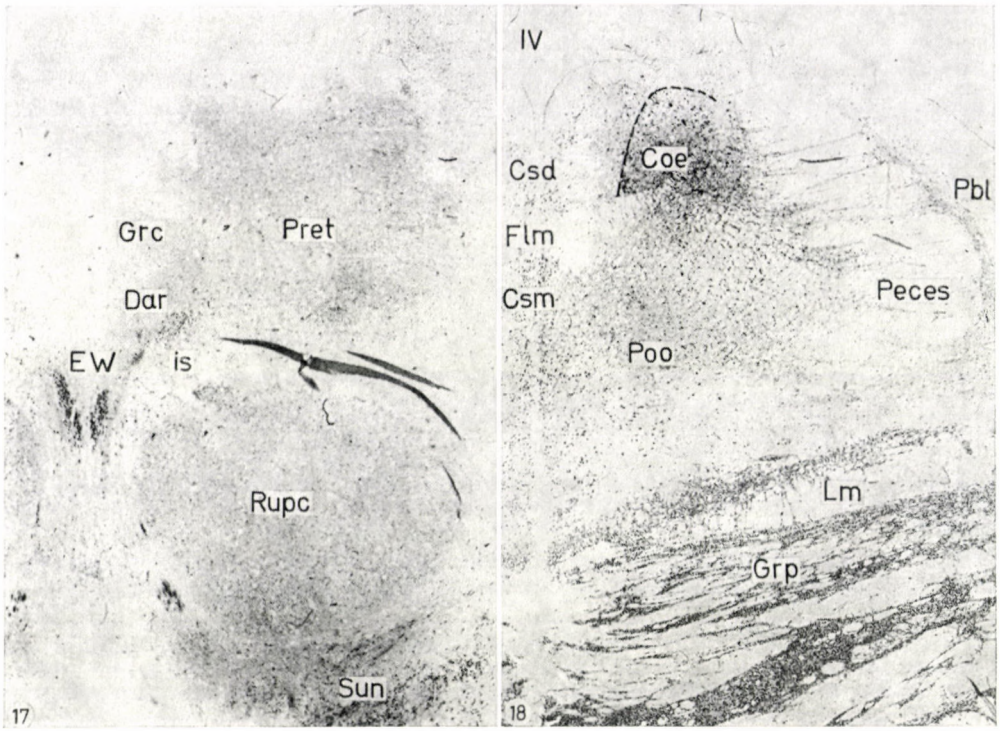


Fig. 17. AChE activity in the mesencephalon at the level of the oral part of the Edinger-Westphal nucleus. pH 6.0; magnification 6×

Fig. 18. Low power view of the lower brain stem at the level of the *n. pontis centralis oralis.* Nerve cells of high enzyme activity in the dorsolateral part of *n. pontis centralis oralis* and in the *locus coeruleus.* pH 6.0; magnification 8.5×

4. Variable, but on the average very high AChE activity was shown by the dorsolateral cell column, including the dorsolateral part of the n. pontis centralis oralis, the ventral part of the locus coeruleus and the dorsal part of the n. tegmenti pedunculopontinus. Beside nerve cells of very high AChE activity, cells of moderate and low activity and cells without staining for AChE (Fig. 1) were found. Among them nerve cells with external AChE activity were seen (Fig. 12). There were many cells showing high activity (Fig. 18).

5. The n. papilliformis differs from the other cell groups of the reticular formation in displaying a significant activity in the neuropil (Figs 15, 16).

IV. AChE activity of nerve fibres

A certain correlation has been found between the course and AChE activity of nerve fibres in the lower brain stem in specimens incubated for 20 hours. Ascending and descending fibres running longitudinally in the brain

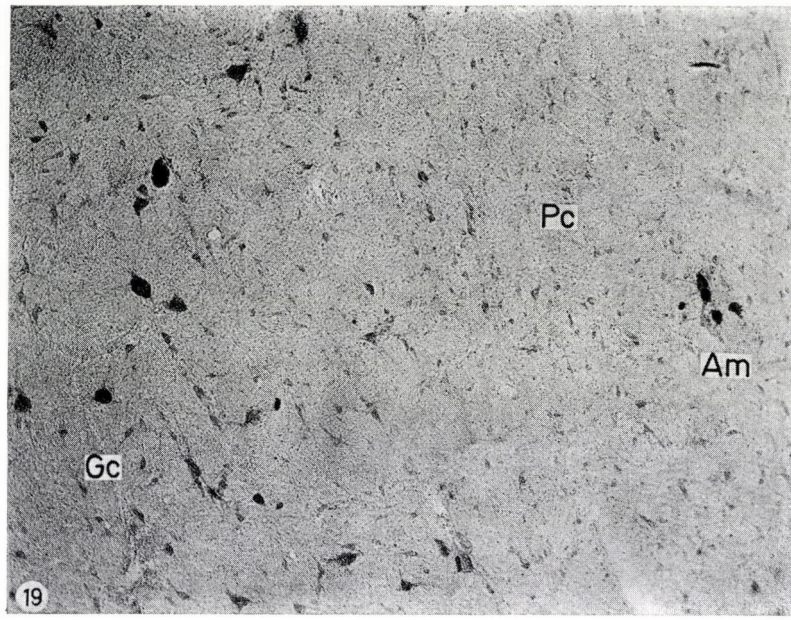


Fig. 19. Distribution of AChE activity at the borderline of *n. gigantocellularis subnucleus caudalis* and *n. parvocellularis*

stem showed in most cases no or very low AChE activity. Axons passing in this direction exhibiting strong activity were found in a notable number exclusively in the dorsolateral part of the lower brain stem at the level of the n. pontis centralis oralis. No significant staining for AChE was in the corticospinal and corticopontile fibres, lemniscus medialis, fasciculus longitudinalis medialis, fibres of the mesencephalic root of the trigeminal nerve, of the corpus restiforme, the brachium conjunctivum, and in many other longitudinally running fibres. The majority of fibres staining intensely were those passing perpendicularly or nearly perpendicularly to the longitudinal axis of the brain stem. Thus a more distinctive AChE activity was shown in addition to the axons of the visceral and somatic efferent cranial nerve nuclei also by the transverse fibres of the pons (Fig. 6), fibres linking the dorsolateral part of the n. pontis centralis oralis with the n. parabrachialis lateralis (Fig. 18), many raphe fibres and the ventral external arcuate fibres. This activity distribution has been supported by observations made on sections cut in the sagittal plane. Here again it cannot be excluded that in certain fibre systems the enzyme content might have decreased below the histochemically demonstrable threshold during the time elapsed until fixation.

Discussion

In the human material studied by us, AChE activity of most specific nerve cells and the neuropil were weaker than those found in fresh animal brain. Although fixation in formaldehyde reduces the amount of cholinesterases (TAXI 1952, CHESSICK 1954, HARDWICK and PALMER 1961), it improves the correctness of the localization of enzyme activity (COUTEAOUX 1958). Manometric studies in Warburg apparatus have shown that the lower brain stem of a sheep, removed 6 hours after death and fixed for 17 hours in 10% neutral formalin maintained approximately 50% of its cholinesterase content (HARDWICK and PALMER 1961) which was sufficient for being demonstrated histochemically (PALMER and ELLERKER 1961). Hence the comparable weakness of the activity found in the human material was, apart from the fixing procedure, presumably due to the long time passed between death and fixation, which was considerably longer than that in earlier animal materials. In certain areas of the reticular formation (n. gigantocellularis subnucleus caudalis) the staining for AChE of nerve cells was nevertheless not significantly lower than that found in fresh animal brain fixed in formalin for 15—18 hours (PAPP and BOZSIK 1966).

Thus, according to our observations, nerve cells with AChE activity in the human lower brain stem belong to the following types.

1. High AChE activity in the entire perikaryon.

Although the role of internal or intracellular AChE is unknown, this type of activity may be regarded as of a cholinergic type, since it characterizes the nerve cells of the ventral and lateral horns of the spinal cord as well as the cholinergic nerve cells of the peripheral ganglia (KOELLE 1963).

2. Moderate AChE activity occurred in these nerve cells in a distribution similar to the former. These nerve cells are supposed to utilize beside ACh also other substances for neurotransmission (KOELLE 1962).

3. Nerve cells of low AChE activity in a distribution similar to the former. This indicates a presumably non-cholinergic nature of the cells which however, require a small amount of intracytoplasmic AChE for their function.

4. Cholinergic nerve cells are characterized by high AChE contents not only in the perikaryon and axon but also in the axon terminal (HEBB 1961). Therefore a non-cholinergic cell group surrounded by the terminals of cholinergic cells may show a marked AChE activity. However, unlike the true cholinergic cells, the activity of the perikaryon is weak or negative, AChE activity being strong in its surroundings. In fact, the AChE activity of the neuropil has been ascribed to cholinergic afferentation (LEWIS and SHUTE 1959), or recurrent cholinergic innervation (ECCLES, FATT and KOKETSU 1954).

In the rostral part of the n. tractus solitarii exclusively this type of enzyme activity has been observed (Fig. 10). This resembles the AChE activity demonstrated in presynaptic localization in the stellate and superior cervical

ganglion of the cat. The latter activity has been designated external or functional, and is supposed to participate directly in neurotransmission. It disappears after transection of the preganglional fibres (KOELLE 1957, KOELLE and KOELLE 1959, MCISAAC and KOELLE 1959).

This non-cholinergic cholinoceptive type AChE activity found in the cells of the n. tractus solitarii could not be due to a recurrent cholinergic innervation, as no AChE was demonstrable in the perikaryon. Thus, one either has to accept the observation made by MATSAMURA and KOELLE (1961) that, in contrast to the findings by HEBB on choline acetylase (1963), a cholinergic mechanism has also a role in the neurotransmission of the vagal afferent cells, or it must be supposed that these cells receive a cholinergic innervation from some other cell group of the brain stem.

5. AChE-negative nerve cells. It cannot be excluded that in certain of these cells, too, the enzyme content had fallen below the histochemically demonstrable threshold.

Clinical pathological and physiological data indicate that in the area of the reticular formation, functional units larger than the cytoarchitectonic units described by OLSZEWSKI and BAXTER (1954) are present (BRODAL 1957, HORÁNYI 1963). This is suggested also by the distribution of AChE activity in several areas of the lower brain stem. Thus, in the cat and rabbit a variable but significant AChE activity was demonstrable in the medial effector area of the reticular formation of the medulla and pons, while a uniformly weaker activity in their lateral associative receptive areas (PAPP and BOZSIK 1966). A similar distribution was found also in man, up to the level of the caudal part of the n. pontis centralis oralis; from here rostrally, the high activity is gradually shifting to the dorsolateral areas of the pons (Figs 13, 18).

Cell counts in sections cut frontally at the level of the caudal part of the nucleus nervi facialis have shown the predominance of small nerve cells of low AChE activity in both the effector and the receptive areas of the reticular formation. It has therefore been supposed that the presence of such small cells of low AChE activity is related to some basic function in both functional areas of the reticular formation.

Many authors have observed AChE activity of variable intensity in the reticular formation of both mammals (KOELLE 1954, GEREBTZOFF 1959, LEWIS and SHUTE 1959, SNELL 1961, PALMER and ELLERKEL 1961) and man (OKINAKA and YOSHIKAVA 1962, DE GIACOMO 1962). According to our observations, AChE activity in the cell groups of the reticular formation may be of two types. 1. Activity of the individual cells appear to be of the same type by light microscopy (uniformly low — n. parvocellularis and n. cuneiformis; uniformly moderate — column of raphe nuclei). 2. Activity of the individual cells is variable (ventromedial medium and the dorsolateral high activity cell columns). As shown by biochemical and histochemical examinations, in the various functional areas of

the peripheral and central nervous system there is a certain parallelism between the acetylcholine, cholinacetylase and AChE contents and the degree of AChE activity may indicate the extent of the participation of acetylcholine in neurotransmission (MacIntosh 1941, Feldberg and Vogt 1948, Hebb and Silver 1956, Giacobini 1959, Koelle 1955). Physiological and histochemical studies (Burn and Rand 1960, Koelle 1961) have shown that one and the same nerve cell may release various neurotransmitter substances, and the term "cholinergic" may refer actually to the principal but not necessarily the only agent participating in transmission. Microiontophoretic examinations have shown that the lower brain stem reticular formation contains beside purely cholinoceptive and monoaminoceptive cells also cells responsive to both substances (Bradley and Wolstencroft 1962). Thus, in contrast to systems constituted predominantly by cholinergic nerve cells (e.g. somatic and visceral efferent systems) as well as to systems containing very little or no AChE (e.g. pyramidal system, primary somatic afferent neurons, Purkinje cells, n. parvocellularis and cuneiformis in the reticular formation), the variable AChE activity of nerve cells in the ventromedial moderate active, and the dorsolateral highly active columns of the human lower brain stem reticular formation may be regarded as suggestive of the varying degree of the participation of acetylcholine in neurotransmission.

Symbols

Am	nucleus ambiguus
Ci	colliculus inferior
Cn	nucleus medullae oblongatae centralis
Coe	locus coeruleus
Cp	corpus pineale
Csd	nucleus centralis superior subnucleus dorsalis
Csm	nucleus centralis superior subnucleus medialis
Cu	nucleus cuneiformis
Dar	nucleus Darkschewitch
EW	nucleus Edinger-Westphal
Flm	fasciculus longitudinalis medialis
Gc	nucleus gigantocellularis subnucleus caudalis
Grc	griseum centrale
Grp	griseum pontis
Is	nucleus interstitialis Cajal
Lm	lemniscus medialis
Oi	oliva inferior
Os	oliva superior
Pbl	nucleus parabrachialis lateralis
Pc	nucleus parvocellularis
Peces	pedunculus cerebelli superior
Pgd	nucleus paragigantocellularis dorsalis
Pgl	nucleus paragigantocellularis lateralis
Poc	nucleus pontis centralis caudalis
Poo	nucleus pontis centralis oralis
Ppl	nucleus papilliformis
Pret	nucleus praetectalis
Prg	processus griseum pontis

Prp	nucleus praepositus hypoglossi
Raer	nucleus raphe obscurus subnucleus extraraphalis
Rair	nucleus raphe obscurus subnucleus intraraphalis
Rapa	caudal end of nucleus raphe pallidus
Rf	nucleus ruber subnucleus parvocellularis
Scv	nucleus subcoeruleus subnucleus ventralis
Sol	nucleus tractus solitarii
SpV	nucleus spinalis nervi trigemini
Sun	substantia nigra
Tg	nucleus tegmenti pedunculopontinus
Th	thalamus
Tr	nucleus trapesoidalis
Trcf	tractus corticofugalis
Trp	trasverse fibres of pons
IV.	fourth ventricle
Vm	nucleus motoricus nervi trigemini
Vpr	nucleus nervi trigemini sensibilis principalis
VIIIm	nucleus vestibularis medialis
VIIIs	nucleus vestibularis superior
VIIIsp	nucleus vestibularis spinalis
X.	oral part of the dorsal nucleus of the vagal nerve

REFERENCES

1. Bradley, P. B., Wolstencroft, J. H.: (1962) Excitation and Inhibition of Brain Stem Neurons by Noradrenaline and Acetylcholine. Nature (Lond.) **196,** 840—873. — 2. Brodal, A.: (1957) The Reticular Formation of the Brain Stem. Anatomical Aspects and Functional Correlations. Oliver and Boyd, Edinburgh. — 3. Burn, J. H., Rand, M. J.: (1960) Sympathetic Postganglionic Cholinergic Fibres. Brit. J. Pharmacol. **15,** 56—66. — 4. Chessick, R. D.: (1954) Cit. Couteaux, R.: (1958) Morphological and Cytochemical Observations on the Post-synaptic Membrane at Motor End-plates and Ganglionic Synapses. Exp. Cell Res. (Suppl.) **5,** 294—322. — 5. Couteaux, R.: (1958) Morphological and Cytochemical Observation on the Post-synaptic Membrane at Motor End-plate and Ganglionic Synapses. Exp. Cell Res. (Suppl.) **5,** 294—322. — 6. Eccles, J. C., Fatt, P., Koketsu, K.: (1954) Cholinergic and Inhibitory Synapses in a Pathway from Motor-axon Collaterals to Motoneurones. J. Physiol. (Lond.) **126,** 524—562.—7. Feldberg, W., Vogt, M.: (1948) Acetylcholine Synthesis in Different Regions of the Central Nervous System. J. Physiol. (Lond.) **107,** 372—381. — 8. Foldes, F. F., E. K. Zsigmond, V. M. Foldes, and E. G. Erdős: (1962) The Distribution of Acetylcholinesterase and Butirylcholinesterase in the Human Brain. J. Neurochem. **9,** 559—572. — 9. Fukuda, T., Koelle, G. B.: (1959) The Cytological Localization of Intracellular Neuronal Acetylcholinesterase. J. biophys. biochem. Cytol. **5,** 433—440. — 10. Gerebtzoff, M. A.: (1959) Cholinesterases. A Histochemical Contribution to the Solution of Some Functional Problems. Pergamon Press, London. Chapter IV. Pp. 74—103. — 11. Giacobini, E.: (1959) The Distribution and Localization of Cholinesterases in Nerve Cells. Acta physiol. scand. **45,** (Suppl. 156) 1—45. — 12. de Giacomo, P.: (1962) Distribution of Cholinesterase Activity in the Human Central Nervous System. IV. International Congress of Neuropathology. München 1961. Ed. H. Jacob, Vol. I. Histochemistry and Biochemistry. Thieme, Stuttgart. Pp. 198—205. — 13. Hamberger, B., Norberg, K. A., Sjöqvist, F.: (1965) Correlated Studies of Monoamines and Acetylcholinesterase in Sympathetic Ganglia, Illustrating the Distribution of Adrenergic and Cholinergic Neurons. Second International Pharmacological Meeting, 1963. Vol. 3. Pharmacology of Cholinergic and Adrenergic Transmission. Ed. G. B. Koelle, W. W. Douglas and A. Carlsson. Pergamon Press, Czechoslovak Medical Press, Praha. pp. 41—53. — 14. Hardwick, D. C., Palmer, A. C.: (1961) Effect of Formalin Fixation on Cholinesterase Activity in Sheep Brain. Quart. J. exp. Physiol. **46,** 350—352. — 15. Hebb, C. O., Silver, A.: (1956) Cit. Hebb, C., 1963. Formation, Storage and Liberation of Acetylcholine. In Handbuch der Experimentellen Pharmakologie, XV. Band. Cholinesterases and Anticholinesterase Agents. Sub-editor G. B. Koelle. Springer, Berlin. Pp. 55—58. — 16. Hebb, C. O.: (1961) Cholinergic Neurons in Vertebrates. Nature (Lond.) **192,** 527—529. — 17. Horányi, B.: (1963) Az agytörzsi integráló rendszer. Orv. Hetil. **104,** 2401—2411. — 18. Koelle, G. B.: (1954) The Histochemical Localization of Cholinesterases in the Central

Nervous System of the Rat. J. comp. Neurol. **100,** 211—228. — 19. KOELLE, G. B.: (1955) The Histochemical Identification of Acetylcholinesterase in Cholinergic, Adrenergic and Sensory Neurons. J. Pharmacol. exp. Ther. **114,** 167—184. — 20. KOELLE, G. B.: (1957) Histochemical Demonstration of Reversible Anticholinesterase Action at Selective Cellular Sites in vivo. J. Pharmacol. exp. Ther. **120,** 488—503. — 21. KOELLE, G. B. :(1961) A Proposed Dual Neurohumoral Role of Acetylcholine; its Functions at the Pre- and Postsynaptic Sites. Nature (Lond.) **190,** 208—211. — 22. KOELLE, G. B.: (1962) A New General Concept of the Neurohumoral Functions of Acetylcholine and Acetylcholinesterase. J. Pharm. Pharmacol. **14,** 65—90. — 23. KOELLE, G. B., FRIEDENWALD, J. S.: (1949) A Histochemical Method for Localizing for Cholinesterase Activity. Proc. Soc. exp. Biol. (N. Y.) **70,** 617—622. — 24. KOELLE, G. B., STEINER, E. C.: (1956) The Cerebral Distribution of a Tertiary and a Quaternary Anticholinesterase Agent Following Intravenous and Intraventricular Injection. J. Pharmacol. exp. Ther. **118,** 420—434. — 25. KOELLE, W. A., KOELLE, G. B.: (1959) The Localization of External or Functional Acetylcholinesterase at the Synapses of Autonomic Ganglia. J. Pharmacol. exp. Ther. **126,** 1—8. — 26. LANDS, A. M., KARCZMAR, A. G., HOWARD, J. W., ARNOLD, A.: (1955) An Evaluation of the Pharmacologic Actions of Some Bis-quaternary Salts of Basically Substituted Oxamides (WIN 8077 and Analogs). J. Pharmacol. exp. Ther. **115,** 185—198. — 27. LEWIS, P. R., SHUTE, C. D.: (1959) Selective Staining of Visceral Efferents in the Rat Brain Stem by Modified Koelle-technique. Nature (Lond.) **183,** 1743—1744. — 28. MACINTOSH, F.: (1941) The Distribution of Acetylcholine in the Peripheral and Central Nervous System. J. Physiol. (Lond.) **99,** 436—442. — 29. MCISAAC, R. J., KOELLE, G. B.: (1959) Comparison of the Effects of Inhibition of External, Internal and Total Acetylcholinesterase upon Ganglionic Transmission. J. Pharmacol. exp. Ther. **126,** 9—20. — 30. MATSUMURA, M., KOELLE, G. B.: (1961) The Nature of Synaptic Transmission in the Superior Cervical Ganglion Following Reinnervation by the Afferent Vagus. J. Pharmacol. exp. Ther. **134,** 28—46. — 31. OKINAKA, S., YOSHIKAVA, M.: (1962) Histochemical Studies on the Autonomic Nervous System. IV. International Congress of Neuropathology. 4—8 September 1961. München. Ed. H. Jacob. Vol. I. Histochemistry and Biochemistry. Thieme, Stuttgart. Pp. 180—187.—32 a) OLSZEWSKI, J., BAXTER, D.: (1954) Cytoarchitecture of the Human Brain Stem. S. Karger, Basel—New York. b) OLSZEWSKI, J.: (1956) Brain Mechanisms and Consciousness. A Symposium, Ed. E. D. Adrian, F. Bremer and H. H. Jasper, pp 54—76. Blackwell, Sci. Publ. Oxford, 1956. —33. ORD, M. G., THOMPSON, R. H. S.: (1952) Pseudo-cholinesterase Activity in the Central Nervous System. Biochem. J. **51,** 245—251. — 34. PALMER, A. C., ELLERKER, A. R.: (1961) Histochemical Localization of Cholinesterases in the Brain Stem of Sheep. Quart. J. exp. Physiol. **46,** 344—350. — 35. PAPP, M., BOZSIK G.: (1966) Comparison of the Cholinesterase Activity in the Reticular Formation of the Lower Brain Stem of Cat and Rabbit. J. Neurochem. **13,** 697—703. — 36. SNELL, R. S.: (1961) The Histochemical Localization of Cholinesterase in the Central Nervous System. (A Preliminary Report.) Histochemistry of Cholinesterase, Symposium, Basel, 1960. Bibl. anat. (Basel) **2,** 50—58. Karger, Basel—New York. — 37. TAXI, J.: (1952) Cit.: COUTEAUX, R.: (1958) Morphological Cytochemical Observations on the Post-Synaptic Membrane at Motor End-plate and Ganglionic Synapses. Exp. Cell Res. (Suppl.) **5,** 294—322.

HISTOCHEMISCHE UNTERSUCHUNGEN IN BEZUG AUF DIE AZETYLCHOLINESTERASE-AKTIVITÄT DES MENSCHLICHEN UNTEREN STAMMHIRNS MIT BESONDERER RÜCKSICHT AUF DIE FORMATIO RETICULARIS

M. PAPP

Die Azetylcholinesterase-Aktivität des unteren Stammhirns von 10 plötzlich verstorbenen, vorher gesunden Personen wurde mit besonderer Rücksicht auf die Formatio reticularis untersucht. Auf Grund der intrazellulären, an der Zellmembran bzw. in der Nähe der Zelle beobachtbaren Azetylcholinesterase-Aktivität wurden die Nervenzellen in 5 Gruppen eingeteilt:

1. Die Azetylcholinesterase-Aktivität der zur ersten Gruppe gehörenden Nervenzellen stimmte mit der Azetylcholinesterase-Aktivität der wohlbekannten cholinergischen Nervenzellen überein (motorische Zellen in dem Vorderhorn, präganglionäre Neuronen, Nervenzellen einzelner Ganglien): Diese Zellen enthalten in einer, der *Nissl*schen Struktur ähnlichen oder körnigen Verteilung eine bedeutende Menge zytoplasmatischer Azetylcholinesterase.

2. In anderen Zellen ist in ähnlicher Verteilung eine mittelstarke Azetylcholinesterase-Aktivität ersichtlich.

3. Bei einem großen Teil der Nervenzellen ist die Azetylcholinesterase-Aktivität im gesamten Perikaryon und in den Dendriten außerordentlich schwach.

4. Eine weitere Gruppe der Nervenzellen weist eine Azetylcholinesterase-Aktivität cholinozeptiven Typs auf. Diese Zellen werden in den histochemischen Präparaten durch externale Azetylcholinesterase-Aktivität charakterisiert.

5. Azetylcholinesterase-negative Nervenzellen.

In der Formatio reticularis des menschlichen unteren Stammhirns ist die Azetylcholinesterase-Aktivität der Nervenzellen in den einigen Zellgruppen (ventromediale Zellgruppen mit mittelstarker und dorsolaterale mit sehr starker Azetylcholinesterase-Aktivität) der einzelnen Zellen derartig verschieden, daß sich sämtliche Typen der Azetylcholinesterase-Aktivität erkennen lassen.

Sämtliche Zellgruppen der Formatio reticularis des menschlichen unteren Stammhirns (auch in den Gruppen mit starker Enzymaktivität) bestehen überwiegend aus kleinen Nervenzellen mit außerordentlich schwacher Azetylcholinesterase-Aktivität.

ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ АЦЕТИЛХОЛИНЭСТЕРАЗНОЙ АКТИВНОСТИ КАУДАЛЬНОГО ОТДЕЛА СТВОЛА МОЗГА ЧЕЛОВЕКА С ОСОБЫМ УЧЕТОМ СЕТЕВИДНОЙ ФОРМАЦИИ

М. ПАПП

У 10 здоровых, внезапно умерших лиц автором была исследована ацетилхолинэстеразная активность каудального отдела ствола мозга с особым учетом сетевидной формации. Нервные клетки он распределил на основе ацетилхолинэстеразной активности, наблюдаемой в перикарион и в окрестности его на 5 групп:

1. Ацетилхолинэстеразная активность части нервных клеток была идентичной с активностью хорошо известных и изученных холинергических нервных клеток (двигательные клетки переднего рога, преганглионарные нейроны). *Ацетилхолинэстеразная активность холинергического типа.*

2. В других нервных клетках отмечается *ацетилхолинэстеразная активность средней интенсивности* подобного распределения.

3. В большистве нервных клеток в околоядерной области и в дендритах видна *очень слабая ацетилхолинэстеразная активность.*

4. *Нервные клетки с ацетилхолинэстеразной активностью холиноцептивного типа.* Для этих клеток характерна „external" ацетилхолинэстеразная активность, наблюдаемая на гистохимических препаратах.

5. Ацетилхолинэстеразно-отрицательные нервные клетки.

В етевидной формации каудального отдела мозгового ствола человека ацетилхолинэстеразная активность нервных клетов в некоторых клеточных групп в общем и целом одинакова (равномерно среднесильная в ядрах raphe, равномерно очень слабая в n. parvocelluraris, и n. cuneiformis). В других клеточных группах (вентромедиальные клеточные группы с активностью средней силы и дорсолатеральные клеточные группы с очень сильной ацетилхолинэстеразной активностью) ацетилхолинэстеразная активность отдельных клеток настолько меняется, что обнаруживаются все типы ацетилхолинэстеразной активности.

В каудальном отделе мозгового ствола человека во всех клеточных группах сетевидиой формации (даже в группах с сильной активностью) в решающем количестве присутствуют нервные клетки очень слабой ацетилхолинэстеразной активностью.

Dr. Mátyás Papp; Budapest VIII. Balassa u. 6, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(4), pp. 391—401, 1968*

Institute of Anatomy (Director: Prof. J. SZENTÁGOTHAI), University Medical School, Budapest

VOLUME RECEPTORS IN THE DIENCEPHALON

M. PALKOVITS, L. ZÁBORSZKY and P. MAGYAR

(Received April 19, 1967)

Karyometric measurements have revealed the presence of volume receptors in the diencephalon of rats. In isosmotic hypovolaemia 30 min. after bleeding, a significant increase of nuclear volume was found in the subcommissural and the subfornical organs, and a less marked one in the paraventricular nucleus. In isosmotic hypervolaemia 30 min. after saline administration, a significant decrease of nuclear volume was registered in the subcommissural and the subfornical organs, and a less marked one in the supraoptic and paraventricular nucleus. These phenomena were direct effects of changes in volume and not due to aldosterone mobilization, since no significant change in nuclear volume occurred in these areas 30 min. after the administration of aldosterone. The pineal body responded to aldosterone with a decrease in nuclear volume but was not affected by changes in volume.

The subcommissural and the subfornical organs may, therefore, be regarded as the volume receptors of the central nervous system. The magnocellular nuclei of the hypothalamus — known as osmoreceptors — either react, to a certain extent, also to changes in volume or, else, the observed changes should be regarded as being caused by the upset of osmotic equilibrium following changes in volume.

Osmotic equilibrium of the organism is maintained by the ADH-producing regions of the hypothalamus, the neurosecretory magnocellular nuclei, the supraoptic and the paraventricular nucleus. Hyperosmosis increases and hyposmosis decreases the secretion of ADH in these nuclei [56]. It is, however, not enough to ensure osmotic equilibrium in order to maintain undisturbed salt and water balance: the volume of blood in the extracellular space — although closely correlated with osmosis — may be changed by other factors also. Blood volume is regulated by aldosterone. The secretion of this hormone is promoted by hypovolaemia and diminished by hypervolaemia. The efferent path of volume regulation, the excretion of sodium, depends on changes in aldosterone secretion. It is, on the other hand, doubtful whether there are receptors sensitive to changes in volume. Osmoreceptors are known to respond to numerous stimuli (pain, stress, drugs), but changes in the blood's osmotic pressure constitute nevertheless the afferent path of the reflex which regulates osmosis under physiological conditions. It seems therefore reasonable to assume that changes in blood volume (hyper- or hypovolaemia) should be regarded as constituting the afferent path of volume regulation, and that the corresponding areas of the nervous system are sensitive to such changes. These areas at the same time are centres of aldosterone regulation and have not yet been located precisely.

Material and methods

Twenty-four white rats of the Wistar strain with body weights from 150 to 180 g were divided into three groups of 6 test animals and 2 controls each. The members of the first group received a 10 μg subcutaneous dose of aldosterone; 2 ml of blood was drawn from the femoral artery of the animals of the second group, while 8 ml of physiol. saline was administered through a gastric tube to the members of the third group. The animals were decapitated 30 min. after the intervention, their brain was fixed in Bouin's solution for 48 hrs and embedded in paraffin. Serial frontal section of 10 μ were stained with haematoxylin-eosin. The nucleus supraopticus, nucleus paraventricularis, nucleus habenulae medialis, the ependyma of the third ventricle, the subcommissural and subfornical organs, further the pineal body were subjected to karyometric examination. The nuclei of the cells (200 to 300 nuclei per animal and area) were measured by a modified version [42] of JAKOBJ's method [29]; their volume was determined on the basis of FISCHER and INKE's nomogram [18].

Results

In general, isosmotic hypovolaemia had the effect to increase and isosmotic hypervolaemia to decrease nuclear volume in the examined areas (Fig. 1,

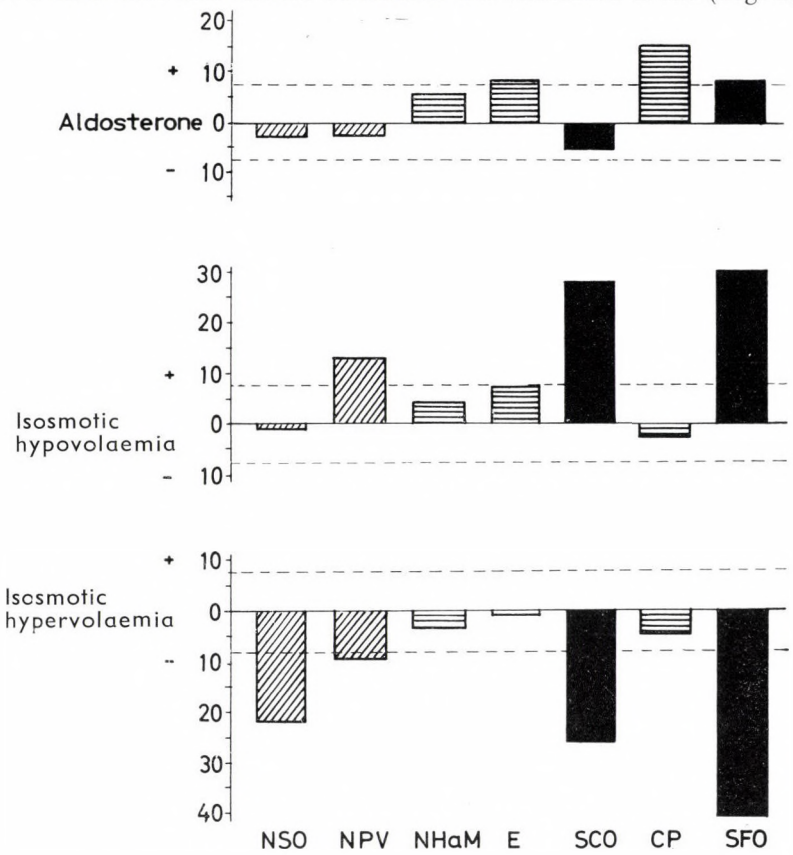


Fig. 1. Average values of changes in nuclear volume after different interventions. Deviations are expressed in per cent of control values. Dotted line indicates standard deviation of the controls

Table I). These effects were particularly marked in the subcommissural organ (SCO) and the subfornical organ (SFO). Increase caused by isosmotic hypovolaemia amounted to 28.2% in the SCO and to 30.2% in the SFO, while the decrease in nuclear volume due to isosmotic hypervolaemia amounted to 26.1% in the SCO and to 41.1% in the SFO. Decrease due to isosmotic hypervolaemia was 22.1% in the supraoptic nucleus, while hypovolaemia caused no change in this organ. The paraventricular nucleus underwent a 13.8% increase in hypovolaemia, and a 9.5% decrease in hypervolaemia. Nuclear volume remained unchanged in the medial habenular nucleus, in the ependyma and in the pineal body.

It was only in the pineal body that aldosterone induced a significant (15.7%) change in nuclear volume. A slight increase of nuclear volume was registered in the ependyma and the SFO, while changes were negligible in the other examined organs.

Discussion

Experimental observations [6, 7, 12], autoradiographic [8, 40, 50, 51, 52, 53] and interference microscopic [33] examinations have shown that the nuclei of cells become larger when protein synthesis is increased and smaller when the production of protein is reduced; phenomena that might be utilized for the demonstration of increased or decreased cellular activity.

Care was taken to examine the volume receptors under experimental conditions in which a change of volume was not accompanied by osmotic changes. As blood loss induces isosmotic hypovolaemia [5, 9, 23, 39, 54, 60] and the administration of saline induces isosmotic hypervolaemia [59], thus, an osmotic change was inevitable to occur. This is why the examinations were performed 30 minutes after the intervention.

A problem arising in this connection was that the change of volume affects the synthesis of aldosterone so that the observed phenomena may not have been direct consequences of the change in blood volume but responses to fluctuations in the aldosterone level. It was to decide this problem that the effect of aldosterone was studied in one of the test groups. Of course, the possibility still existed that the administration of aldosterone would modify the volume of fluid so that we would not observe the direct effect of aldosterone but only the consequences of the effect, (so to say, the secondary effect) of the drug. It is, however, known that it takes some 60 to 120 min. for aldosterone to cause sodium retention [2, 11, 36, 57]. The cause of this delay is obscure, but it warrants in any case that changes found 30 minutes after the administration of aldosterone cannot yet represent the secondary effect of the drug but that it must be the affected area itself which is directly sensitive to aldosterone.

Table I

Average values of nuclear volume per μ^3 (300 cell nuclei) group

	Control			Aldosterone		
	μ^3	average	%	μ^3	average	%
Supraoptic nucleus	249.00 287.05 238.80 286.95 248.15 255.70	260.94	—	249.50 250.80 230.90 278.42 257.90 260.75	254.71	−2.4%
Paraventricular nucleus	342.90 352.60 345.50 317.00 313.50 348.87	336.73	—	362.10 308.55 302.90 300.46 341.54 358.80	329.06	−2.3%
Nucl. habenulae med.	222.45 204.97 179.55 184.73 187.72 211.13	198.42	—	164.33 177.00 223.35 215.95 235.85 235.40	208.65	+5.2%
Ependyma	104.50 104.65 109.47 92.55 91.73 107.20	101.68	—	105.65 104.82 111.15 110.80 116.53 113.63	110.43	+8.6%
Subcommissural organ	46.61 51.07 54.83 46.47 53.51 53.23	51.12	—	43.47 51.70 44.26 50.45 50.60 50.61	48.51	−5.1%
Pineal body	94.27 78.55 83.62 98.85 73.80 71.85	83.49	—	112.55 79.64 115.21 82.38 90.27 99.59	96.61	**+15.7%**
Subfornical organ	151.32 147.23 137.40 142.73 143.60 146.75	144.84	—	163.30 145.05 162.75 167.18 142.60 157.67	156.42	+8.0%

Percentages printed in bold type are statistically

averages, and their percentual deviation from control values

Isosmotic hypovolaemia			Isosmotic hypervolaemia		
μ^3	average	%	μ^3	average	%
253.21			205.25		
271.41			179.96		
264.57	256.03	−1.9%	224.16	203.12	**−22.1%**
236.27					
242.68					
267.99					
359.03			274.87		
386.43			300.97		
419.22			330.52		
362.52	383.29	+13.8%	311.69	304.48	−9.5%
386.95					
385.62					
212.89			210.89		
238.14			159.39		
172.34	207.21	+4.4%	203.20	191.02	−3.7%
202.04			190.62		
202.15					
215.72					
105.52			99.68		
111.12			108.32		
120.17	108.96	+7.2%	103.23	100.68	−0.9%
99.34			91.97		
105.00					
112.63					
63.75			37.93		
67.00			42.20		
70.44	65.55	**+28.2%**	35.59	37.79	**−26.1%**
62.96			35.44		
67.11					
62.02					
82.66			76.43		
87.11			80.52		
77.49	81.46	−2.5%	69.85	79.31	−5.0%
81.14			81.16		
80.62			83.30		
79.72			85.12		
165.23			96.80		
223.90			107.49		
175.20	188.45	**+30.2%**	65.29	102.67	**−41.1%**
190.75			103.88		
207.20			128.80		
168.45			113.76		

significant at the 5% level of confidence.

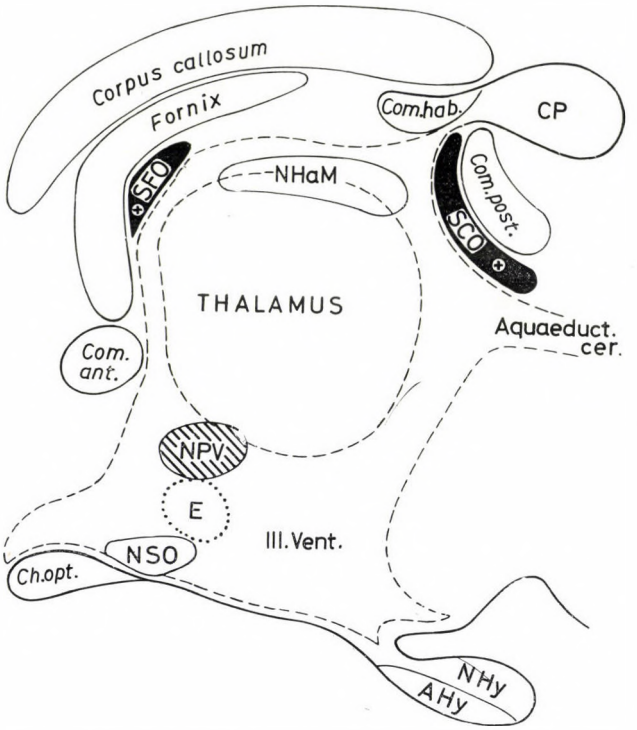


Fig. 2. Changes of nuclear volume in the diencephalon 30 min. after bleeding (isosmotic hypovolaemia). ■ = deviation exceeding 15%; ▨ = deviations of 7.5 to 15%

We examined those areas which earlier investigations had shown to be involved in volume regulation. The experiments revealed the SCO and the SFO to be the areas most sensitive to changes in blood volume (Figs 2, 3). GILBERT [22] was the first to point to the SCO in this connection, and our investigations proved this organ to be a factor in the regulation of aldosterone synthesis. Its extract promotes the activity of the absorption of sodium and water more than twofold [19]. Production of aldosterone abates after the electrocoagulation of the SCO [10, 49, 56], and a diet poor in sodium and potassium affects the organ [20]. It was only two hours after the administration of aldosterone that a change was observed by us [44]. The SCO reacted at that time (i.e. after two hours) presumably no longer to the drug itself but to the change in the fluid milieu caused by the aldosterone. Examinations were performed 30 min. after the administration in the present experiments: while the organ revealed no change, a significant change in volume was registered.

The function of the SFO is rather obscure. LEGAIT's investigations [35] point to the possibility that it is involved in the maintenance of the organism's salt and water balance, but this theory has not been proved. What we observed

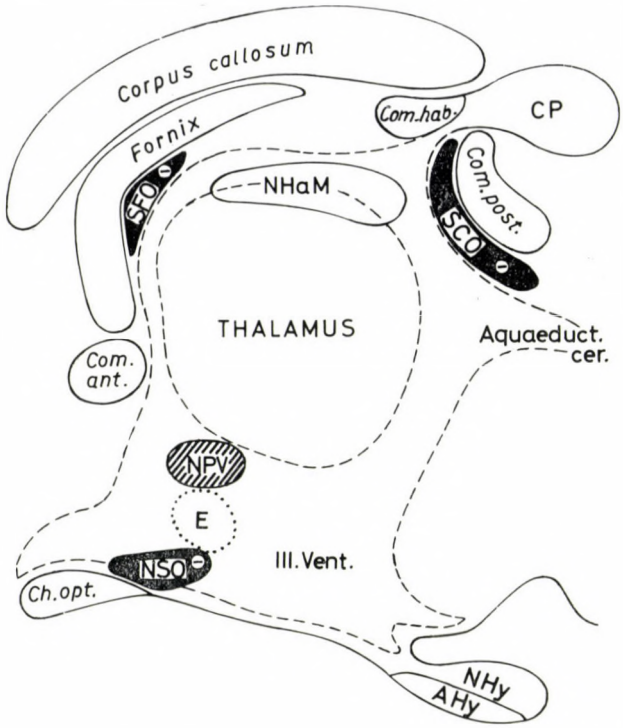


Fig. 3. Changes of nuclear volume in the diencephalon 30 min. after the administration of physiological saline (isosmotic hypervolaemia). Signs as in Fig. 2

in earlier experiments [45] was a chronologically increasing adequate change in nuclear volume 10, 20, and 30 min. after bleeding or the uptake of fluid. In the present experiments, a pronounced change in nuclear volume was registered in both hypervolaemia and hypovolaemia, while no direct aldosterone action could be observed (Fig. 4).

The supraoptic and the paraventricular nuclei are known as areas of hypothalamic magnocellular neurosecretion and as centres of osmotic regulation [3, 4, 24, 25, 26, 27, 28, 41]. Osmotic changes affect the volume of nuclei and nucleoli in these areas [1, 12, 28, 30, 31, 37, 41]. It was supposed — though not proved — that the supraoptic and paraventricular nuclei are the centres which regulate both osmosis and volume. Our observations lead to the conclusion that these areas are not (or at least nor primarily) volume receptors, for changes in nuclear volume following interventions were less pronounced than those observed in the SCO and the SFO. Certain changes were registered also in these areas: hypervolaemia caused a 22% reduction of nuclear volume in the areas of the supraoptic, and one of 9.5% in that of the paraventricular nucleus. Hypovolaemia induced a 13.5% increase in nuclear volume in the latter area.

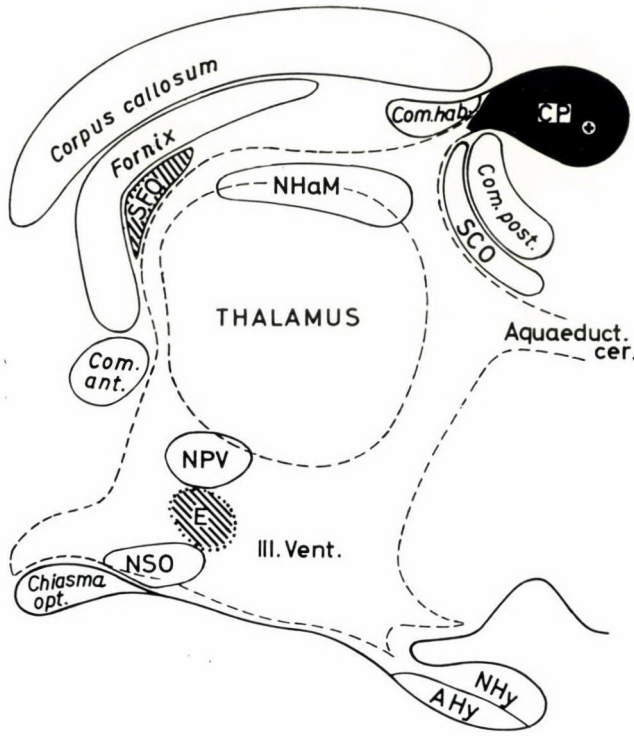


Fig. 4. Changes of nuclear volume in the diencephalon 30 min. after the administration of aldosterone

These areas may be sensitive to changes in osmosis and volume alike, but it is more probable that the observed changes in nuclear volume were due to the upset of osmotic equilibrium.

It has been suggested that the pineal body may play a part in the secretion of aldosterone. This organ produces, according to FARRELL et al. [3 to 17], substances which, respectively, promote and inhibit the synthesis of aldosterone. GIACOMELLI [21] found that the extract of the organ increased the activity of the zona glomerulosa; KOVÁCS et al. [30], using pineal-body extract, succeeded in increasing the amount of aldosterone produced by rat adrenals in vitro. In our earlier experiments we failed to produce any change in the zona glomerulosa by means of such extract [47]; its capacity to promote the absorption of sodium and water was decidedly inferior to that of SCO-extract [19]. A diet poor in sodium and potassium failed to change the volume of nuclei in the cells of the pineal body, nor did adrenalectomy lead to any change in this respect [46]. The present results justify the statement that the pineal body is not a volume receptor. It was, on the other hand, only in this organ that a change was observed 30 min. after the administration of aldosterone. We are not able to

interpret this phenomenon, for changes occurring in the late phases of aldosterone effect have not been elucidated.

The function of the medial habenular nucleus is unknown; all we know is that neither changes in thyroid activity nor adrenalectomy cause a marked change in its nuclear volume [38, 55]. The medial habenular nucleus remained unchanged in our material in cases of both hypovolaemia and hypervolaemia, as also after the administration of aldosterone.

Our reason to examine the ependyma of the third ventricle was that both the SCO and the SFO — areas where changes were most pronounced — are ependymal organs. Neither Kovács et al. [31, 32] nor we did find that changes in osmotic pressure caused any change in its nuclear volume.

REFERENCES

1. Bachrach, D., Szabó, E. B., Baradnay, G., Korpássy, B.: (1961) Histophysiological Changes of the Adrenal Cortex of the Rat in Dehydration and Rehydration. J. Endocr. **23,** 1. — 2. Barger, A. C., Berlin, R. D., Tulenko, J. F.: (1958) Infusion of Aldosterone, 9-alpha-fluorohydrocortisone and Antidiuretic Hormone into the Renal Artery of Normal and Adrenalectomized Unanaesthetized Dogs. Effect on Electrolyte and Water Excretion. Endocrinology **62,** 804. — 3. Bargmann, W.: (1949) Über die neurosekretorische Verknüpfung von Hypothalamus und Neurohypophyse. Z. Zellforsch. **34,** 610. — 4. Bargmann, W., Hild, W., Ortmann, R., Schiebler, T. H.: (1950) Morphologische und experimentelle Untersuchungen über das hypothalamisch-hypophysäre System. Acta neuroveg. (Wien) **1,** 233. — 5. Bartter, F. C., Vogt, M.: (1959) In: P. Vecsei, E. Gláz: Aldosteron. Medicina, Budapest 1964. — 6. Benninghoff, A.: (1949) Funktionelle Kernschwellung und Kernschrumpfung. Anat. Nachr. **1,** 50. — 7. Benninghoff, A.: (1953) Die funktionelle Kernödem als Indicator der Zelltätigkeit. Mon. Zool. Ital. **61** (suppl.) 84. — 8. Citoler, P., Citoler, K., Hempel, K., Schultze, B., Maurer, W.: (1966) Autoradiographische Untersuchungen mit zwölf H^3 und fünf C^{14}-markierten Aminosäuren zur Grösse des nucleären und cytoplasmatischen Eiweisstoffwechsels bei verschiedenen Zellarten von Maus und Ratte. Z. Zellforsch. **70,** 419. — 9. Davis, J. O.: (1961) Mechanism Regulating the Secretion and Metabolism of Aldosterone in Experimental Secondary Hyperaldosteronism. Rec. Progr. Hormone Res. **17,** 293. — 10. De Wied, D.: (1963) Factors Controlling the Regulation of Aldosterone Secretion. Symposium on Aldosterone, Prague. — 11. Dingmann, J. F., Finkerstaedt, J. T., Laidlaw, J. C., Renold, A. E., Jenkins, D., Merrill, J. P., Thorn, G. W.: (1958) Influence of Intravenously Administered Adrenal Steroids on Sodium and Water Excretion in Normal and Addisonian Subjects. Metabolism **7,** 608. — 12. Eichner, D.: (1952) Über funktionelle Kernschwellung in den Nuclei supraoptici und paraventricularis des Hundes bei experimentellen Durstzuständen. Z. Zellforsch. **37,** 406. — 13. Farrell, G.: (1959a) Steroidogenic Properties of Extracts of Beef Diencephalon. Endocrinology **65,** 29. — 14. Farrell, G.: (1959b) Glomerulotropic Activity of an Acetone Extract of Pineal Tissue. Endocrinology **65,** 239. — 15. Farrell, G.: (1960) Adrenoglomerulotropin. Circulation **21,** 1009. — 16. Farrell, G., McIsaac, W. W.: (1961) Adrenoglomerulotropin. Arch. Biochem. **94,** 543. — 17. Farrell, G., Taylor, A. N.: (1962) Neuroendocrine Aspects of Volume Regulation. Ann. Rev. Physiol. **24,** 471. — 18. Fischer, J., Inke, G.: (1956) Nomogramme zur Berechnung des Kernvolumens. Acta morph. Acad. Sci. hung. **7,** 141. — 19. Földvári, I. P., Czeizel, E., Simon, G., Palkovits, M., Kertai, P.: (1962) The Influence of the Subcommissural Organ on the Resorption of Water and Electrolytes from the Small Intestine. Acta physiol. Acad. Sci. hung. **22,** 43. — 20. Földvári, I. P., Palkovits, M.: (1964) Effect of Sodium and Potassium Restriction on the Functional Morphology of the Subcommissural Organ. Nature (Lond.) **202,** 905. — 21. Giacomelli, F.: (1962) Über Veränderungen der Nebennierenrinde nach Pinealektomie. Endokrinologie **42,** 144. — 22. Gilbert, G. J.: (1956) The Subcommissural Organ. Anat. Rec. **126,** 253. — 23. Gláz, E., Sugár, K.: (1963) Gesichtspunkte bei der Behandlung kardialer Oedeme und sonstiger sekundärer Hyperaldosteronismen mit Progesteron. Acta Secundi Conventus Medicinae Internae Hungaricae. Akadémiai Kiadó, Budapest, P. 277. — 24. Hild, W.: (1951) Experimentell-morphologische Untersuchungen über das Verhalten der neurosekretorischen Bahn

nach Hypophysenstieldurchtrennungen, Eingriffen in den Wasserhaushalt und Belastung der Osmoreceptoren. Virchows Arch. path. Anat. **319,** 526. — 25. HILD, W., ZETLER, G.: (1951a) Über das Vorkommen der drei sog. »Hypophysenhinterlappenhormone« Adiuretin, Vasopressin und Oxytocin im Zwischenhirn als wahrscheinlicher Ausdruck einer neurosekretorischen Leistung der Ganglienzellen der Nuclei supraopticus und paraventricularis. Experientia (Basel) **7,** 189. — 26. HILD, W., ZETLER, G.: (1951b) Über das Vorkommen der Hypophysenhinterlappenhormone im Zwischenhirn. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **213,** 139. — 27. HILD, W., ZETLER, G.: (1953) Über die Funktion des Neurosekrets im Zwischenhirn-Neurohypophysensystem als Trägersubstanz für Vasopressin, Adiuretin und Oxytocin. Z. exp. Med. **120,** 236. — 28. HILLARP, N. A.: (1949) Studies on the Localization of Hypothalamic Centres Controlling the Gonadotrophic Function of the Hypophysis. Acta endocr. (Kbh.) **2,** 11. — 29. JAKOBJ, W.: (1925) Über das rhythmische Wachstum der Zellen durch Verdoppelung ihres Volumens. Arch. Entwickl. Mech. Org. **106,** 124. — 30. KOVÁCS, K., BACHRACH, D., JAKOBOVITS, A., HORVÁTH, E., KORPÁSSY, B.: (1954a) Über die Beziehungen der Systeme vorderer Hypothalamus-Neurohypophyse und Adenohypophyse-Nebennierenrinde. Endokrinologie **31,** 149. — 31. KOVÁCS, K., BACHRACH, D., JAKOBOVITS, A., HORVÁTH, É., KORPÁSSY, B.: (1954b) Hypothalamo-hypophyseal Relations of Experimentally Induced Changes in Salt and Water Metabolism. Acta morph. Acad. Sci. hung. **4,** 417. — 32. KOVÁCS, K., DÁVID, M., WEISZ, P.: (1960) Aldosteronotrophic and Corticosteronotrophic Substances in Normal Human Brain. Med. exp. **3,** 113. — 33. LAMPERT, F., HOFMANN, D., SANDRITTER, W.: (1966) Interferenzmikroskopische Trockengewichtsbestimmungen an Kern, Cytoplasma und Nucleolus von Hela- und Amnionzellen der Gewebekultur. Histochemie **6,** 370. — 34. LEGAIT, H.: (1959) Contribution à l'étude morphologique et expérimentale du système hypothalamo-neurohypophysaire de la poule rhode-island. Thèse Nancy. — 35. LEGAIT, H.: (1962) Étude caryométrique de la pars intermedia de l'hypophyse, de l'épiphyse et de l'organe subfornical au cours d'états d'hyperactivité de l'hypothalamus neurosécrétoire chez quelques mammifères. C. R. Soc. Biol. (Paris) **156,** 1662. — 36. LENYS, R.: (1965) Contribution à l'étude de la structure et du rôle de l'organe sous-commissural. Thèse Nancy. — 37. MACHER, E.: (1952) Zellkernschwellungen der Nuclei supraopticus und paraventricularis bei Dursttieren. Anat. Anz. **99,** 95. — 38. MESS, B., KUTAS, J.: (1959) Zell- und Zellkernveränderungen in den Nuclei habenulae und einigen Hypothalamus-Kernen bei experimentellen Eingriffen an der Schilddrüse. Z. Zellforsch. **50,** 307. — 39. MÜLLER, A. F.: (1962) Regulation der Aldosteronsekretion. Verh. dtsch. Ges. Inn. Med. **68,** 599. — 40. OEHLERT, W., SCHULTZE, B.: (1960) Die Kerngrösse als Ausdruck der synthetischen Aktivität des Kerns. Beitr. path. Anat. **123,** 101. — 41. ORTMANN, R. (1951) Über experimentelle Veränderungen der Morphologie des Hypophysenzwischenhirnsystems und die Beziehung der sog. »Gomorisubstanz« zum Adiuretin. Z. Zellforsch. **36,** 92. — 42. PALKOVITS, M.: (1961) Angaben und Hilfsmittel zur Auswertung von Kernvariationsuntersuchungen. Z. mikr.-anat. Forsch. **67,** 343. — 43. PALKOVITS, M.: (1965) Participation of the Epithalamo-epiphyseal System in the Regulation of Water and Electrolyte Metabolism. Progr. Brain Res. **10,** 627—634. — 44. PALKOVITS, M.: (1966a) Morphology and Function of the Subcommissural Organ. Akadémiai Kiadó, Budapest. — 45. PALKOVITS, M.: (1966b) The Role of the Subfornical Organ in the Salt and Water Balance. Naturwissenschaften **53,** 336. — 46. PALKOVITS, M.: (1968) To be published. — 47. PALKOVITS, M., FÖLDVÁRI, I. P.: (1962) Effect of the Subcommissural Organ and the Pineal Body on the Adrenal Cortex. Endocrinology **72,** 28. — 48. PALKOVITS, M., LUKÁCS, G.: (1963) Karyometric Examination of the System Subcommissural Organ-Adrenal Cortex in the Rat. Acta biol. Acad. Sci. hung. **13,** 361. — 49. PALKOVITS, M., MONOS, E., FACHET, J.: (1965) The effect of Subcommissural Organ Lesions on Aldosterone Production in the Rat. Acta endocr. (Kbh.) **48,** 169. — 50. STÖCKER, E.: (1962a) Autoradiographische Untersuchungen zur Deutung der funktionellen Kernschwellung am exocrinen Pankreas. Z. Zellforsch. **57,** 47. — 51. STÖCKER, E.: (1962b) Autoradiographische Untersuchungen zur Ribonucleinsäure- und Eiweiss-Synthese im nukleären Funktionsformwechsel der exocrinen Pankreaszelle. Z. Zellforsch. **57,** 145. — 52. STÖCKER, E., ALTMANN, H. W.: (1963) Die Grösse des Nucleolus und die Nucleolus-Karyoplasma Relation als Ausdruck synthetischer Aktivität. Z. Krebsforsch. **65,** 351. — 53. STÖCKER, E., MAURER, W., ALTMANN, H. W.: (1961) Autoradiographische Untersuchungen der Eiweiss- und RNS-Synthese mit H^3-Leucin und H^3-Cytidin zur Deutung der funktionellen Kernschwellung während des Funktionsformwechsels im exocrinen Pankreas. Klin. Wschr. **39,** 926. — 54. STURCZ, J., SÓLYOM, J., KOTRA, Z., SALAMON, A., UJJ, M.: (1963) Role of the Renin-Angiotensin System in the Regulation of Aldosterone Excretion. Second int. Congr. Nephrology. Excerpta med. Int. Congr. Ser. **67,** 378. — 55. SZENTÁGOTHAI, J., FLERKÓ, B., MESS, B., HALÁSZ, B.: (1962) Hypothalamic Control of the Anterior Pituitary. Akadémiai Kiadó, Budapest. — 56. TAYLOR, A. N., FARRELL, G. L.: (1962) Effect of Brain Stem Lesions on Aldosterone and Cortisol Secretion. Endocrinology **70,** 556. — 57. VECSEI, P., GLÁZ, E.:

1964) Aldosteron. Medicina, Budapest. — 58. VERNEY, E. B.: (1947) The Antidiuretic Hormone and the Factors which Determine its Release. Proc. roy. Soc. Med. **135,** 25. — 59. VESIN, P.: (1958) L'aldostérone. Régulation de la sécrétion chez le sujet normal et oedémateux, Presse méd. **66,** 507. — 60. VOGT, M.: (1959) In: VECSEI, P., GLÁZ, E.: Aldosteron. Medicina, Budapest, 1964.

VOLUMENREZEPTOREN IM ZWISCHENHIRN

M. PALKOVITS, L. ZÁBORSZKY und P. MAGYAR

Im Diencephalon von Ratten lassen sich mit karyometrischen Methoden Volumenrezeptoren nachweisen. Bei isosmotischer Hypovolämie 30 min. nach Entblutung kann im subkommissuralen Organ und im subfornikalen Organ eine signifikante, ferner im Nucleus paraventricularis eine geringere Kernvolumenvergrößerung festgestellt werden, während bei isosmotischer Hypervolämie 30 min. nach Verabreichung einer physiologischen Kochsalzlösung in den subkommissuralen und subfornikalen Organen eine starke, im Nucleus supraopticus eine geringere Abnahme des Kernvolumens beobachtet wurde. Der Effekt ist die direkte Folge der Volumenveränderung und nicht der Aldosteronmobilisation, da 30 min. nach Aldosteronverabfolgung an den obigen Stellen keine signifikante Kernvolumenveränderung erfolgte. Das Corpus pineale reagiert auf Aldosteron mit Kernvolumenabnahme, bleibt nach diesen Volumenveränderungen jedoch unverändert.

Das subkommissurale und das subfornikale Organ können demnach als Volumenrezeptoren des zentralen Nervensystems betrachtet werden.

Die magnozellulären Kerne des Hypothalamus — die bekanntlich Osmorezeptoren sind — reagieren in geringerem Grade auch auf Volumenveränderungen, oder ihre Veränderungen sind durch die Veränderung des osmotischen Gleichgewichts bedingt.

РЕЦЕПТОРЫ ОБЪЕМА В ПРОМЕЖУТОЧНОМ МОЗГЕ

М. ПАЛЬКОВИЧ, Л. ЗАБОРСКИ и П. МАДЬЯР

Кариометрическим методом исследования в промежуточном мозге крысы можно выявить наличие рецепторов объема. При изосмотической гиповолемии (через 30 минут после обескровливания животных) выявляемо значительное увеличение объема ядер в субкоммиссуральном и субфорникальном органах, а меньшее — в паравентрикулярном ядре, в то время как при изосмотической гиперволемии (через 30 минут после введения физиологического раствора соли) в субкоммиссуральном и субфорникальном органах наблюдается сильное уменьшение объема ядер, а меньшее уменьшение — в супраоптическом и паравентрикулярном ядрах. Этот эффект представляет собой непосредственное последствие изменения объема, а не последствие мобилизации альдостерона, так как через 30 минут после введения альдостерона на указанных местах не наблюдалось достоверного изменения объема ядер. Шишковидная железа реагировала на дачу альдостерона уменьшением объема ядер, но после изменений объема она оставалась неизменной.

Из сказанного можно заключить, что субкоммиссуральный и субфорникальный органы можно рассматривать как рецепторы объема центральной нервной системы, и что магноцеллулярные ядра гипоталамуса — о которых известно, что они представляют собой осморецепторы — или реагируют в незначительной мере также и на изменения объема, или полученные ответы являются последствием нарушения осмотического равновесия, вызванного изменением объема.

Dr. Miklós PALKOVITS
Dr. László ZÁBORSZKY
Dr. Pál MAGYAR

Budapest IX. Tűzoltó u. 58, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(4), pp. 403—412, 1968*

Institute of Anatomy (Director: Prof. J. SZENTÁGOTHAI), University Medical School, Budapest

CORRELATION BETWEEN PINEAL BODY AND SUBCOMMISSURAL ORGAN*

M. PALKOVITS

(Received July 19, 1967)

Karyometric measurements performed on the pineal gland of white rats have shown that this organ plays no role in the regulation of salt and water balance, since hyperosmosis, hypervolaemia and adrenalectomy failed to induce changes in this respect. As the same factors elicited changes in the subcommissural organ, there seems to be no functional correlation between the two organs, although a suspension prepared from the subcommissural organ produced an inhibitory effect not only on this organ itself but on the pineal gland as well.

A morphological and embryological correlation seems to exist between the subcommissural organ (SCO) and the corpus pineale (CP). The supracommissural part of the SCO forms the lower wall of the pineal recess and reaches into the substance of the CP (Fig. 1) as is readily demonstrable in lower mammals [41] and in young human subjects [21]. The ependymal elements later appear as islets or rosettes in the CP [1, 4, 5, 10, 27, 43, 50, 51, 52, 53]. A direct neural connection between the two organs has been described in lower animals [33, 34]. Vessels constitute the main pathways of communication.

Both organs develop from the same site and anlage, viz. the matrix ependyma of the epiphyseal projection of the primary cephalic saccus. At first, the pineal recess presses downward; the cells of the CP are invaginated in this area, while the SCO is formed behind it. The CP develops thereafter together with the hypendyma of the SCO [21, 41]. These morphological and embryological connections gave rise to speculations regarding a possible functional identity of the two organs. Following the findings of FARREL et al. who demonstrated the role of the pineal complex in the regulation of aldosterone [11 to 15], workers began to examine if, within the complex, it was the CP, the SCO or both that were responsible for the observed effect. While it is now accepted that the SCO is involved in the regulation of salt and water balance [36], the role of the CP is still unelucidated. Earlier investigators suggested that the organ played some part in sexual activity, that — by way of perceiving photic stimuli — it was involved in diurnal and seasonal rhythmicity, while, of recent, it was thought to contribute to the regulation of melanin synthesis. Its role in

* Report at the 1st Roumanian Endocrinological Congress, Bucharest 1967.

water balance is still a matter of contention. Several authors found that pineal body extract enhanced the production of aldosterone [7, 11, 12, 13, 14, 23, 26, 31, 44, 45], while others failed to observe such an effect [30, 38, 55]. By karyometry, GIACOMELLI [19] registered changes in the zona glomerulosa after pinealectomy, whereas others [8, 9, 13, 24, 41, 48, 54, 56] found no change either in adrenal activity or aldosterone production in pinealectomized animals.

The functional correlation between SCO and CP has been assumed [33, 36] but in epiphysectomized chick embryos STALSBERG [48] found no change in the nuclear volume of the cells of the SCO. We are now faced with the problem whether — relying on the morphological picture alone, further in consideration of the topographical, neural and vascular affinities — it is justified to accept the existence in a physiological sense of an epithalamo-epiphyseal system as postulated by THIÉBLOT and LE BARS [49].

Material and methods

Laboratory-bred white rats of the Wistar strain were used in the experiments. The animals were decapitated at the termination of the experiments, their brain was fixed in Bouin's fluid for 48 hrs, and frontal serial sections 10 μ thick were prepared, then the nuclear diameters of 300 cells were measured in the CP of each animal by a modified version [35] of JAKOBJ's [22] method. A table, prepared on the basis of FISCHER and INKE's formula [16], served for the computation of nuclear volume: vol $= \pi/6$ $(LB)^{3/2}$. Experimental observations [2, 3, 36], autoradiographic examinations [6, 32, 46, 47] and interference-microscopic measurements [28] have shown that an increase in nuclear volume points to an enhanced, while a decrease to a reduced, cellular activity.

Experiment No. 1. Effect of acute hyperosmosis and hypervolaemia. Six animals received through a gastric tube 2 ml of 2.5% NaCl and 8 ml of physiological saline, and were killed 30 min. later. Only the gastric tube was introduced into the controls.

Experiment No. 2. Effect of adrenalectomy. The animals were bilaterally adrenalectomized and sacrificed 8 and 14 days later. Between operation and death they received slightly salted drinking water, a standard diet, further 0.5 mg/100 g DOCA on the first postoperative day. The controls were subjected to a sham operation.

Experiment No. 3. Effect of SCO- *and* CP-*extract.* The whole of an SCO and the half of a CP of a calf were homogenized in 5 ml of physiological saline at a temperature below 4°C. The test animals received intraperitoneally 2.5 ml of the homogenate every other day over a period of 20 days and were killed on the 21st day. The controls received the same amounts of a homogenate prepared from cerebral cortex of a similar size.

Results

ad 1. Acute hyperosmosis and hypervolaemia caused no significant change of nuclear volume in the CP (Table I, Fig. 1).

ad 2. No change of nuclear volume was observed either 8 or 14 days after adrenalectomy (Table II, Fig. 2).

Fig. 1. Subcommissural organ and pineal body of newborn rat. Sections made in the frontal plane at 100 μ intervals in oro-caudal direction (A—D). PR = pineal recess; R = Reissner's fibre. Haematox.-eosin. 120 ×

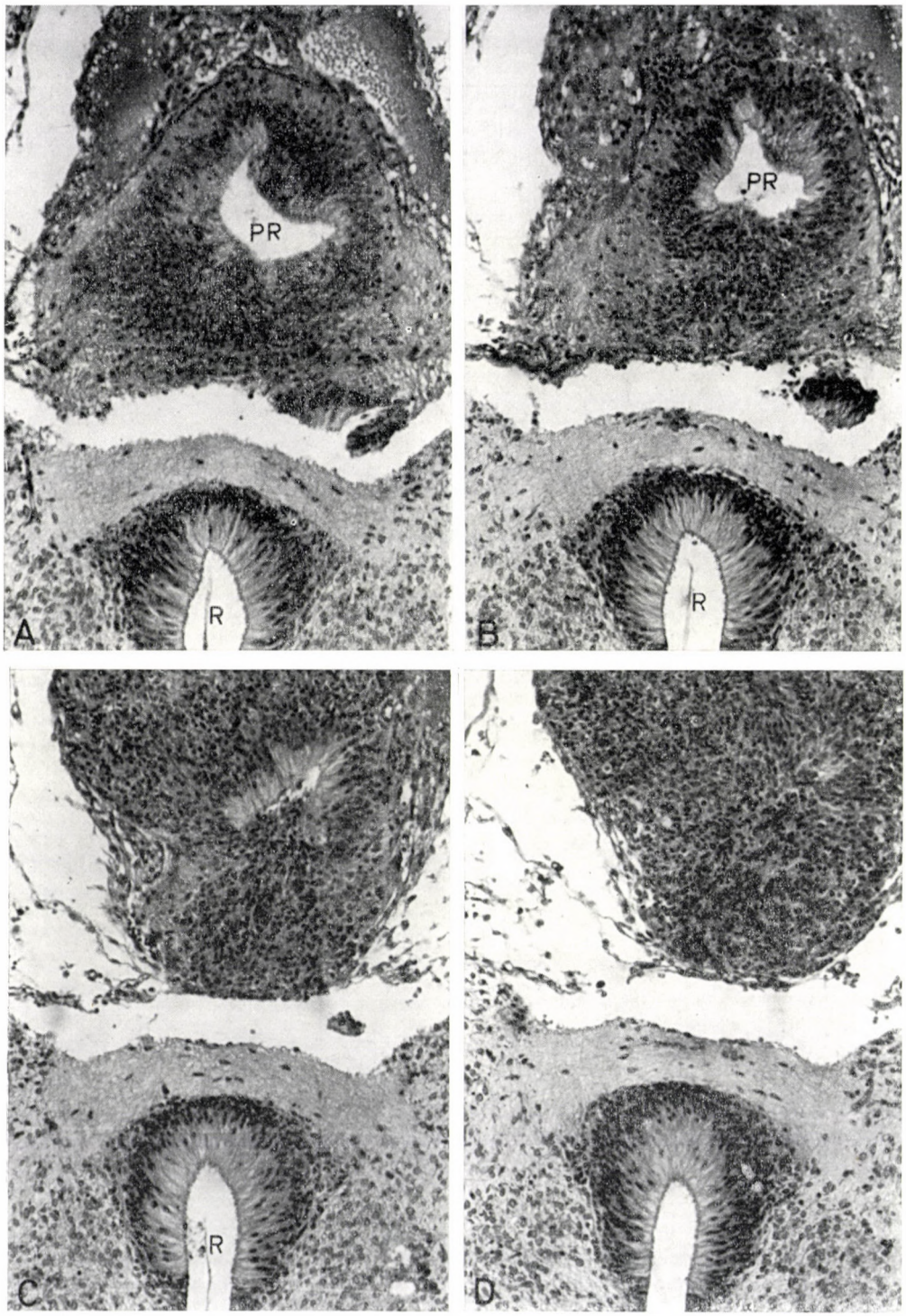

PR
R
A
PR
R
B
R
C
D

Table I

Nuclear volume in cells of pineal body, after acute hyperosmosis and hypervolaemia

	μ^3	Average μ^3	Deviation from control value per cent
	94.27		
	78.55		
	83.62		
Control	98.85	83.49	—
(2 ml of physiological	73.80		
saline)	71.85		
	97.23		
	75.78		
2 ml of 2.5% NaCl 30′	88.12	84.65	+1.4
	76.96		
	79.84		
	89.97		
	76.43		
	80.52		
8 ml of physiological saline	69.85	79.31	−5.0
30′	81.16		
	83.30		
	85.12		

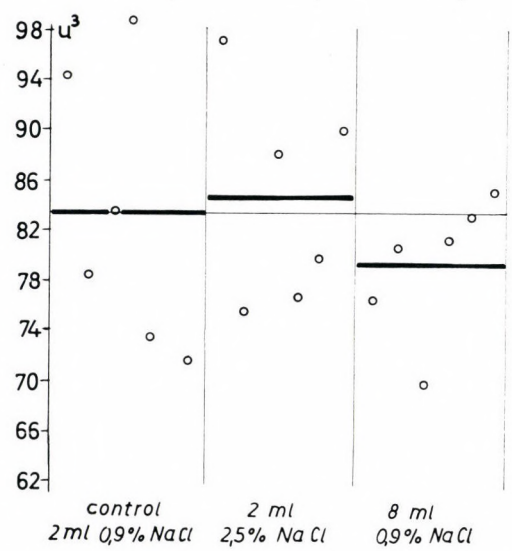


Fig. 2. Mean nuclear volume in cells of pineal body. Each point indicates the average of 300 nuclei in a single animal; horizontal line shows group average

Table II

Nuclear volume in cells of pineal body, 8 and 14 days after adrenalectomy

	μ^3	Average μ^3	Deviation from control value per cent
Control	79.68 85.10 80.89 88.46 82.54 75.22	81.98	—
Adrenalectomy (8 days)	89.59 62.63 76.67 91.50 64.53 88.65	78.93	−3.7
Adrenalectomy (14 days)	86.32 84.22 86.60 70.97 77.19 81.10	81.07	−1.1

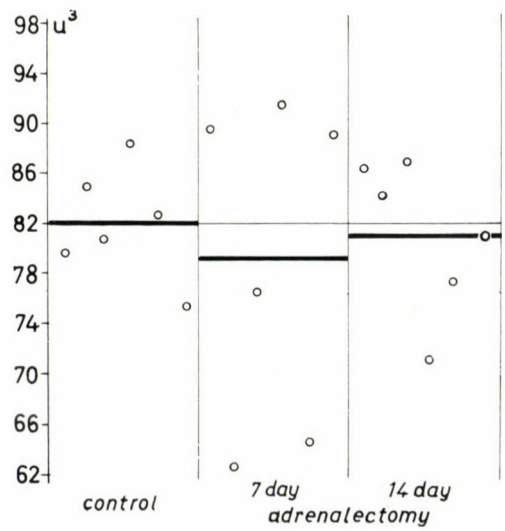


Fig. 3. Mean nuclear volume in cells of pineal body following adrenalectomy. For notations see Fig. 2

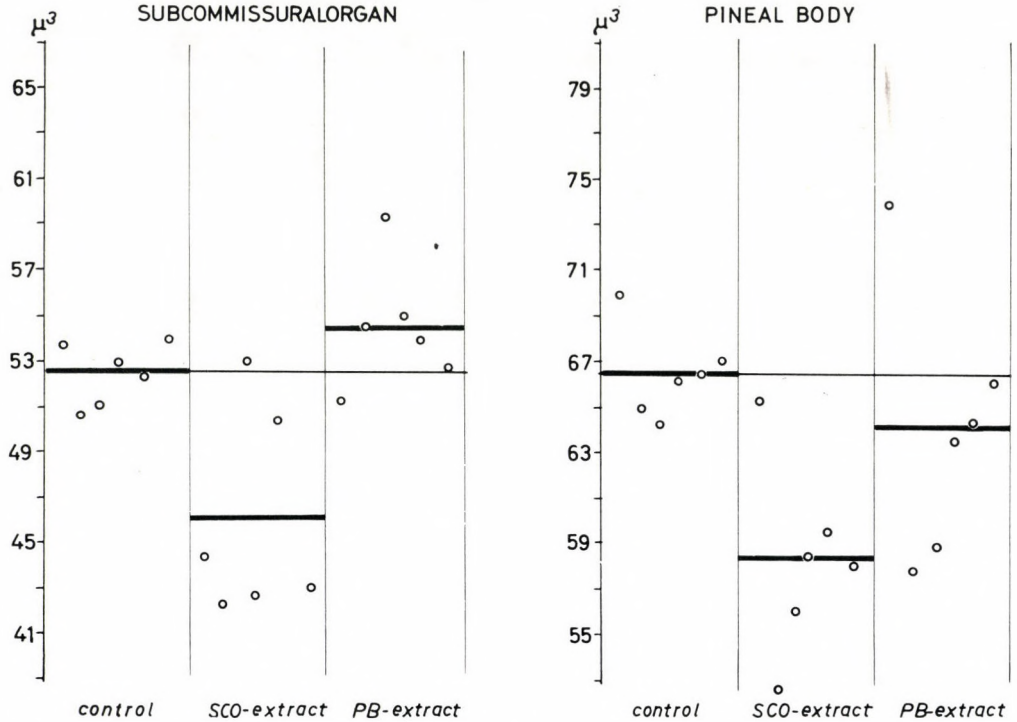


Fig. 4. Mean nuclear volume in cells of subcommissural organ and pineal body after 20-day treatment with extracts of these organs. For notations see Fig. 2

ad 3. Homogenized CP caused no change of nuclear volume either in the SCO or in the CP, whereas the SCO suspension induced in both organs a decrease of nuclear volume of approximately the same degree (12.3% and 12.1%, respectively). (Table III, Fig. 3).

Differences in the control values, in the three experiments must have been due to that the specimens had been worked up at different times.

Discussion

The present results support the view that the pineal body is not involved in the regulation of salt and water balance. This is in contrast with GIACOMELLI's karyometric findings but in agreement with our earlier experiments [38] in which CP extract failed to affect the nuclear volume of cells in the zona glomerulosa, while SCO extract produced a perceptible effect in this respect. Sodium absorption in the small intestine was increased by 150% under the effect of SCO extract, while CP-extract had a negligible effect [17]. Extract of

Table III

Nuclear volume in cells of subcommissural organ and pineal body, after treatment with the suspension of these organs

	Subcommissural organ			Pineal body		
	μ^3	Average μ^3	Deviation from control value per cent	μ^3	Average μ^3	Deviation from control value per cent
	53.89			69.98		
	50.79			64.96		
	51.18			64.42		
Control	53.00	52.58	—	66.19	66.52	—
	52.55			66.45		
	54.07			67.11		
	44.75			65.37		
	42.35			52.66		
Extract of subcommissural	53.14	46.12	−12.3	56.10	58.41	−12.1
organ	42.88			58.57		
	50.50			59.69		
	43.08			58.08		
	51.35			73.91		
Extract of pineal body	54.61	54.44	+3.7	57.89	64.11	−3.6
	59.25			58.85		
	55.07			63.55		
	54.03			64.36		
	52.97			66.13		

intact pineal body failed to prevent the reduction of aldosterone production following isolated lesion of the SCO [40].

Adrenalectomy induced gross changes in the SCO [37] but had no effect on the CP. Opinions are divided in this respect. While PANAGIOTIS and HUNGERFORD [42] registered changes in the CP after adrenalectomy, LINGJAERDE et al. [29] failed to do so. In our opinion a change ought to take place 7 or 14 days after adrenalectomy were the two organs in functional connection.

The CP suspension had no effect on the SCO and the CP also remained unchanged, a phenomenon which argued against hormonal activity of any kind. Judging from the decrease of nuclear volume, SCO suspension inhibited the activity of the SCO, causing a negative feedback effect. It is more difficult to understand why the SCO suspension decreased nuclear volume in the cells of the pineal body. The rate of aldosterone production was not determined after

such treatment in the present experiments, but earlier investigations justify the assumption that the aldosterone level was above normal. It is known that, through some unelucidated mechanism, aldosterone changes nuclear volume in the cells of the pineal gland, a phenomenon which failed to appear under the effect of hypervolaemia or hypovolaemia. Although some of the present findings seemed to point to a role of the CP in the regulation of salt and water balance, those examinations which would have definitely verified such an effect, yielded negative results. Since the SCO gave a positive reaction in the same experiments, it is safe to conclude that there is no functional interconnection between the two organs.

Considering that not only the SCO and the CP but also the nucleus habenulae forms part of the epithalamus, the question arises as to whether it is justified to speak of an epithalamo-epiphyseal system in the functional sense of the term. Further investigations will, on the other hand, have to clarify whether the nucleus habenulae is in some connection either with the SCO or with the CP.

REFERENCES

1. Bargmann, W.: (1943) Die Epiphysis cerebri. In: Möllendorf, v. W. Handbuch der mikroskopischen Anatomie des Menschen. Springer, Berlin. — 2. Benninghoff, A.: (1949) Funktionelle Kernschwellung und Kernschrumpfung. Anat. Nachr. **1,** 50—53. — 3. Benninghoff, A.: (1953) Die funktionelle Kernödem als Indikator der Zelltätigkeit. Mon. zool. ital. **61,** Suppl. 84—89. — 4. Canepa, G.: (1949) Contributi alla conoscenza delle relazioni tra organo subcommessurale ed epifisi in alcuni mammiferi. Arch. Anat. Antropol (Lisboa) **26,** 513—518. — 5. Capurro, S.: (1942) Osservazioni sull'acquedotto di Silvio e sull'organo subcommessurale nell'uomo e in alcuni mammiferi. Riv. sper. Freniat. **66,** 101—110. — 6. Citoler, P., Citoler, K., Hempel, K., Schultze, B., Maurer, W.: (1966) Autoradiographische Untersuchungen mit zwölf H^3- und fünf C^{14}-markierten Aminosäuren zur Grösse des nucleären und cytoplasmatischen Eiweissstoffwechsels bei verschiedenen Zellarten von Maus und Ratte. Z. Zellforsch. **70,** 419—448. — 7. Clementi, F., Fraschini, F., Müller, E., Zanoboni, A.: (1965) The Pineal Gland and the Control of Electrolyte Balance and of Gonadotropic Secretion: Functional and morphological observations. Progr. Brain Res. **10,** 585—603. — 8. Coghlan, J. P. Denton, D. A., Goding, J. R., Wright, R. D.: (1960) The Control of Aldosterone Secretion. Postgrad. med. J. **36,** 76—102. — 9. Davis, J. O.: (1961) Mechanisms Regulating the Secretion and Metabolism of Aldosterone in Experimental Secondary Hyperaldosteronism. Recent Progr. Hormone Res. **17,** 237—247. — 10. Dendy, A.: (1907) On the Parietal Sense Organs and Associated Structures in the New Zealand Lamprey. Quart. J. micr. Sci. **51,** 1—11. — 11. Farrell, G.: (1959a) Steroidogenic Properties of Extracts of Beef Diencephalon. Endocrinology **65,** 29—33. — 12. Farrell, G.: (1959b) Glomerulotropic Activity of an Acetone Extract of Pineal Tissue. Endocrinology **65,** 239—241. — 13. Farrell, G.: (1960) Adrenoglomerulotropin. Circulation **21,** 1009—1015. — 14. Farrell, G., McIsaac, W. M.: (1961) Adrenoglomerulotropin. Arch. Biochem. **94,** 543—544. — 15. Farrell, G., Taylor, A. N.: (1962) Neuroendocrine Aspects of Blood Volume Regulation. Ann. Rev. Physiol. **24,** 471—490. — 16. Fischer, J., Inke, G.: (1956) Nomogramme zur Berechnung des Kernvolumens. Acta morph. Acad. Sci. hung. **7,** 141—165. — 17. Földvári, I. P., Czeizel, E., Simon, G., Palkovits, M., Kertai, P.: (1962) The Influence of the Subcommissural Organ on the Resorption of Water and Electrolytes from the Small Intestine. Acta physiol. Acad. Sci. hung. **22,** 43—50. — 18. Funquist, H.: (1912) Zur Morphogenie und Histogenese des Pinealorgans bei den Vögeln und Säugetieren. Anat. Anz. **42,** 111—130. — 19. Giacomelli, F.: (1962) Über Veränderungen der Nebennierenrinde nach Pinealektomie. Endokrinologie **42,** 144—150. — 20. Gusek, W., Buss, H., Wartenberg, H.: (1965) Weitere Untersuchungen zur Feinstruktur der Epiphysis cerebri normaler und vorbehandelter Ratten. Progr. Brain Res. **10,** 296—316. — 21. Inke, G., Palkovits, M.: (1963) Die embryonale Entwicklung des

Subcommissuralkomplexes (Subcommissuralorgan und seine Komponenten) beim Menschen. Anat. Anz. **113,** 240—254. — 22. JAKOBJ, W.: (1925) Über das rhythmische Wachstum der Zellen durch Verdoppelung ihres Volumens. Arch. entw. Mech. **106,** 124—192. — 23. JOUAN, P., SAMPEREZ, S.: (1965) Étude de la sécrétion des corticostéroides et de l'hormone adrénocorticotrope hypophysaire chez le rat épiphysectomisé. Progr. Brain Res. **10,** 604—611. — 24. KEELER, R.: (1961) The Effect of Mesencephalic Lesions or Pinealectomy on the Sodium Retention Produced by Suprahepatic Constriction of the Inferior Vena cava in Rats. J. Physiol. (Lond.) **159,** 78—79. — 25. KELLER, A., PIOTTI, L. E., ROMANI, J. D.: (1961) Nouvelles preuves de l'activité adréno-glomérulotrope de l'extrait épiphysaire. Ann. Endocr. (Paris) **22,** 82—86. — 26. KOVÁCS, K., DÁVID, M., WEISS, P.: (1960) Aldosteronotrophic and Corticosteronotrophic Substances in Normal Human Brain. Med. exp. (Basel) **3,** 113—116. — 27. KRABBE, K.: (1925) L'organe sous-commissural du cerveau chez les mammifères. Kungl. Dansk Vidensk. Selks. biol. med. **5,** 1—83. — 28. LAMPERT, F., HOFMANN, D., SANDRITTER, W.: (1966) Interferenzmikroskopische Trockengewichtsbestimmungen an Kern, Cytoplasma und Nucleolus von HeLa- und Amnionzellen der Gewebekultur. Histochemie **6,** 370—381. — 29. LINGJAERDE, P., MALM, O. J., NATVIG, R. A., SKAUG, O. E.: (1958) The Effect of Adrenalectomy on the Uptake of Radioactive Phosphorus in the Rat. Acta endocr. (Kbh.) **28,** 558—565. — 30. LUCIS, O. J., DYRENFURTH, I., VENNING, E. H.: (1961) Effect of Various Preparations of Pituitary and Diencephalon on the in-vitro Secretion of Aldosterone and Corticosterone by the Rat Adrenal Gland. Canad. J. Biochem. **39,** 901—913. — 31. MACHADO, A. B. M., DA SILVA, C. R.: (1963) Pineal Body and Urinary Sodium Excretion in the Rat. Experientia (Basel) **19,** 264—266. — 32. OEHLERT, W., SCHULTZE, B.: (1960) Die Kerngrösse als Ausdruck der synthetischen Aktivität des Kerns. Beitr. path. Anat. **123,** 101—113. — 33. OKSCHE, A.: (1954) Über die Art und Bedeutung sekretorischer Zelltätigkeit in der Zirbel und im Subkommissuralorgan. Anat. Anz. **101,** 88—96. — 34. OKSCHE, A.: (1956) Funktionelle histologische Untersuchungen über die Organe des Zwischenhirndaches der Chordaten. Anat. Anz. **102,** 404—419. — 35. PALKOVITS, M.: (1961) Angaben und Hilfsmittel zur Auswertung von Kernvariationsuntersuchungen. Z. mikr-anat. Forsch. **67,** 343—355. — 36. PALKOVITS, M.: (1966) Morphology and Function of the Subcommissural Organ. Academic Press, Budapest. — 37. PALKOVITS, M.: (1968) Karyometrische Untersuchungen zur Klärung der osmo- bzw. volumenregulatorischen Rolle des Subcommissuralorganes und seiner funktionellen Verbindung mit der Nebennierenrinde. Z. Zellforsch. **84,** 59—71. — 38. PALKOVITS, M., FÖLDVÁRI, I. P.: (1962) Effect of the Subcommissural Organ and the Pineal Body on the Adrenal Cortex. Endocrinology **72,** 28—32. — 39. PALKOVITS, M., INKE, G., LUKÁCS, G.: (1962) Topographische Beziehungen des menschlichen Subcommissuralorgans zur Epiphyse und ihre funktionelle Bedeutung. Endokrinologie **42,** 194—202. — 40. PALKOVITS, M., MONOS, E., FACHET, J.: (1965) The Effect of Subcommissural-lesions on Aldosterone Production in the Rat. Acta endocr. (Kbh.) **48,** 169—176. — 41. PALKOVITS, M., WETZIG, H.: (1962) Untersuchungen über das Subcommissuralorgan und den Recessus mesocoelicus bei der Sturmmöve (Larus canus L.). Z. mikr.-anat. Forsch. **68,** 612—626. — 42. PANAGIOTIS, N. M., HUNGERFORD, G. F.: (1961) Response of the Pineal and Adrenal Glands to Sodium Restriction. Endocrinology **69,** 217—224. — 43. PASTORI, G.: (1927) Pineali accessorie e relazioni tra gli organi pineale e subcommissurale. Cervello **6,** 1—16. — 44. ROMANI, D., KELLER, A., PIOTTI, L. E.: (1960a) Action d'un extrait épiphysaire sur la distribution des lipides et des phosphatases alcalines du cortex surrénal. Ann. Endocr. (Paris) **21,** 79—85. — 45. ROMANI, J. D., KELLER, A., PIOTTI, L. E.: (1960b) Étude de l'action d'un extrait épiphysaire sur l'excrétion du sodium chez le rat. Ann. Endocr. (Paris) **21,** 612—616. — 46. STÖCKER, E.: (1962a) Autoradiographische Untersuchungen zur Deutung der funktionellen Kernschwellung am exokrinen Pankreas. Z. Zellforsch. **57,** 47—62. — 47. STÖCKER, E.: (1962b) Autoradiographische Untersuchungen zur Ribonukleinsäure- und Eiweiss-Synthese im nukleären Funktionsformwechsel der exokrinen Pankreaszelle. Z. Zellforsch. **57,** 145—171. — 48. STALSBERG, H.: (1965) Effects of Extirpation of the Epiphysis cerebri in 6-day Chick Embryos. Acta endocr. (Kbh.) **97,** suppl. 1—119. — 49. THIÉBLOT, L., LE BARS, H.: (1955) La glande pinéale ou épiphyse. Maloine, Paris. — 50. TRETJAKOFF, D. (1913) Die zentralen Sinnesorgane bei Petromyzon. Arch. mikr. Anat. **83,** 69—115. — 51. TRETJAKOFF, D.: (1915) Die Parietalorgane von Petromyzon fluviatilis. Z. wiss. Zool. **113,** 1—112. — 52. TURKEWITSCH, N.: (1937a) Eigentümlichkeiten der embryologischen Entwicklung des Epiphysengebiets des Schafes (Ovis avies L.) Morph. Jb. **79,** 305—330. — 53. TURKEWITSCH, N.: (1937b) Eigentümlichkeiten in der Entwicklung des Epiphysengebietes des Kaninchens (Lepus cunuculus L.). Morph. Jb. **79,** 634—649. — 54. VAN DER WALL, B., MOLL, J., DE WIED, D.: (1965) The Effect of Pinealectomy and of Lesions in the Subcommissural Body on the Rate of Aldosterone Secretion by Rat Adrenal Glands in-vitro. Progr. Brain Res. **10,** 635—645. — 55. WURTMAN, R. J., ALTSCHULE, M. D., GREEP, R. O., FALK, J. L., GRAVE, G.: (1960) The Pineal Gland and Aldosterone. Amer.

J. Physiol. **199,** 1109—1111. — 56. YAMADA, T.: (1961) The Effect of Electrical Ablation of the Nuclei habenulae, Pineal Body and Subcommissural Organ on Endocrine Function, with Special Reference to Thyroid Function. Endocrinology **69,** 706—711.

ZUSAMMENHANG ZWISCHEN CORPUS PINEALE UND SUBKOMMISSURALEM ORGAN

M. PALKOVITS

Nach den Ergebnissen der karyometrischen Untersuchung des Corpus pineale weißer Ratten spielt dieses Organ in der Regulation des Salz- und Wasserhaushalts keine Rolle, da weder nach Hyperosmose noch nach Hypervolämie oder Adrenalektomie eine Veränderung beobachtet werden kann. Da im subkommissuralen Organ hingegen nach den obigen Einwirkungen eine Veränderung vor sich geht, besteht zwischen den beiden Organen kein funktionaler Zusammenhang, ungeachtet dessen, daß die aus dem subkommissuralen Organ hergestellte Suspension nicht nur auf das subkommissurale Organ selbst, sondern auch auf das Corpus pineale eine Hemmwirkung ausübt.

СВЯЗЬ МЕЖДУ ШИШКОВИДНОЙ ЖЕЛЕЗОЙ И СУБКОММИССУРАЛЬНЫМ ОРГАНОМ

М. ПАЛКОВИЧ

Согласно кариометрическим исследованиям, проведенным на шишковидной железе у белых крыс, этот орган не участвует в регулировании солевого и водного обмена, так как при гиперосмозе, гиперволемии или адреналектомии не удалось выявить изменений. Ввиду того, что после вышеуказанных воздействий субкоммиссуральный орган изменяется, по мнению автора между двумя органами не существует функциональной связи, вопреки тому, что взвесь из субкоммиссурального органа оказывает тормозящее действие не только на сам субкоммиссуральный орган, но и на шишковидную железу.

Dr. Miklós PALKOVITS; Budapest IX. Tűzoltó u. 58, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(4), pp. 413—424, 1968*

Department of Pathology and Pathohistology (Head: K. LAPIS),
Postgraudate School of Medicine, Budapest and Department of Biochemistry,
National Korányi Tuberculosis Institute (Scientific Director: I. FÖLDES)

ACUTE, SUBACUTE AND CHRONIC EFFECT OF ALPHA-NAPHTHYL-ISOTHIOCYANATE ON THE LIVER OF THE RAT

M. BALÁZS and E. MEDVECZKY

(Received October 2, 1967)

A single oral dose of alpha-naphthyl-isothiocyanate was found to cause biliary duct changes beginning in the septal ducts near the porta. No mechanical obstruction of the biliary ducts has been observed.

On the third day of the acute experiment the epithelium of the interlobar ducts and intralobular ductules was so swollen that the lumen was almost totally obliterated. At the same time there was a slight increase in the serum bilirubin level which up to that time had been normal.

On subacute treatment, biliary duct proliferation dominated; it was associated with marked fibroblastic activity and during the third to fifth weeks severe perilobular fibrosis developed.

The changes were reversible, and in spite of the continuous administration no primary biliary cirrhosis had developed by the end of the 5th month, and proliferation and fibrosis diminished. At the same time the parenchymal cells showed vacuolation and fatty degeneration.

Thus, in the acute stage ANIT has a cholangiotoxic, in the subacute stage a cholangioplastic and in the chronic stage a hepatotoxic effect.

The liver lesion produced by chronic alpha-naphthyl-isothiocyanate (in the following: ANIT) treatment is considered to represent an experimental model of human biliary cirrhosis.

The exact mode of action of ANIT is not known, but there is reason to believe that it damages the intrahepatic biliary ducts. The most consistent and persistent change is the proliferation of ductular epithelial cells. The effect of ANIT differs from that of other agents inducing bile duct proliferation (ethionine, thioacetamide, butter yellow), because it is not carcinogenic even on prolonged administration [3, 7, 8, 11, 20, 21].

Material and methods

Eighty rats of both sexes, weighing about 150 g each, were used, with 10 rats serving as normal controls.

ANIT was prepared the following way:

In a flask with reflux cooler 20 g of alpha-naphthyl-thiourea were heated in 220 ml of chlorobenzene for 10 hours. Subsequently about 180 to 190 ml of the chlorobenzene were distilled off. The crude product, precipitating in the form of crystals, was filtered and washed with chlorobenzene. The yellowish crystals were shaken five times in 40 ml warm hexane. Distillation till the onset of crystallization was followed by cooling in a refrigerator, filtration of the crystals and recrystallization twice from 150 ml of hexane.

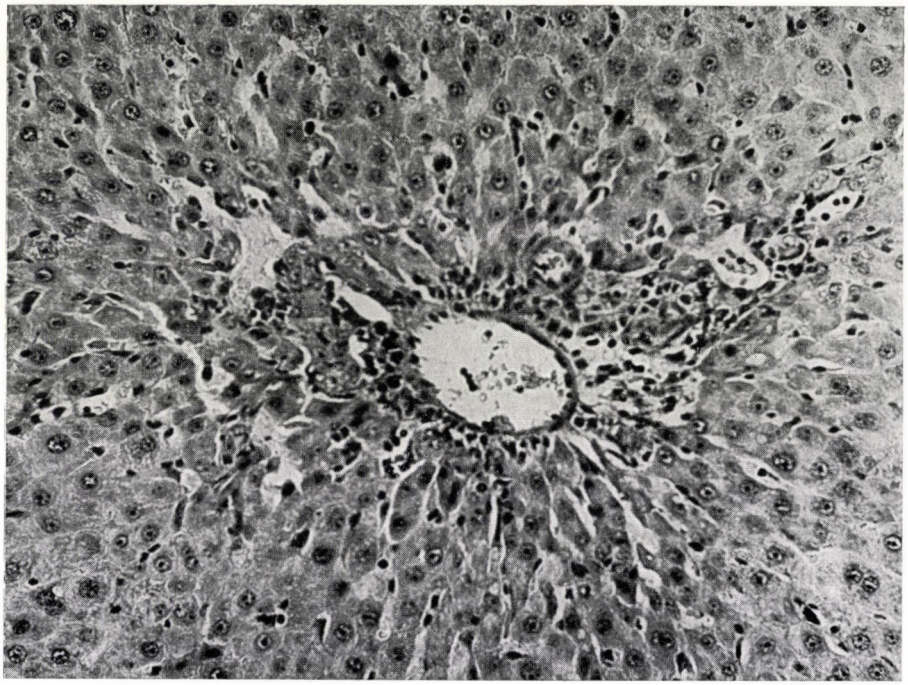


Fig. 1. Animal killed at 24 hours. Oedema and inflammation in periportal connective tissue. Haematoxylin-eosin, ×160

ANIT dissolved in sunflower oil, was administered through a gastric tube.

In the acute experiment involving 40 rats the single dose was 100 mg/kg body weight, then subgroups of 4 animals each were killed daily over a period of 10 days.

In the subacute experiment (20 rats) 100 mg/kg body weight of ANIT was administered twice a week, and 3 or 4 animals were killed each week till the 6th week.

In the chronic experiment 20 animals were treated with 100 mg/kg body weight of ANIT twice a week, over a period of 5 months.

The animals were killed by exsanguination and were tested for serum bilirubin according to van den Bergh.

At autopsy no changes of importance were detected. There was no evidence of jaundice. Liver tissue specimens were subjected to histologic and electronmicroscopic study. The sections were fixed in formaldehyde and stained with haematoxylin-eosin, PAS, Mallory, and Foot's silver impregnation. In the following, light microscopic changes will only be described.

Results

Throughout the experiments the serum bilirubin level was normal, except on the third day of the acute experiment, when slightly elevated (2.3 to 2.8 mg per 100 ml) values were obtained.

Group I. Acute experiment

At 24 hours the lobular and trabecular structure of the liver was maintained. The liver cells showed no lesion, only the increase in the number of bi-

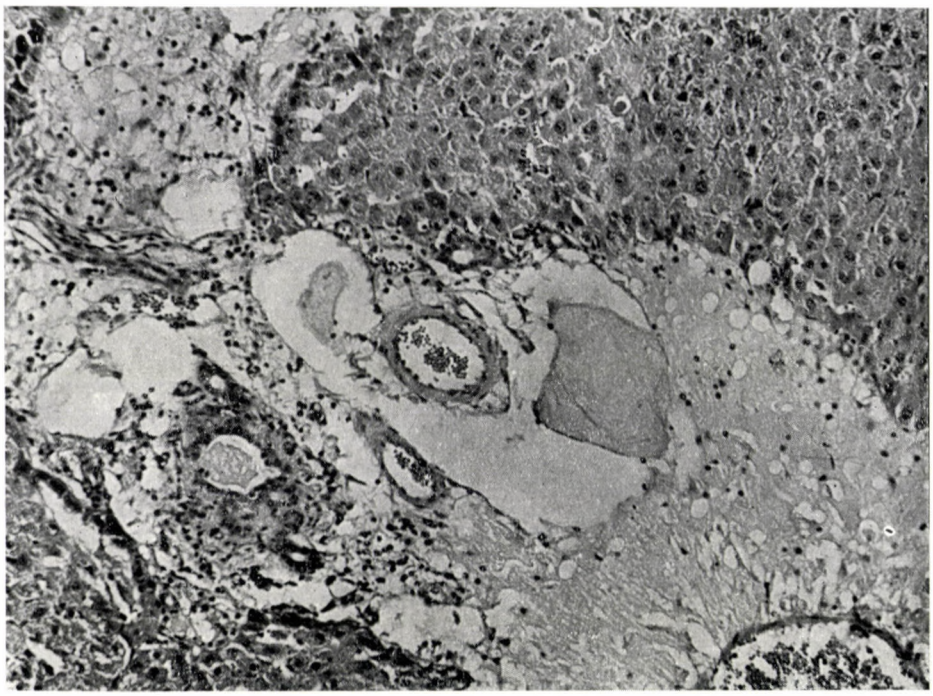


Fig. 2. Animal killed at 24 hours. Oedema and destruction of septal biliary ducts near the porta. Haematoxylin-eosin, ×120

nucleated cells indicated an increased regenerative activity. Kupffer's cells were swollen basophilic. The sinusoids were narrower than normally.

The epithelium of the interlobular bile ducts was swollen, light, slightly narrowed. The branches of the portal vein were distended, filled with blood. A slight inflammatory reaction could be seen in the portal connective tissue (Fig. 1).

Near the hepatic porta in the septal bile ducts severe destruction, partial necrosis and irregular epithelium were visible. The connective tissue showed oedema, around the bile ducts infiltration composed of lymphocytes and plasma cells was seen. In the lumen of the affected bile ducts at sites detritus and eosinophilic homogeneous material were visible (Figs 2, 3).

At 48 hours the liver structure was unchanged, the parenchymal cells appeared to be normal, but regenerative activity and the swelling of Kupffer's cells were more intensive.

Inflammation and oedema around the interlobular bile ducts increased. Beginning proliferation of biliary duct epithelium could be noted around both the interlobular ducts and intralobular ductules.

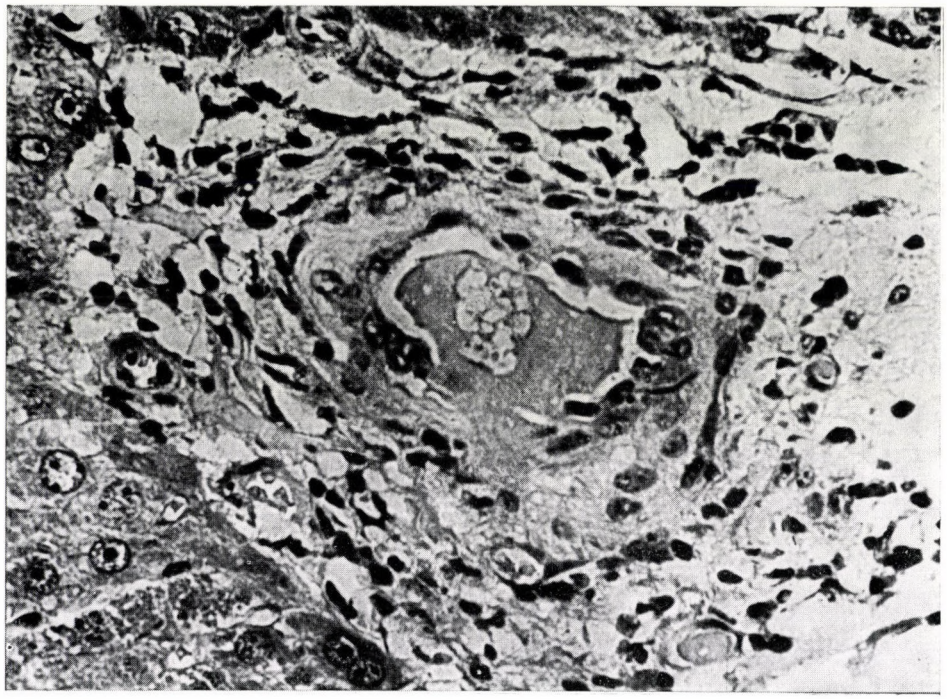


Fig. 3. High-power view of septal biliary duct. The epithelial cells are partly destroyed and partly irregular. In the lumen there are detritus and homogeneous fluid. Haematoxylin-eosin, ×230

As compared to the 24-hour pattern, the alteration of the septal biliary ducts was more severe, the inflammatory cellular infiltration and oedema had increased and the destruction of the epithelium of the bile ducts was even more severe. Satellite biliary ducts appeared at junction of connective tissue and parenchyma.

At 72 hours the liver cells were still intact, Kupffer cell activity had become more marked.

The biliary ducts were more severely affected; epithelial oedema in both the septal and interlobular biliary ducts was excessive, so that at sites the lumen seemed to be obstructed. Proliferation of biliary ducts was intense, dense inflammatory infiltration and fibroblasts were visible around the biliary ducts (Figs 4, 5).

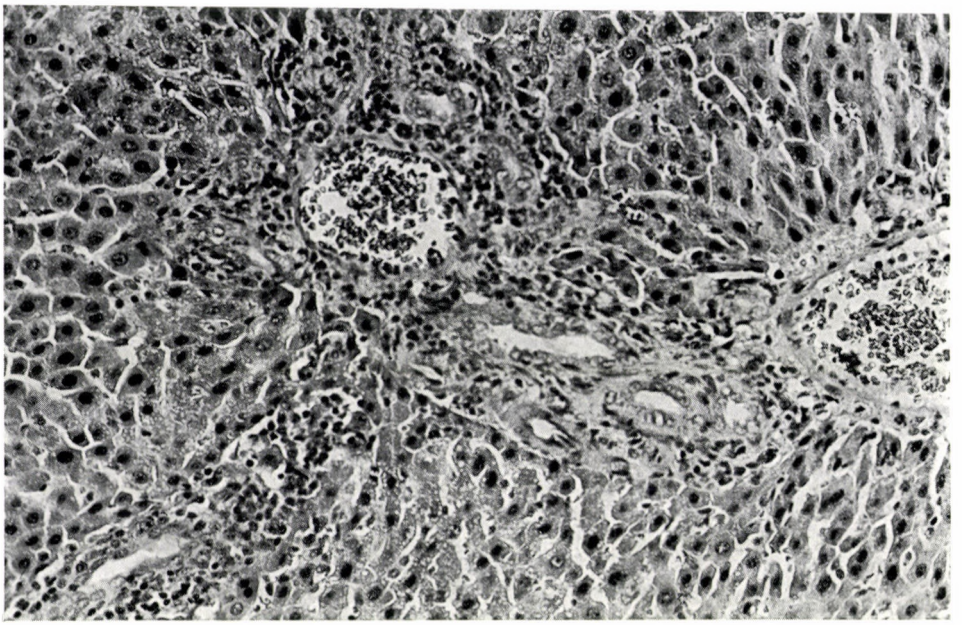


Fig. 4. After 72 hours there is intensive bile duct proliferation with pericholangitis. Haematoxylin-eosin, ×140

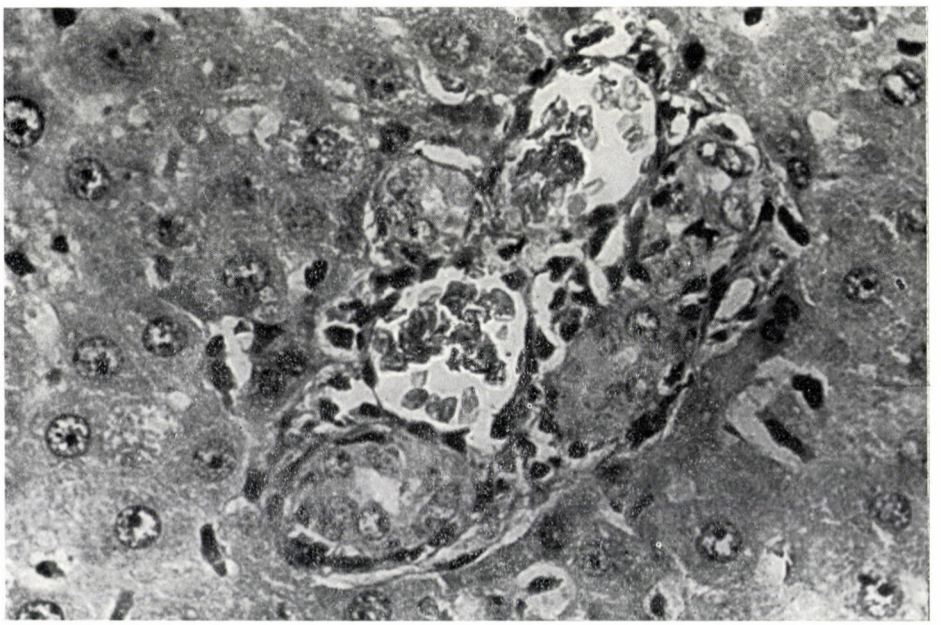


Fig. 5. Swelling of epithelial lining of bile ducts, narrowing of lumen numerous fibroblasts around the bile ducts. Haematoxylin-eosin, ×240

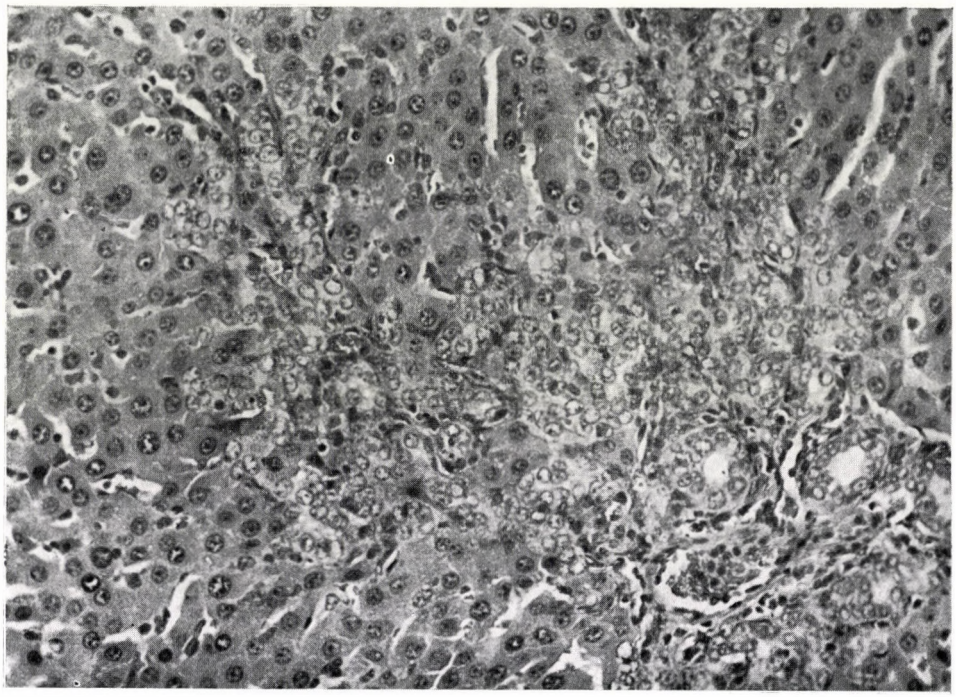


Fig. 6. After 3 weeks treatment the biliary duct proliferation is marked, extending in between the parenchymal cells. Haematoxylin-eosin, ×140

At 4 days no progression could be observed.

From the 5th day on the changes gradually regressed. The epithelium of the septal biliary ducts normalized, the lumen was free. Proliferation of the interlobular biliary ducts, inflammation of same and Kupffer cell activity diminished, but persisted till the 6th to 8th days.

On the 10th day groups of proliferating biliary duct epithelial cells marked the previous damage, but no other change could be observed.

Group II. Subacute experiment

At the end of the first and second weeks proliferation of the biliary duct epithelium dominated the pattern.

The changes culminated the in third or fourth week, when the epithelial proliferation in the bile ducts completely surrounded the liver lobules and extended to the periphery of the parenchyma. The epithelial proliferation was partly trabecular, partly showed a tendency to forming a lumen (Fig. 6).

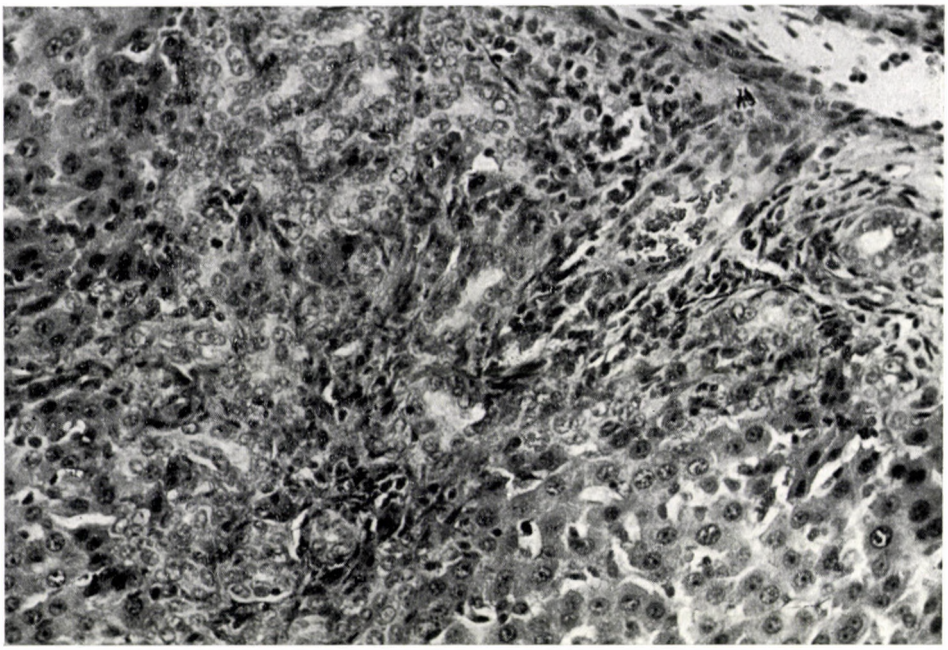


Fig. 7. During the fifth week the bile duct proliferation shows a tendency to lumen formation. Fibroblasts around the ducts. Haematoxylin-eosin, ×140

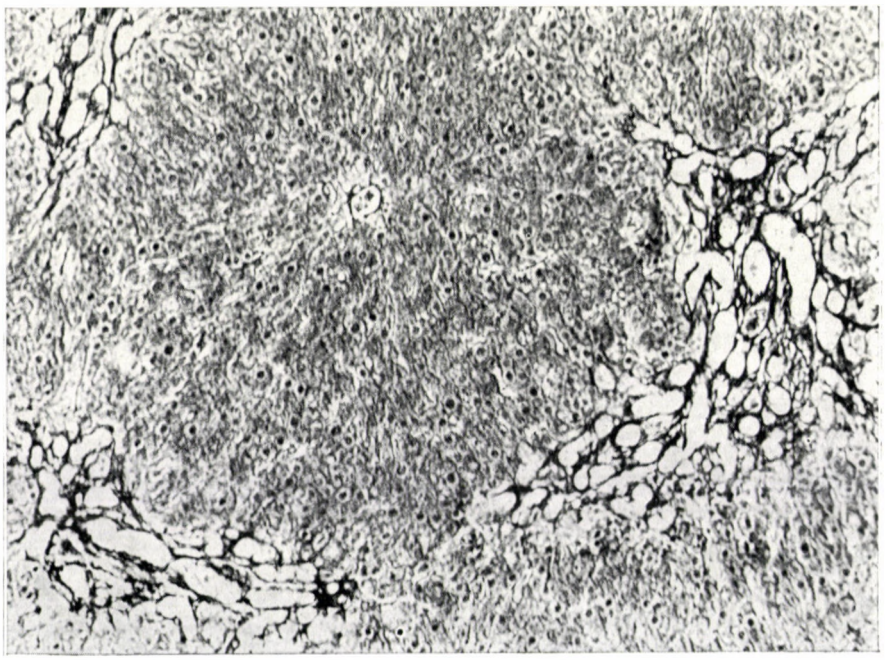


Fig. 8. Perilobular fibrosis. Foot's silver impregnation, ×90

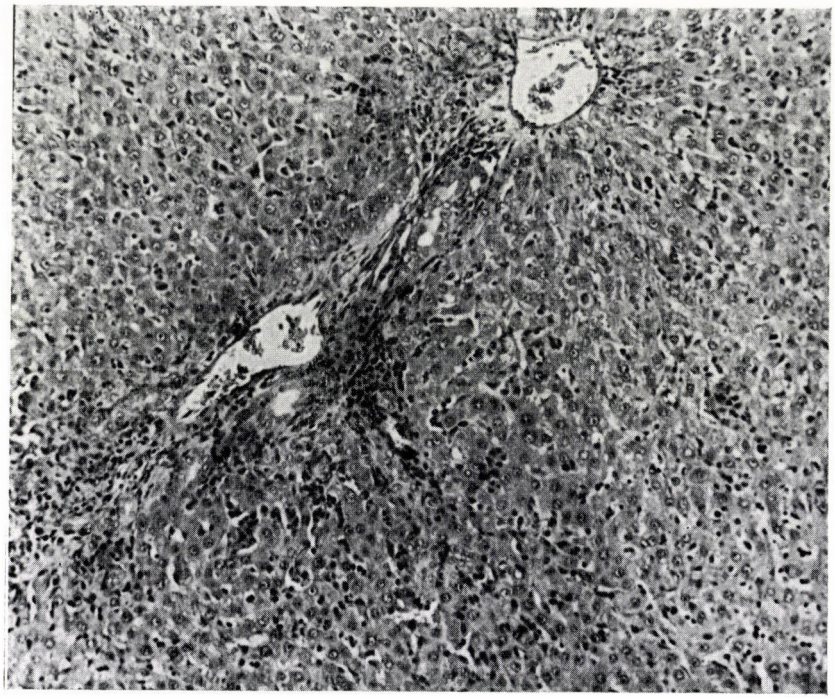


Fig. 9. After 5 months treatment slight proliferation of biliary ducts, pericholangitis and interlobular fibrosis. Haematoxylin-eosin, ×120

During the fifth and sixth weeks, around the proliferating biliary duct epithelium marked fibroblast cell activity was visible, with ample fibre formation. The septa were invariably perilobular, no true cirrhotic changes could be observed (Figs 7, 8).

Group III. Chronic experiment

In the third month of treatment 5 rats were subjected to laparotomy and liver biopsy. The changes were similar to those found at 6 weeks in the subacute group.

In the liver of animals killed at the end of the fifth month a marked regression of changes was visible. Biliary duct proliferation diminished, the previous damage was marked exclusively by a slight inflammation, perilobular septum formation and cholangiosclerosis restricted to a few areas (Fig. 9).

On the other hand, the liver parenchyma showed fatty and parenchymal degeneration and necrosis of single liver cells. There was an increased regenerative activity at the periphery of the lobules.

Discussion

Since the effect of ANIT on the liver had been described [6, 22], many reports have dealt with its light microscopic [1, 2, 3, 4, 7, 8, 11, 12, 14, 21], electronmicroscopic [15, 17, 18, 19] and biochemical [5, 9, 10, 13, 16, 22] aspects. The mode of action is far from being clarified, the above papers contain many contradictory data.

In our experiments in response to a single dose the earliest change occurred at 24 hours, in the big septal biliary ducts near the porta, as it was observed also by MORAN and UNGAR [8]. On the 2nd and 3rd days the epithelium of the septal biliary ducts suffered more severe destruction; eosinophilic fluid and detritus were visible in their lumen, but this change was not so generalized and wide-spread that a mechanical occlusion could have been concluded upon, as suggested by GOLDFARB et al. [4].

The oedema and inflammation of the epithelium of the interlobular ducts and intralobular ductules reached the peak on the third day, when the epithelial lining was so swollen that the lumen of the ducts appeared to be almost completely obstructed. At the same time, the serum bilirubin level was slightly elevated (2.3 to 2.8 mg per 100 ml). This agreed with the results obtained by GOLDFARB et al. [4] who, too, have found the severest changes on the third day of the acute experiment.

The proliferation of the biliary duct epithelium began on the second day, increased on the third and fourth days, then gradually subsided, but was still observable on the sixth to eighth days.

Summing up our findings it may be stated that in the acute experiment the compound's effect was mainly cholangiotoxic.

In response to subacute administration the most prominent histologic change was an intensive proliferation of the biliary duct epithelium. This was marked at the end of the first week and reached the maximum in the third to fifth week, as observed also by GARAY et al. [3]. At that time the biliary duct epithelium proliferated not only in bundles but showed a tendency to lumen formation, and almost one-third of the liver substance was occupied by the proliferating cells.

Thus, in the subacute experiment the most conspicuous effect was a cholangioplastic one.

The proliferating biliary ducts surrounded the lobules and penetrated in between the liver cells, but no true cirrhotic transformation was visible, as opposed to the claim made by MORAN and UNGAR [8] that true biliary cirrhosis developed after 8 weeks of treatment.

In spite of the serious changes the process was reversible. Still under the chronic treatment the biliary duct proliferation substantially diminished at the end of the 5th month, the pericholangitis was less severe, no cirrhosis de-

veloped, the only changes being septum formation at some sites and cholangiosclerosis at others. The development of resistance to the compound during administration was described also by MORAN and UNGAR [8], who carried out intermittent treatment at 1-week intervals. We found resistance to develop even on administration carried out twice weekly.

In our acute and subacute experiments the liver parenchymal cells showed only a slight regenerative hyperactivity and Kupffer cell activity, but on chronic treatment signs of parenchymal and fatty degeneration were visible in the cytoplasm of the liver cells and a few cells underwent necrosis. This is at variance with the data according to which the agent is not hepatotoxic even on chronic administration [4].

The pathomechanism of the liver lesion caused by ANIT is unclear. In some of its features it resembles the changes induced by biliary duct ligation, but has different effects in different species. In the guinea pig for instance extensive necrosis of the liver cells develops [11], while in the rat no such change has been found either by most workers or by us. According to GOLDFARB et al. [4] with the bile some agent is excreted which diffuses through the lesioned biliary ducts, causing periductal inflammation and secondary proliferation. On the basis of electronmicroscopic studies STEINER et al. [19, 20] assumed that the fundamental change is a disturbance of bile excretion.

REFERENCES

1. CAMERON, G. H., MCLEAN, M. R., PRASAD, L. B.: (1960) Recovery from the Bile Duct Hyperplasia Induced by Feeding Alphanaphtyl Isothiocyanate to Rats. J. Path. Bact. **80,** 137. — 2. ELIAKIM, M., EISNER, M., UNGAR, H.: (1959) Experimental Intrahepatic Obstructive Jaundice Following Ingestion of Alpha-naphthyl Isothiocyanate. Bull. Res. Counc. Israel. **8E.** 7. — 3. GARAY, E. R., PEREZ, V., NOIR, B., ROYER, M.: (1965) Chronic Intoxication Induced by Alpha-naphthyl Isothiocyanate. Arch. Path. **80,** 127. — 4. GOLDFARB, S., SINGER, E. J., POPPER, H.: (1962) Experimental Cholangitis due to Alpha-napthyl Isothiocyanate. (ANIT) Amer. J. Path. **40,** 685. — 5. ITO, N., MARUGAMI, M., NAKAMURA, H., SASAKI, S., OKAJIMA, E., KITAMURA, H.: (1962) On the Relationship between the Functional Activity of Reticuloendothelial System and Quantitative Histology in the Liver of Rats Ingesting dl-ethionine, Thioacetamide or Alpha-naphthyl Isothiocyanate. Gann **53,** 235. — 6. LOPEZ, M., MAZZANTI, I.: (1955) Experimental Investigation on Alpha-naphthyl Isothiocyanate as a Hyperplastic Agent of the Biliary Ducts in the Rat. J. Path. Bact. **69,** 243. — 7. MCLEAN, M. R., REES, K. R.: (1958) Hyperplasia of Bile-ducts Induced by Alpha-naphthyl Isothiocyanate. Experimental Biliary Cirrhosis Free from Biliary Obstruction. J. Path. Bact. **76,** 175. — 8. MORAN, E., UNGAR, H.: (1964) The Effect of Intermittent Administration of Alpha-naphthyl Isothiocyanate to Rats. Amer. J. Path. **44,** 947. — 9. MORAN, E., ELIAKIM, M., SUCHOWOLSKI, A., UNGAR, H.: (1961) Serum Vitamin B 12 and Glutamic-oxalacetic Transaminase in Experimental Intrahepatic Obstructive Jaundice. Gastroenterology **40,** 408. — 10. MORAN, E. M., ELIAKIM, M., UNGAR, H.: (1965) The Effect of Cortisone on Cholangitis Induced by Alpha-naphthyl Isothiocyanate in Rats. Gastroenterology **48,** 773. — 11. PHILLIPS, M. J., STEINER, J. W.: (1964) Comparative Study of Alpha-naphthyl Isothiocyanate Induced Liver Injury. Lab. Invest. **13,** 779. — 12. ROBERTS, R. J., PHARM, B., PLAA, G. L.: (1966) The Effect of Bile Duct Ligation, Bile Duct Cannulation and Hypothermia on Alpha-naphthyl Isothiocyanate Induced Hyperbilirubinemia and Cholestasis in Rats. Gastroenterology **50,** 768. — 13. ROBERTS, R. J., PLAA, G. L.: (1965) Potentiation and Inhibition of Alpha-naphthyl Isothiocyanate Induced Hyperbilirubinemia and Cholestasis. J. Pharmacol. exp. Ther. **150,** 499. — 14. ROBERTS, R. J., PLAA, G. L.: (1967) Effect of Norethandrolone, Aceto-

hexamide and Enovid on Alpha-naphthyl Isothiocyanate-induced Hyperbilirubinaemia and Cholestasis. Biochem. Pharmacol. **16,** 827. — 15. ROUILLER, CH.: (1964) The Liver. Academic Press, New York. Vol. 2, P. 421. — 16. STEFENELLI, N., HOLZNER, J. H.: (1965) Cholestasis in the Rat by One Dosage Alpha-naphthyl Isothiocyanate (ANIT). Histological and Enzyme Histochemical Changes in the Liver. Z. ges. exp. Med. **139,** 762. — 17. STEINER, J. W., BAGLIO, C. M.: (1963) Electron microscopy of the Cytoplasm of Parenchymal Liver Cells in Alpha-naphthyl Isothiocyanate Induced Cirrhosis. Lab. Invest. **12,** 765. — 18. STEINER, J. W., CARRUTHERS, J. S.: (1963) Electron Microscopy of Hyperplastic Ductular Cells in Alpha-naphthyl Isothiocyanate Induced Cirrhosis. Lab. Invest. **12,** 471. — 19. STEINER, J. W., PHILLIPS, M. J., BAGLIO, C. M.: (1963) Electron Microscopy of the Excretory Pathways in the Life in Alpha-naphthyl Isothiocyanate Intoxication. Amer. J. Path. **43,** 677. — 20. STEINER, J. W., PHILLIPS, M. J., MIYAI, K.: (1964) Alpha-naphthyl Isothiocyanate (ANIT) Poisoning. Int. Rev. exp. Path. **3,** 65. — 21. UNGAR, H., MORAN, E., EISNER, M., ELIAKIM, M.: (1962) Rat Intrahepatic Biliary Tract Lesions from Alphanaphthyl Isothiocyanate. Arch. Path. **73,** 427. — 22. ZLOTNICK, A., HOROWITZ, A., UNGAR, H.: (1965) Alteration in Rat Liver Antigenicity by the Administration of Alpha-naphthyl Isothiocyanate. Israel J. Med. **1/2,** 244.

DIE WIRKUNG VON ALPHA-NAPHTHYL ISOTHIOCYANAT (ANIT) AUF DIE RATTENLEBER IM AKUTEN, SUBAKUTEN UND CHRONISCHEN EXPERIMENT

M. BALÁZS und E. MEDVECZKY

Einmalige orale Dosen von alpha-Naphthyl-isothiocyanat (ANIT) verursachen schwere Veränderungen der Gallenwege, die am frühesten in den septalen Gallengängen, nahe der Leberpforte, manifest werden. Zeichen von mechanischer Obstruktion können nicht beobachtet werden.

Am dritten Tag des akuten Versuchs ist das Epithel der interlobären Ducti und der intralobären Ductuli dermaßen geschwollen, daß das Lumen obstruiert oder sehr eingeengt wird. In Übereinstimmung hiermit zeigen die Serumbilirubinwerte einen Anstieg.

Im subakuten Experiment ist die Proliferation der Gallengänge vorherrschend, die von einer stark ausgeprägten Fibroblasten-Tätigkeit begleitet ist und in der 3—5 Woche eine schwere perilobuläre Fibrose herbeiführt.

Die schwere Veränderung erweist sich als reversibel, am Ende des 5. Monats entwickelt sich — ungeachtet der ununterbrochenen Verabreichung des Präparats — keine primäre biliäre Zirrhose, die Proliferation der Gallengänge sowie die Fibrose zeigen sogar einen erheblichen Rückgang. Parallel damit läßt sich indessen am Ende des chronischen Versuchs eine vakuoläre und fettige Degeneration der Leberparenchymzellen wahrnehmen.

Demnach übt das Präparat im akuten Stadium eine cholangiotoxische, im subakuten Stadium eine cholangioplastische und im chronischen Stadium eine hepatotoxische Wirkung aus.

ДЕЙСТВИЕ АЛЬФА-НАФТИЛ-ИЗОТИОЦИАНАТА (АНИТ) НА ПЕЧЕНЬ КРЫС В ОСТРЫХ, ПОДОСТРЫХ И ХРОНИЧЕСКИХ ОПЫТАХ

М. БАЛАЖ и Э. МЕДВЕЦКИ

Разовые пероральные дозы альфа-нафтил-изотиоцианата (АНИТ) вызывают тяжелую альтерацию желчных путей, что раньше всего проявляется в перегородочных желчных путях, близких к воротам печени.

Закупоривания желчных путей, указывающего на механическую обструкцию, не отмечается.

На третий день острого опыта эпителий междолевых протоков и внутридолевых канальцев оказывается до того набухшим и отечным, что их просвет или почти полностью исчезает или в значительной мере суживается. В соответствии с этим, в этот же срок величины сывороточного билирубина несколько повышены по сравнению с нормальной величиной, измеренной до этого срока.

В подостром эксперименте доминирует пролиферация желчных путей, сопровождающаяся очень выраженной деятельностью фибробластов, а на 3—5-ой неделе возникает тяжелый перилобулярный фиброз.

Тяжелое изменение обратимое. К концу 5-го месяца, вопреки непрерывной даче препарата — не развивается первичного билиарного цирроза, а даже пролиферация желчных путей и фиброз в существенной мере уменьшаются. Однако, параллельно с этим, к концу хронического опыта наблюдается вакуольное и жировое перерождение паренхиматозных клеток печени.

Следовательно, препарат оказывает в острой стадии холангиотоксическое, в подострой стадии—холангиопластическое, а в хронической стадии—гепатотоксическое действие.

Dr. Márta Balázs; Budapest XIII. Szabolcs u. 33, Hungary
Dr. Endre Medveczky; Budapest XI. Pihenő út 1., Hungary

Acta Morphologica Acad. Sci. hung. **16** *(4), pp. 425—430, 1968*

Institute of Pathophysiology, University Medical School, Budapest

MYOCARDIAL ENZYME ACTIVITY IN EXPERIMENTAL CARDIOMYOPATHY

J. Pucsok, J. Sós, T. Gáti and I. Szelényi

with the technical assistance of Anna Szabó, Margit Jóna and Mária Schnell

(Received October 5, 1967)

The cardiovasopathogenic diet representing a combination of pathogenic factor[s] affects the enzymes playing an important role in heart muscle metabolism. A marked decrease in succinic dehydrogenase activity was found, presumably owing to a detrimental effect of the diet on mitochondria. The decrease of alkaline phosphatase activity was indicative of a disturbance in anaerobic glycolysis. These changes signify the detrimental effect of the diet earlier than the classical histological changes [15].

It has been demonstrated in recent years that the same alimentary factors are involved in the focal heart muscle changes induced in animals by dietary means and in the pathogenesis of myocardial infarction of man. Selye et al. [22, 23, 24] used the term infarctoid cardiomyopathy to denote the heart muscle lesion induced by highly active corticosteroids in animals overloaded with sodium and phosphate salts. Suzuki [31] and Gresham [10] observed infarctoid changes on feeding fat-rich diets. Chappel et al. [6] induced myocardial necrosis by the administration of isoproterenol.

At our Institute Sós et al. [25, 26, 27] have confirmed the damaging effect on the heart muscle of a cardiovasopathogenic diet in the rat [28], the cock [29], the dog [30] and in the pig [15, 16, 17]. The diet contains a variety of noxious factors (Table 1), of which overloading with salt proved to represent the most decisive pathogenic factor. In this, in addition to the partial potassium and magnesium deficiency, the shift in the K : Na and Mg : Ca ratios played a significant role. Potassium deficiency is known to induce significant changes in heart muscle, especially with an associated chloride excess [11, 14]. Administration of magnesium prevents the development of such changes, and also those elicited by the cardiovasopathogenic diet [7, 8, 18, 19, 20, 28].

In the lesioned heart muscle the activity of certain enzymes involved in cardiac metabolism decreases while as a result of their liberation from disintegrating tissues the activity increases in serum. For example, at the site of a fresh infarction LDH activity decreases to about half the normal while in the serum LDH activity increases.

Continuing the investigations the changes in enzyme activity in response to feeding the cardiovasopathogenic diet were studied [12, 15]. In the present

work, attempts have been made to draw conclusions as to the metabolic changes produced by the diet by assaying the enzymes succinyl dehydrogenase (SDH) and alkaline phosphatase (AFA).

Experimental

The experiments were conducted on 160 male white rats of the same stock. One hundred rats were fed the cardiovasopathogenic diet and 60 were given a normal one in a daily dose of 10 g. Water was allowed ad libitum. Six weeks later the animals were anaesthetized by ether and exsanguinated from the abdominal aorta. The right and left halves of the heart were tested for enzyme activity histochemically, the rest of the body by biochemical methods.

Table I

Composition of cardiovasopathogenic (cvp) diet

Composition of cvp diet	g/kg	Salt mixture	μg/g	Trace salts	μg/g
Ground wheat	350	NaCl	270	$FeSO_4$	50
Casein	120	NaH_2PO_4	170	$MnCl_2$	20
Starch	296	Na_2HPO_4	170	$ZnCl_2$	17
Fat (suet)	150	$CaCO_3$	152	NaF	10
Dried yeast	20	Ca lactate	150	$CuSO_4$	2
Cod liver oil	10	$NaClO_4$	80	Arsenic acid	0.5
Cholesterol	2	Trace salts	8	Alum	0.5
Dried bile	2				
Lucerne extract	10				
Salt mixture	40				
Vitamin D_2	3 mg				

Cryostatic sections (10 μ) were tested for SDH according to CASCARANO and ZWEIFACH [5] and for AFA according to GROGG and PEARSE [9].

For the biochemical SDH assay the hearts were homogenized with quartz dust in cold distilled water, centrifuged at 3000 r.p.m. for 10 minutes and the supernatant was tested for SDH activity by the method of KUN and ABOOD [13], and at the same time for protein content according to BÜRGI et al. [4]. Enzyme activity was expressed as related to protein content.

Results

SDH activity of the hearts is shown in Fig. 1. As compared to normal activity, the decrease was marked in cardiopathy.

The state of cardiopathy, as compared to the normal controls, is illustrated in Figures 2a, b, c. When the experiment was terminated, in the cardiopathy group the heart showed degeneration, round cell infiltration (b) and at sites fibrosis (c).

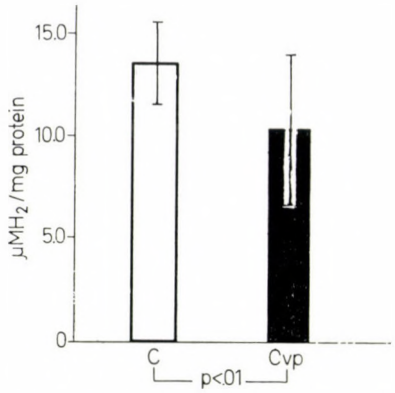


Fig. 1. Succinic dehydrogenase (SDH) activity of heart muscle following feeding a normal diet (C) and the cvp diet. As compared to the controls, SDH activity is significantly lower in the cvp group ($p < 0.01$)

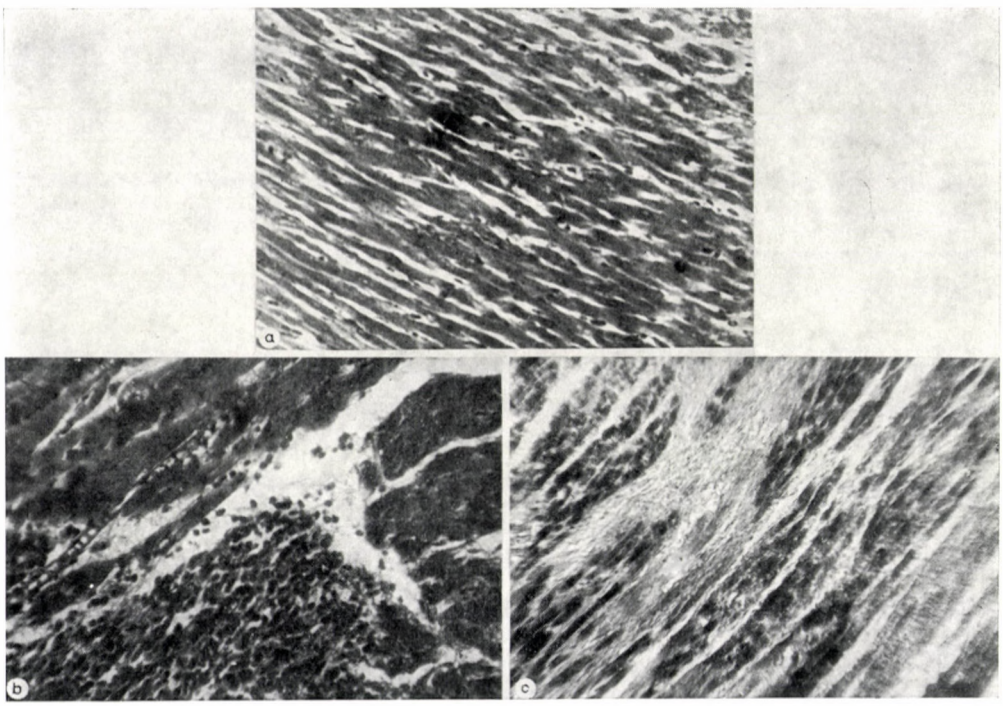


Fig. 2. Histologic appearance of hearts after staining with haematoxylin-eosin (HE). a: normal rat heart muscle. ×100. b: rat fed the cvp diet. Extensive myocardial damage, leucocytic infiltration. ×100. c: extensive fibrosis. ×100

Histochemical SDH activity is illustrated in Figures 3d, e and f. As related to normal activity (d, e), in the dietary group there was a focal decrease in the formazane granules indicative of SDH activity (f).

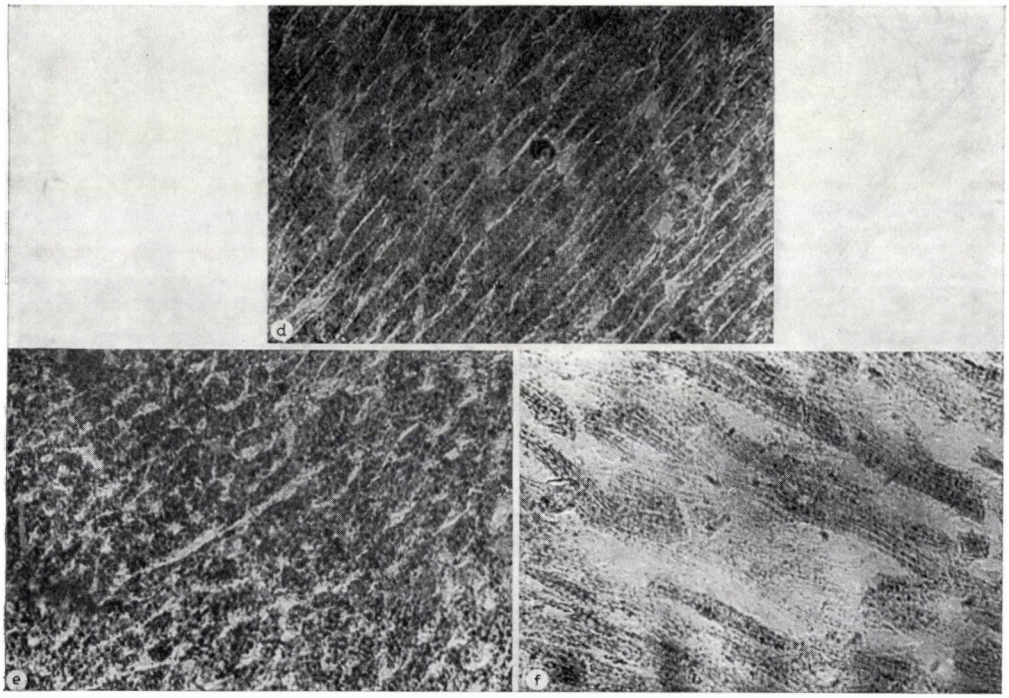


Fig. 3. Myocardial SDH activity. The formazane granules appearing as the end product of the histochemical reaction signify enzyme activity. d: normal SDH activity. ×100. e: normal SDH activity. ×150. f: SDH activity following feeding the cvp diet. Focal decrease of activity. ×100

AFA activity in heart muscle and in the coronaries is shown in Figures g, h. AFA activity in the muscle and the coronary wall (h) was lower than in the controls. AFA activity was decreased also in the aortic wall (Fig. 5i, j).

Discussion

The changes in enzyme activity in the heart muscle in infarctoid cardiomyopathies are known from the investigations of BAJUSZ and JASMIN [1, 2, 3]. In our own experiments the cardiovasopathogenic diet caused a decrease of SDH and AFA activities. Both these enzymes play an important role in myocardial metabolism.

SDH is an important mitochondrial enzyme. Its decrease can be understood in the knowledge of the mechanism of terminal oxidation. The coenzyme of SDH, which couples the hydrogen taken off from the foodstuffs is FAD. Re-oxidation of $FADH_2$ is carried out by the cytochromes activated with molecular O_2. In the absence of molecular O_2 the cytochromes are unable to re-oxidize

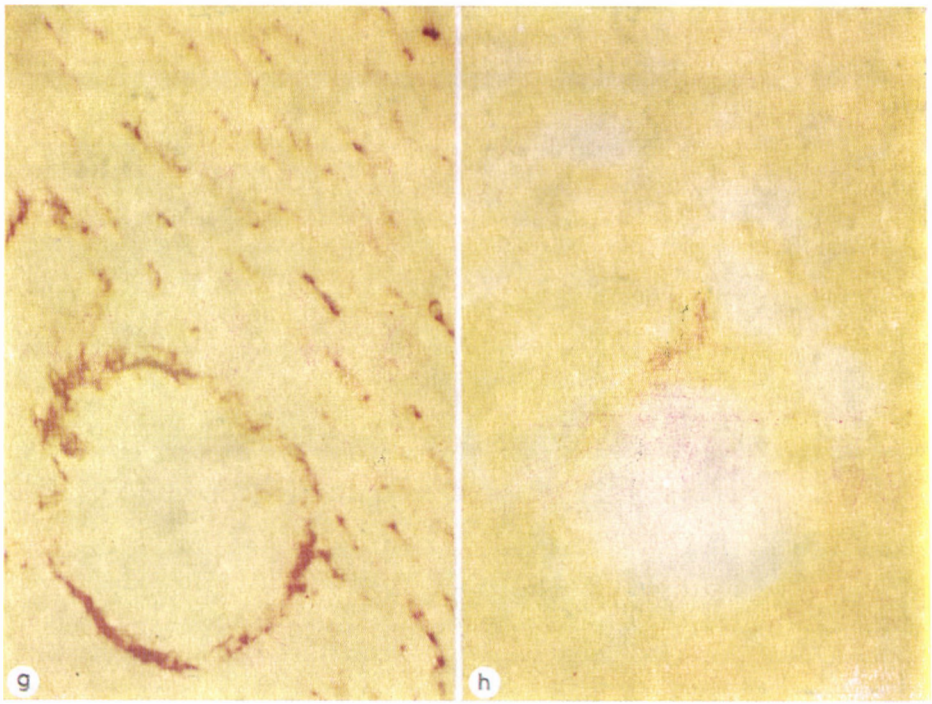


Fig. 4. Myocardial alkaline phosphatase (AFA) activity. g: AFA activity of normal heart muscle. ×150. h: Alkaline phosphatase (AFA) activity in the cvp group is hardly visible either in the myocardium or in the coronaries. ×150

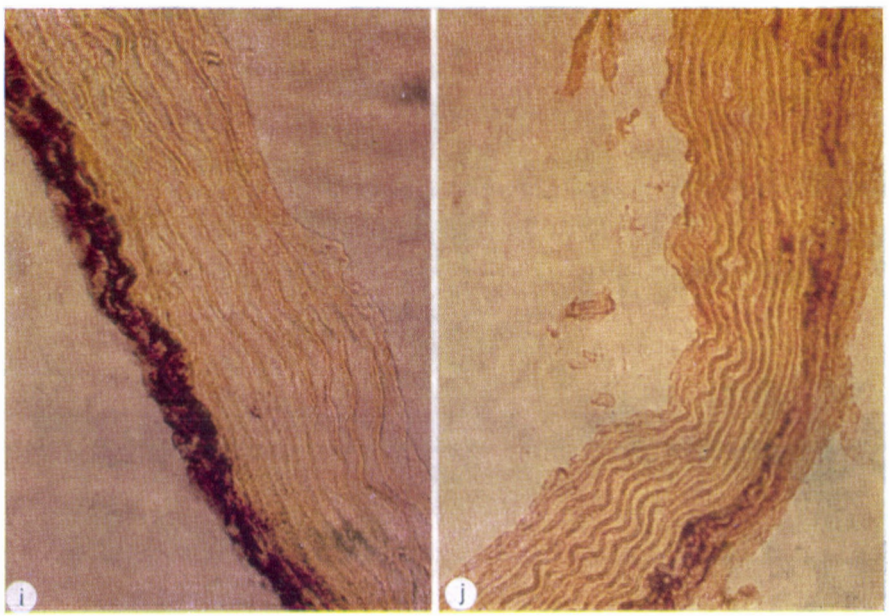


Fig. 5. Alkaline phosphatase (AFA) activity in aorta. i: normal activity. ×200. j: marked decrease of alkaline phosphatase activity in aorta in the cvp group. ×200

$FADH_2$ and thus SDH activity ceases. By histochemical methods a decrease in cytochrome activity is observable in hypoxia or anoxia.

AFA activity decreased both in heart muscle and the endothelium of the coronaries. This means that the diet had damaged not only the oxidative processes, but also anaerobic glycolysis.

The changes in the activity of other enzymes, e.g. acid phosphatase, esterase, lactate dehydrogenase, have been discussed in other papers [15, 21].

REFERENCES

1. Bajusz, E., Jasmin, G.: (1964) Enzyme Histochemistry of Various Necrotizing Cardiopathies. Fed. Proc. **22,** 253. — 2. Bajusz, E.: (1964) Monoamine Oxidase and Myocardial Infarction. Naturwissenschaften **51,** 389. — 3. Bajusz, E., Jasmin, G.: (1965) Observations on Histochemical Differential Diagnosis between Primary and Secondary Cardiomyopathies; Behavior of Monoamine Oxidase, Phosphorylase and Glycogen in Heart Muscle. Amer. Heart J. **69,** 83. — 4. Bürgi, W., Richterich, R., Brinet, M.: (1966) UV-photometric Determination of Total Cerebrospinal Fluid Proteins with a Modified Biuret Reagent. Clin. chim. Acta **15,** 181. — 5. Cascarano, J., Zweifach, B. W.: Comparative Histochemical and Quantitative Study of Adrenal and Kidney Tissue by Tetrazolium Technique. J. Histochem. Cytochem. **3,** 369. — 6. Chappel, C. I., Rona, Gy., Gaudry, R.: (1959) The Influence of Adrenal Cortical Steroids on Cardiac Necrosis Produced by Isoproterenol. Acta endocr. (Kbh.) **32,** 419. — 7. Gáti, T., Rigó, J., Gyenge, K., Sós, J.: (1964) Wirkung der kardiopathogenen Diät, von Ca und Mg auf die Grösse des Cold-pressor-Reflexes bei Ratten. Z. Kreisl. Forsch. **53,** 518. — 8. Gáti, T., Gyenge, K., Sós, J.: (1965) Die Wirkung des Monokalium + Monomagnesium-DL-Asparaginats sowie verschiedener Na-, K-,-Ca-, und Mg-Salze auf die Grösse des Cold-pressor-Reflexes bei Ratten. Arzneimittel-Forsch. **15,** 87. — 9. Grogg, E., Pearse, A. E. G.: (1952) Coupling Azo-Dye Methods for Histochemical Demonstration of Alkaline Phosphatase. Nature (Lond.) **170,** 578. — 10. Gresham, G. A., Howard, A. N.: (1960) Atherosclerosis and Coronary Heart Disease. Lancet **2,** 527. — 11. Grudner-Culemann, A.: (1952) Experimentelle und morphologische Untersuchungen über Veränderungen des Herzmuskels von Ratten bei Kalium-Mangelernährung. Arch. Kreisl.-Forsch. **18,** 185. — 12. Harmos, Gy., Pucsok, J., Romics, L., Csémi, A., Sós, J.: (1963) Histochemistry of Dietary Cardiac Lesions. Acta physiol. Acad. Sci. hung. **22,** Suppl. 15. — 13. Kun, E., Abood, G. L.: (1949) Colorimetric Estimation of Succinic Dehydrogenase by Triphenyltetrazolium Chloride. Science **109,** 144. — 14. Perkins, J. G., Petersen, A. B., Riley, J. A.: (1950) Renal and Cardiac Lesions in Potassium Deficiency Due to Chronic Diarrhea. Amer. J. Med. **8,** 115. — 15. Pucsok, J., Gáti, T., Sós, J., Zelles, T.: (1966) Histochemical Observations on Cardiopathic Rats and Miniature Pigs. Acta physiol. Acad. Sci. hung. **30,** Suppl. 338. — 16. Pucsok, J., Sós, J., Jáky, M.: (1966) Experimental Modification of Lipid Metabolism by Dietary Means. Med. u. Ernähr. **12,** 313. — 17. Pucsok, J., Sós, J., Jáky, M.: (1967) A lipoidforgalom diétás befolyásolása. Kísérl. Orvostud. **19,** 651. — 18. Rigó, J., Simon, Gy., Hegyváry, Cs., Sós, J.: (1963) Effect of Magnesium on Dietary Infarctoid Changes of the Heart. Acta med. Acad. Sci. hung. **19,** 231. — 19. Rigó, J., Szelényi, I.: (1963) Magnézium szerepe az idegrendszeri terheléssel súlyosbított alimentaris cardiopathiákban. Kísérl. Orvostud. **15,** 587. — 20. Rigó, J., Szelényi, I.: (1966) Magnesium bei nervös belasteten, alimentären Kardiomyopathien. Vitalst. Zivilis.-Krankh. **11,** 139. — 21. Selmeci, L., Pósch, E., Farkas, E., Szelényi, I., Sós, J.: (1966) Effect of Hypoxia on Lactic Acid Dehydrogenase (LDH) Activity in the Serum and Cardiac Muscle of the Rat. Acta physiol. Acad. Sci. hung. **30,** Suppl. 339. — 22. Selye, H., Bajusz, E.: (1959) Die Bedeutung von Elektrolyten bei der Prophylaxe experimenteller Herznekrosen. Arzneimittel-Forsch. **9,** 281. — 23. Selye, H., Bajusz, E.: (1960) A stress-kutatás újabb eredményei és a stress-elmélet szerepe a modern kórtani kutatómunkában. II. Kondicionáló tényezők az infarktoid cardiopathiák pathomechanizmusában. Orv. Hetil. **101,** 145. — 24. Selye, H., Bajusz, E.: (1959) Role of Sodium in Production of Myocardial Necroses by Stress. Proc. Soc. exp. Biol. (N. Y.) **100,** 11. — 25. Sós, J.: (1961) Az experimentális hypertonia és cardiopathia alimentáris tényezői. Magyar. Tud. Akad. Biol. orv. Oszt. Közl. **12,** 91. — 26. Sós, J.: (1962) Szervezeti károsodások alimentáris tényezői. Magy. Tud. Akad. Biol. orv. Oszt. Közl. **13,** 41. — 27. Sós, J.: (1965) An Investigation into the Nutritional Factors of Experimental Cardiopathy. In: Electrolytes and Cardiovascular Diseases, ed. E. Bajusz.

Karger, Basel. P. 161. — 28. Sós. J., Rigó, J., Gáti, T.: (1964) Die Wirkung von Asparaginat-Salzen auf die experimentelle Hypertonie, die myocardiale Veränderung und den Serum-Elektrolyt-Gehalt von Ratten. Arzneimittel-Forsch. **14,** 1134. — 30. Sós, J., Gáti, T., Kemény, T., Rigó, J.: (1964) Infarctoid Cardiac Lesions Induced by Dietetic Factors in the Dog. Acta med. Acad. Sci. hung. **20,** 1. — 31. Suzuki, M.: (1959) Plasma Cholesterol Levels in Rats Fed "Infarct-producing Diets". Proc. Soc. exp. Biol. (N. Y.) **102,** 544.

FERMENTAKTIVITÄT IM MYOKARD BEI EXPERIMENTELLER KARDIOPATHIE

J. PUCSOK, J. SÓS, T. GÁTI und I. SZELÉNYI

Eine kombinierte Kardiopathogene Diät setzt die Aktivität der myokardialen Fermente herab. Die ausgeprägte Verminderung der SDH-Aktivität ist wahrscheinlich ein Hinweis auf die mitochondrien-schädigende Wirkung der Diät. Die Herabsetzung der alkalischen Phosphatase-Aktivität zeigt eine Störung der anaeroben Glykolyse an. Die in der Aktivität der obigen Enzyme vor sich gehenden Veränderungen sind Anzeichen der schädigenden Wirkung der Diät, die zu einem früheren Zeitpunkt, als die klassische histologische Veränderung manifest werden.

ИЗМЕНЕНИЯ ФЕРМЕНТАТИВНОЙ АКТИВНОСТИ В МИОКАРДЕ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ КАРДИОПАТИИ

Й. ПУЧОК, Й. ШОШ, Т. ГАТИ, и И. СЕЛЕНЬИ

На основе многолетных наблюдений диета, содержащая комбинацию патогенных для кардио-васкулярной системы агентов, нарушает активность энзимов, играющих важную роль в обмене веществ сердечной мышцы. Выраженно снижается активность сорбитодегидрогеназы, что по всей вероятности указывает на поражение митохондриев под влиянием диеты. Уменьшение активности щелочной фосфатазы, по мнению авторов, указывает на расстройство анаэробного гликолиза. Изменения активности вышеуказанных энзимов выявляют вредное действие диеты раньше, чем классическое гистологическое изменение.

Dr. József Pucsok
Dr. József Sós
Dr. Tibor Gáti
Dr. István Szelényi
Budapest IX. Hőgyes Endre u. 9, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(4), pp. 431—438, 1968*

Institute of Pathology (Director: Prof. P. ENDES)
University Medical School, Debrecen, Hungary

DEHYDROGENASE AND NUCLEOPROTEID HISTOCHEMISTRY OF THE JUXTAGLOMERULAR COMPLEX

SZ. GOMBA, M. B. SOLTÉSZ, V. SZOKOLY and P. ENDES

(Received January 2, 1968)

The dehydrogenase and nucleoproteid histochemical investigation of the juxtaglomerular complex revealed a relative hypoactivity of the Krebs-cycle enzymes and a marked pyroninophilia in the cells of the macula densa. The granulated juxtaglomerular cells exhibited the same dehydrogenase reactions as the arteriolar smooth muscle cells. The granules themselves did not show any pyroninophilia. The relation between the enhanced macular pentose-phosphate cycle activity and the pyroninophilia and low Krebs-cycle activity is discussed.

The importance of the juxtaglomerular complex in controlling renal sodium excretion is becoming more and more evident [11]. It is supposed that the macula densa transmits information to the juxtaglomerular granulated cells (JGC) about the sodium content of the distal tubular fluid, this information influences the secretory activity of JGC, and the released renin and angiotensin change the glomerular filtration rate [30] or the proximal tubular sodium reabsorption [20]. The Goormaghtigh cells are probably also involved in this function [24].

The exploration of the biochemical aspects of this supposed feedback mechanism is difficult owing to the limited possibilities for the isolation of the above-mentioned cell groups. At present, histochemical methods seem to be more informative about this field than are the biochemical ones. The dehydrogenase histochemical investigation of the JG complex has detected an enhanced activity of the enzymes of the hexose monophosphate shunt in the mitochondria of the macular cells [13, 23, 31] and a moderate alpha glycerophosphate, lactate, and malate dehydrogenase activity of the JGC [14]. Considering the connection between the hexose monophosphate shunt and RNA production, HESS and PEARSE [13] investigated the RNA content of the macula cells, but the methyl green–pyronine stain did not reveal a higher degree of pyroninophilia.

In this paper we present further observations made on the dehydrogenase and RNA histochemistry of the JG complex.

Material and methods

Wistar rats of both sexes weighing about 200 g and albino mice of our own strain weighing about 20 g were used. The animals were kept on a normal diet and killed by opening the heart ventricles under superficial ether anesthesia. About 2 mm thick coronal slices from

the kidneys were immediately frozen by immersion into isopentane cooled with solid CO_2 and sectioned in a Pearse-SLEE cryostat. The 8 micra sections were mounted on cover glasses and incubated to demonstrate the localization of enzymes.

The following enzyme histochemical reactions were applied. For the demonstration of succinate dehydrogenase (SD) activity, the cobalt-MTT and nitro-BT method; for DPN-diaphorase and glucose-6-phosphate dehydrogenase (G—6—PD), the cobalt-MTT method; for malate (MD), glutamate (GD), isocitrate (ID) and lactate dehydrogenase (LD), the nitro-BT method; and for cytochrome oxidase (CO) Burstone's n-phenyl paraphenylene diamine method was used.

SD, DPN-diaphorase and LD activity were also demonstrated in cryostat sections prepared from blocks fixed for 10—60 minutes in 10% cold Ca formol adjusted to pH 7 with N NaOH. All the reactions were carried out according to PEARSE's prescriptions [26]. The controls were incubated in media containing no substrate.

Ribonucleic acid (RNA) staining was accomplished in Carnoy or cold 6% glutaraldehyde or cold 4% paraformaldehyde fixed, paraffin embedded material. The paraformaldehyde solution contained 0.25 M sucrose and was adjusted to pH 7.0—7.2 with N NaOH. In some cases cold neutral 10% Ca formol was also used as a fixative. The sections were stained with Einarson's gallocyaninchromalum solution [26] or with methyl green-pyronine according to KURNICK [26], diluting the methyl green-pyronine mixture with 30 ml 0.02 M veronal acetate buffer pH 4.4 instead of 30 ml distilled water and using Gurr's pyronine Y. The methyl green—pyronine staining gave good results after 2 hours fixation in paraformaldehyde, dehydration in acetone and paraffin embedding. Fixation, dehydration and imbibition with benzol were accomplished under continuous stirring.

The specificity of the RNA staining reaction was controlled by digestion with crystalline ribonuclease (RNAase, *Reanal*, Budapest) dissolved in distilled water (1—4 mg/ml) for 1—3 hours at 37 °C. Parallel sections were incubated in distilled water without RNAase.

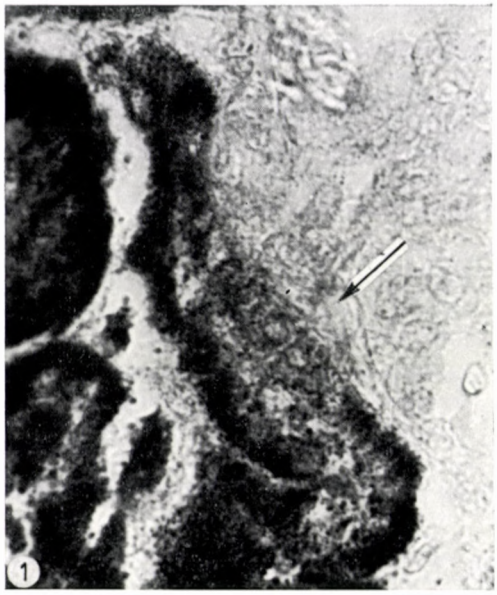
Results

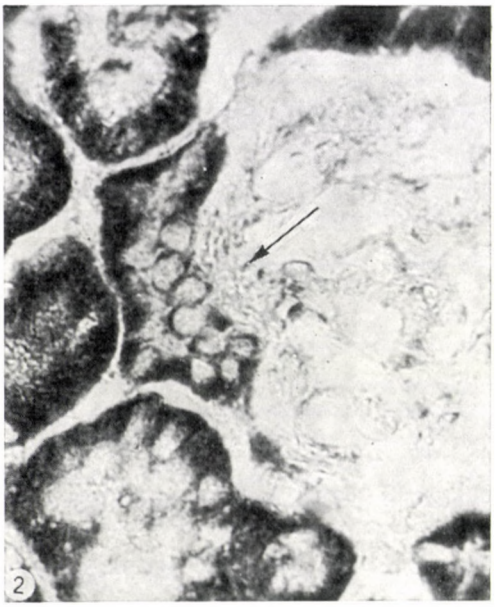
Macula densa. The G-6-PD reaction showed the well-known strong activity of the macular cells as compared to the distal tubular epithelium not being in contact with the hilum of the glomerulus. On the contrary, the SD, MD, GD, ID, LD, CO and DPN diaphorase reactions proved to be less active in the macular cells than in the non-macular ones (Figs 1—4).

The enhanced macular G-6-PD activity was more prominent in the rat than in the mouse. Both species showed the same hypoactivity of the above-mentioned dehydrogenases in the macula densa.

In the material fixed in 4% paraformaldehyde-sucrose for 2 hours, the supra- and infranuclear cytoplasm of the macular cells exhibited an enhanced pyroninophilia (Fig. 5) which was found to be eliminated by previous RNAase digestion. The pyroninophilia was unaltered after incubation for 4 hours in distilled water at 37 °C. Essentially the same pyroninophilia was observed in the glutaraldehyde or 10% neutral Ca formol fixed material, but never in Carnoy fixed tissue. The gallocyanin chromalum staining of the macular cell cytoplasm was also moderately stronger in comparison to the nonmacular cells.

Granulated juxtaglomerular cells (JGC). All the investigated dehydrogenases showed the same activity in the JGC and vascular smooth muscle cells. The SD, DPN-diaphorase (Fig 6.) and LD reactions carried out on fresh or formol fixed tissue revealed the same distribution of these enzymes in the JGC

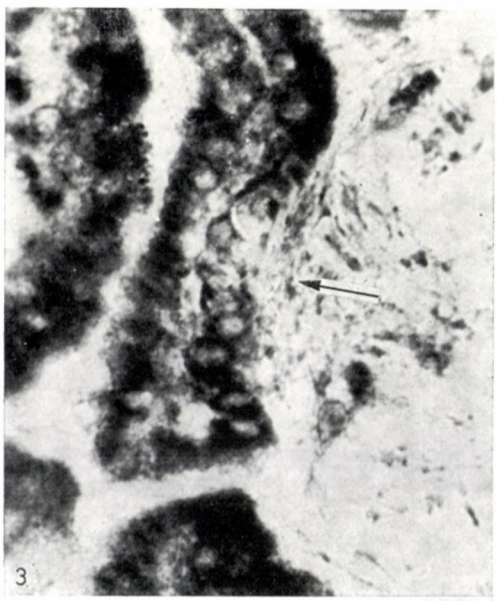




Figs. 1—4. Note the weakness of dehydrogenase reactions in the macular cells (arrows)

Fig. 1. Succinate dehydrogenase, mouse kidney, ×500

Fig. 2. Malate dehydrogenase, mouse kidney, ×500



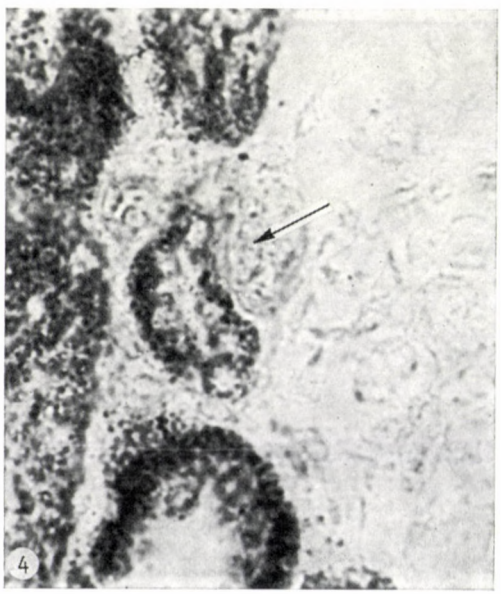


Fig. 3. Lactate dehydrogenase, rat kidney, ×500

Fig. 4. Cytochrome oxydase, rat kidney, ×500

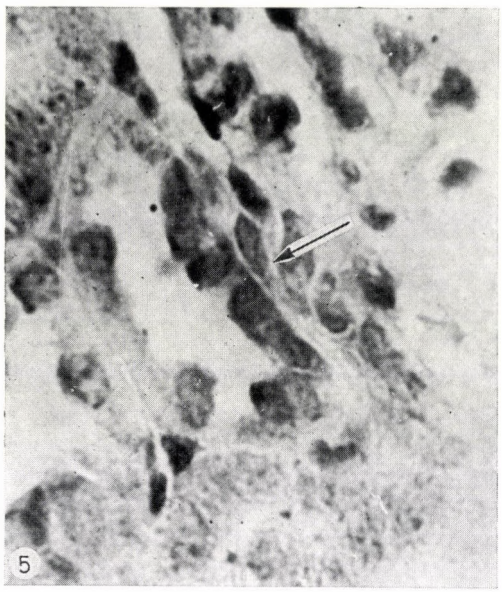


Fig. 5. Strong pyroninophilia in the macula densa cells (arrows). Paraformaldehyde fixed rat kidney, paraffin embedding, ×700

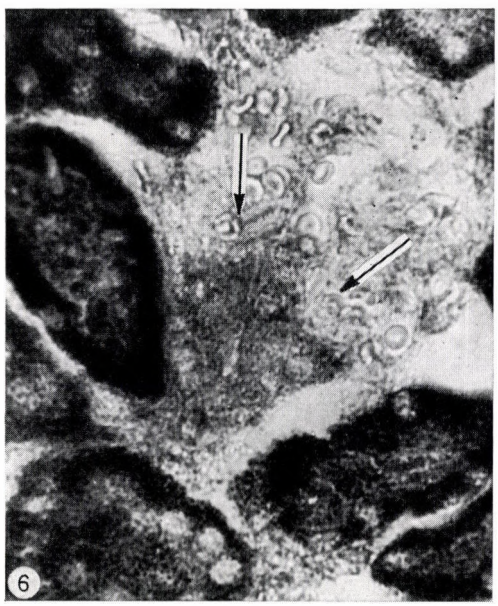


Fig. 6. Weak DPN-diaphorase reaction in the JG cells (arrows). Mouse kidney, 1 hour fixation in formol, frozen section, ×400

and vascular smooth muscle cells, and the picture was much the same as that of the cryostat sections except that the SD reaction was usually weaker.

The intergranular cytoplasm of the JGC exhibited a slight pyroninophilia in sections fixed in glutaraldehyde, paraformaldehyde or formol but was unstained in Carnoy fixed kidneys. The pyroninophilia was abolished by RNAase digestion. The JGC granules did not show any pyroninophilia. The intergranular substance stained faintly with gallocyanin chromalum while the JGC granules were left unstained in such specimens.

Goormaghtigh cells. The dehydrogenase methods revealed a few mitochondria in the cytoplasm but it was rather difficult to recognize them in unfixed cryostat sections. In paraformaldehyde fixed paraffin sections a slight cytoplasmic pyroninophilia was detectable.

Discussion

Since Hess and Pearse [13] detected the enhanced G-6-PD activity of renal macular cells in the normal rat, many investigators have dealt with the problem and showed G-6-PD hyperactivity also in the rabbit, mouse, guinea pig, cat, pig, and man [16, 17, 18].

Macular G-6-PD activity was also tested under different experimental conditions. Hess and Gross [12] and Hess and Regoli [15] demonstrated that constriction of the renal artery by a silver clip was followed by an increase of G-6-PD activity in the macula cells. Fisher [6, 7] and Gross and Hess [10] confirmed this statement. On the contrary, activity decreased almost to zero in rats rendered hypertensive by overdosage with DOC and saline while a reverse effect was obtained in adrenalectomized animals (Hess and Pearse [14], Hess and Gross [12]).

Another factor enhancing enzyme activity in the macula densa is a low sodium diet [21]. This is in harmony with Reeves, Velasco and Caravaca's [27] and Reeves and Sommers' [28] finding that sodium ions exert an inhibitory effect on macular G-6-PD activity *in vitro* while such an effect could not be demonstrated *in vivo*. Chronic administration of angiotensin caused a marked decrease of the reaction [22].

In some of the aforementioned experiments the renal renin content and juxtaglomerular cell index were also estimated. These parameters changed in every case parallel with macular G-6-PD activity; their dissociation was observed only in autotransplanted renal cortex [4, 9].

After various investigators [2, 3, 13, 23, 25, 31] had demonstrated 6-phosphogluconic acid dehydrogenase and TPN diaphorase activity in the macula densa, it was generally accepted that the macular G-6-PD content represented an enhanced pentose phosphate cycle (PPC) activity in that part of the nephron.

The majority of the speculations concerning the role of that cycle in the macula densa was based on BING's [1] view of the renin producing capacity of the macular cells. Knowing the possible role of the PPC in protein synthesis by delivering pentoses for RNA production it was tempting to conclude that the cycle was taking part in the renin synthetizing system of the macular cells. In spite of the fact that the overlapping histochemical localization of a strong G-6-PD reaction and pyroninophilia in rapidly growing (i.e. protein synthetizing) tissues is well demonstrable, HESS and PEARSE [13] were unable to detect histochemically a higher degree of pyroninophilia in the macular cells of material fixed in acetic ethanol. So they concluded that the macular PPC serves renin synthesis not by enhancing RNA synthesis with pentose production but furnishing energy for the synthetic processes by the direct oxidation of glucose [26].

The macular pyroninophilia appearing in our paraformaldehyde fixed material seemed to furnish a more direct proof of an enhanced protein synthetizing capacity. Aldehydes are usually not applied for the preservation of RNA in tissues, and the majority of authors prefers Carnoy's fluid, although e.g. PEARSE [26] recommended formol for that purpose. According to the result of RNAase digestion, the macular staining was not an unspecific one, nor was the RNA rendered RNAase resistant by the aldehydes. Thus, it is not clear why methyl green-pyronine staining and Einarson's method gave a negative result in Carnoy fixed kidney sections. Since electronmicroscopically the macula cells have no more ribosomes than the other distal tubular cells [19, 20], and no sign of any secretory or synthetic activity was observed in the macula densa [5], so it has not been possible to find a clear and unequivocal interpretation of the enhanced macular PPC activity and pyroninophilia.

Few data are available concerning the citric acid cycle (CAC) activity of the juxtaglomerular complex. HESS and PEARSE [14] observed a similar grade of malate and lactate dehydrogenase activity in the JGC and renal vascular smooth muscle cells and thus they concluded that JG cells originate from smooth muscle cells. Our results have confirmed that the CAC has the same activity in both types of cell. The reason why we investigated the dehydrogenases in the JGC also in formol fixed material was that we had observed a destruction of the specific JGC granules in fresh cryostat sections, which could influence and modify the histological picture of the dehydrogenase reaction. The destruction of the granules was well observable also in the course of the present work but the mitochondrial enzyme histochemical reactions of the JGC yielded practically the same results in unfixed and shortly formol-fixed material.

The relative hypoactivity of the CAC in the macular cells has not so far been described. One could assume that it resulted from the low number of mitochondria registered electronmicroscopically in the macula densa. To some extent this may be true but as far as we could estimate, even the individual macular

mitochondria were less active than mitochondria from other parts of the distal tubules. This suggests that the macular mitochondria are in some way "different" ones, having a relatively hyperactive PPC and hypoactive CAC, and makes it likely that macular cells get less energy from CAC than the other tubular cells do. One can speculate whether their energy requirement is comparatively low, or, also, the deficit is counterbalanced by direct oxidation of glucose in their PPC.

The methyl green-pyronine negativity of the JG granules is in accordance with our previous finding [8]. The positivity of the intergranular cytoplasm is in all probability connected with the rough endoplasmic reticulum, known to be engaged in cellular synthetic processes of the JGC.

REFERENCES

1. Bing, J.: (1964) Morphological Aspects of the Renin-angiotensin System. Dan. med. Bull. **11,** 24. — 2. Brown, J. J., Davies, D. L., Lever, A. F., Parker, R. A., Robertson, J. I. S.: (1965) The Assay of Renin in Single Glomeruli in the Normal Rabbit and the Appearance of the Juxtaglomerular Apparatus. J. Physiol. (Lond.) **176,** 418. — 3. Brown, J. J., Davies, D. L., Lever, A. F., Parker, R. A., Robertson, J. I. S.: (1966) The Assay of Renin in Single Glomeruli and the Appearance of the Juxtaglomerular Apparatus in the Rabbit Following Renal Artery Constriction. Clin. Sci. **30,** 223. — 4. Dévényi, I., Schill, H., Tessmann, D.: (1965) Enzymhistochemische und histologische Untersuchungen am Nierenautotransplantat der Ratte zur Frage der Abhängigkeit der Aktivität des juxtaglomerulären Zellapparates von der Nierenfunktion. Virchows Arch. path. Anat. **338,** 315. — 5. Fisher, E. R., Perez-Stable, E., Pardo, V.: (1966) Ultrastructural Studies in Hypertension. I. Comparison of Renal Vascular and Juxtaglomerular Cell Alterations in Essential Hypertension in Man. Lab. Invest. **15,** 1409. — 6. Fisher, E. R.: (1961) Correlation of Juxtaglomerular Granulation, Pressor Activity and Enzymes of Macula Densa in Experimental Hypertension. Lab. Invest. **10,** 707. — 7. Fisher, E. R.: (1961) The Role of Macula Densa in Renin Formation. Fed. Proc. **20,** 404. — 8. Gomba, Sz., Soltész, M. B., Endes, P.: (1962) Studies of the Granulated Cells of the Juxtaglomerular Apparatus. I. Acta morph. Acad. Sci. hung. **11,** 189. — 9. Gomba, Sz.: (1966) Studies on the Renin Content of Renal Cortex Autografts in Rats. Acta path. microbiol. scand. **68,** 206. — 10. Gross, F., Hess, R.: (1960) Histochemical Changes in Kidneys and in Salivary Glands of Rats with Experimental Hypertension. Proc. Soc. exp. Biol. (N. Y.) **104,** 509. — 11. Guyton, A. C., Langston, J. B., Navar, G.: (1964) Theory for Renal Autoregulation by Feedback at the Juxtaglomerular Apparatus. Circulat. Res., Suppl. 1 to Vols **14** and **15,** 187. — 12. Hess, R., Gross, F.: (1959) Glucose-6-phosphate dehydrogenase and Renin in Kidneys of Hypertensive or Adrenalectomized Rats. Amer. J. Physiol. **197,** 869. — 13. Hess, R., Pearse, A. G. E.: (1959) The Significance of Renal Glucose-6-phosphate Dehydrogenase in Experimental Hypertension in the Rat (a Histochemical Study). Brit. J. exp. Path. **40** 243. — 14. Hess, R., Pearse, A. G. E.: (1961) Mitochondrial Alpha-Glycerophosphate Dehydrogenase Activity of Juxtaglomerular Cells in Experimental Hypertension and Adrenal Insufficiency. Proc. Soc. exp. Biol. (N. Y.) **106,** 895. — 15. Hess, R., Regoli, D.: (1964) Correlation of Enzymatic Activity of the Juxtaglomerular Complex with Renin Content in Renal Hypertensive Rats. Brit. J. exp. Path. **45,** 666. — 16. Kazimierczak, J.: (1963) Histochemical Study of Oxidative Enzymes in Rabbit Kidney before and after Birth. Acta anat. (Basel) **55,** 352. — 17. Kazimierczak, J.: (1964) Glucose-6-phosphate Dehydrogenase and Alpha Glycerophosphate (menadion reductase) Dehydrogenase in Juxtaglomerular Apparatus of Various Species. 2nd Int. Congr. Histo-Cytochemistry, P. 207. — 18. Kazimierczak, J.: (1965) Development of the Glomerulus and Juxtaglomerular Apparatus. Acta path. microbiol. scand. **65,** 318. — 19. Latta, H., Maunsbach, A. B.: (1962) The Juxtaglomerular Apparatus as Studied Electronmicroscopically. J. Ultrastruct. Res. **6,** 547. — 20. Leyssac, P. P.: (1967) Intrarenal Function of Angiotensin. Fed. Proc. **26,** 55. — 21. Marx, A. J., Deane, H. W.: (1963) Histophysiologic Changes in the Kidney and Adrenal Cortex in Rats on a Low Sodium Diet. Endocrinology **73,** 317. — 22. Marx, A. J., Deane, H. W., Mowles, T. F., Sheppard, H.: (1963) Chronic Administration of Angiotensin in Rats: Changes in Blood Pressure, Renal and Adrenal Histo-

physiology and Aldosterone Production. Endocrinology, **73,** 329. — 23. NACHLAS, M. M., WALKER, D. G., SELIGMAN, A. M.: (1958) The Histochemical Localization of Triphosphopyridine Nucleotide Diaphorase. J. biophys. biochem. Cytol. **4,** 467. — 24. OBERLING, G. H., HATT, P. Y.: (1960) Étude de l'appareil juxtaglomerulaire du rat au microscope électronique. Ann. Anat. path. **5,** 441. — 25. PARKER, R. A.: (1964) The Microdissection and Staining of the Juxtaglomerular Apparatus in the Rabbit Kidney. J. Physiol. (Lond.) **171,** 31. — 26. PEARSE, A. G. E.: (1960) Histochemistry. Theoretical and Applied. Churchill, London. — 27. REEVES, G., VELASCO, C. A., CARAVACA, J.: (1964) Influence of Electrolytes and Hormones on the Activity of Glucose-6-phosphate Dehydrogenase. J. Histochem. Cytochem. **12,** 21. — 28. REEVES, G., SOMMERS, S. C.: (1965) Sensitivity of the Renal Macula Densa to Urinary Sodium. Proc. Soc. exp. Biol. (N. Y.) **120,** 324. — 29. THOENES, W.: (1961) Zur Feinstruktur der Macula densa im Nephron der Maus. Z. Zellforsch. **55,** 486. — 30. THURAU, K.: (1966) Influence of Sodium Concentration in Macula densa Cells on Tubular Sodium Load. Ann. N. Y. Acad. Sci. **139,** 388. — 31. WACHSTEIN, M., MEISEL, E.: (1959) Enzymatic Staining Reactions in the Kidneys of Potassium-depleted Rats. Amer. J. Path. **35,** 1189.

DEHYDROGENASEAKTIVITÄT UND NUCLEOPROTEIDE IM JUXTAGLOMERULÄREN KOMPLEX

SZ. GOMBA, M. B. SOLTÉSZ, V. SZOKOLY und P. ENDES

Bei der histochemischen Untersuchung der Dehydrogenaseaktivität und der Nucleoproteide im juxtaglomerulären Komplex wurde gesteigerte Pyroninophilie der Macula-densa-Zellen sowie herabgesetzte Aktivität der Enzyme des Krebs-Zyklus festgestellt. Die granulierten juxtaglomerulären Zellen zeigen die gleiche Dehydrogenaseaktivität wie die glatten Muskelzellen der Arteriolen. Die Körnchen sind nicht pyroninophil. Der Zusammenhang zwischen der bekannten gesteigerten Aktivität des Pentose-Phosphat-Zyklus, der Pyroninophilie und der geringen Aktivität der Enzyme des Krebs-Zyklus wird besprochen.

ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ДЕГИДРОГЕНАЗЫ И НУКЛЕОПРОТЕИДОВ ЮКСТАГЛОМЕРУЛЯРНОГО КОМПЛЕКСА

С. ГОМБА, М. Б. ШОЛТЕС, В. СОКОЛИ и П. ЭНДЕШ

При гистохимическом исследовании дегидрогеназы и нуклеопротеидов юкстагломерулярного комплекса было установлено повышение пирониноφилии клеток macula densa и уменьшение активности энзимов цикла Кребса. Активность дегидрогеназы гранулированных юкстагломерулярныхклеток и гладких мышц артериол—одинакова. Зернышки не показывают пирониноφилии. Обсуждается связь между общеизвестной повышенной активностью макулярного пентоз-фосфатного цикла, пирониноφилией и низкой активностью энзимов цикла Кребса.

Dr. Szabolcs GOMBA
Dr. Margit B. SOLTÉSZ
Dr. Viktor SZOKOLY
Dr. Pongrác ENDES

Debrecen, Kórbonctani Intézet, Hungary

Acta Morphologica Acad Sci. Hung. **16** *(4), pp. 439—453, 1968*

Institute of Pathological Anatomy and Pathohistology (Director: Prof. J. ORMOS), University Medical School, Szeged

HISTOENZYMOLOGY OF THE ANTIDIURETIC CENTRES

D. BARA and J. SKALICZKI

(Received January 3, 1968)

The activity of several enzymes involved in cellular metabolism has been studied by histochemical methods in the antidiuretic centres of normal and dehydrated rats.

Resting supraoptic and paraventricular ganglion cells exhibited strong lactic acid dehydrogenase and weak succinic dehydrogenase, glucose-6-phosphate dehydrogenase and cytochrome oxidase activity, indicating that anaerobic glycolysis predominates and the rate of oxidative metabolism is low in the hydroregulator nuclei of normal rats.

The activity of enzymes involved in fermentation or in the Szent-Györgyi—Krebs cycle was negligible in the hypertrophic ganglion cells of dehydrated animals. It was the primary oxidative decomposition of glucose (hexosemonophosphate shunt, pentose cycle) which increased in hyperactive ganglion cells; TPN-diaphorase and glucose-6-phosphate dehydrogenase activity became more pronounced in cases of neuronal hyperfunction.

In the anterior hypothalamo-neurohypophyseal apparatus only the capillaries exhibited alkaline phosphatase activity. Dehydration produced no change in this respect. Acid phosphatase activity which is vigorous in the ganglion cells of magnocellular nuclei even under physiological conditions, became, on the other hand, still more intensive in cases of neuronal hyperactivity, a phenomenon possibly correlated with increased protein synthesis.

Knowledge is scarce regarding details of the enzyme-histochemical structure of the hypothalamo-neurohypophyseal system. Certain enzymes are exceptions in this respect. The presence of acetylcholine esterase and a few other esterases has, for instance, repeatedly been demonstrated in the supraoptic and paraventricular nuclei [1, 2, 12, 15, 18, 22, 25, 32]. The invariable presence of acetylcholine esterase and its considerably increased activity in cases of dehydration [15, 25] support the theory of PICKFORD [26] that acetylcholine represents the chemical transmitter of antidiuretic centres. The negative or slightly positive monoamine oxidase reaction of neurosecretory nuclei [20, 31], too, points to the probability that one is dealing with cholinergic rather than adrenergic neurones.

ARVY [2] demonstrated the presence of a considerable amount of leucyl-β-naphthylamidase in the supraoptic and paraventricular nuclei of numerous vertebrates. Although many authors have studied the alkaline and acid phosphatase activity of magnocellular nuclei [3, 6, 28], results were ambiguous owing to unreliable techniques. MCMANUS et al. [21] observed a conspicuously large amount of 5-nucleotidase in the neurohypophysis, while OSINCHAK [24]

demonstrated thiamine-pyrophosphatase activity in the Golgi plexus of the ganglion cells of the supraoptic nucleus.

Details concerning the metabolism of the ganglion cells of the supraoptic and paraventricular nuclei have still to be elucidated. It was surprising to find that the activity of certain important enzymes, e.g. succinic dehydrogenase, cytochrome oxidase [2, 29, 30], peroxidase [14] and several amino oxidases [2], was weak in these nuclei of the central nervous system which are richly vascularized and have a vigorous metabolism.

We studied the distribution of several enzymes in the anterior hypothalamo-neurohypophyseal system of normal and dehydrated rats. Apart from alkaline and acid phosphatase, the intraneuronal activity of the following metabolically important enzymes was studied:

succinic dehydrogenase (Szent-Györgyi– Krebs cycle);

lactic acid dehydrogenase and α-glycerophosphate dehydrogenase (fermentation);

glucose-6-phosphate dehydrogenase (hexosemonophosphate shunt);

glutamic acid dehydrogenase (decomposition of glutamic acid);

DPN-diaphorase; TPN-diaphorase; cytochrome oxidase (terminal oxidation).

Material and methods

Eighty-five laboratory-bred white rats of both sexes, with body weights of 130 to 270 g, were used; 39 animals served as controls. The latter received compact dry rat food, and drinking water *ad libitum*. The 46 test animals were given 2.5% NaCl with the drinking water for 1 to 17 days. The animals were killed by decapitation, their brain and pituitary were promptly removed, the anterior hypothalamus together with the surrounding tissues as well as the pituitary gland placed on pieces of kidney or liver were frozen, and sections 20 μ thick were prepared in the cryostat.

The simultaneously coupling azo dye method was used for the demonstration of alkaline and acid phosphatase. Beside naphthol AS—TR phosphate, Brentamin Fast Blue and freshly hexazotized pararosaniline served as coupling agents. Beside the corresponding substrates sodium succinate, sodium lactate, Na-α-glycerophosphate, Na-L-glutamate, enzymatically reduced DPN and TPN, and glucose-6-phosphate, Nitro-BT was employed as hydrogen acceptor for the demonstration of succinic dehydrogenase, lactic acid dehydrogenase, α-glycerophosphate dehydrogenase, glutamic acid dehydrogenase, DPN- and TPN-diaphorase, and glucose-6-phosphate dehydrogenase. The incubating fluid contained DPN for the estimation of lactic acid dehydrogenase, α-glycerophosphate dehydrogenase and glutamic acid dehydrogenase, while TPN for the estimation of glucose-6-phosphate dehydrogenase. P-amino-diphenylamine and naphthol AS—L_4G served as substrates for cytochrome oxidase. As regards the technique of the reactions we refer to the textbook of BARKA and ANDERSON [4] which contains a list of reports published by the original authors of the procedures for the demonstration of the said enzymes.* We applied the reactions described in the textbook, most of which were modified by BARKA and ANDERSON and slightly further modified in the present experiments, working up 2 to 3 animals daily. The methods were checked in heat-inactivated sections (90 °C for 10 minutes), in sections incubated in substrate-free solutions or, in the case of cytochrome oxidase, in sections incubated in the presence of potassium cyanide.

* Alkaline and acid phosphatase: BURSTONE, 1958; succinic dehydrogenase: NACHLAS et al., 1957; lactic acid dehydrogenase, α-glycerophosphate dehydrogenase, glutamic acid dehydrogenase and glucose-6-phosphate dehydrogenase: NACHLAS et al. 1958 and HESS et al., 1958; DPN- and TPN-diaphorase: SCARPELLI et al., 1958; cytochrome oxidase: BURSTONE, 1959.

Results and discussion

Alkaline and acid phosphatase. Alkaline phosphatase was found only in the wall of capillaries. The blue azo dye produced by the enzymatic reaction affords a good outline of the supraoptic and paraventricular nuclei that are more richly capillarized than their surroundings (Fig. 1). The ground substance remained negative. The reaction in the vessels of the neurohypophysis was considerably stronger than in the anterior lobe. In the pars intermedia the enzyme activity was demonstrated only in a few but intensely reacting vessels (Fig. 2). Dehydration seemed to have no appreciable effect.

Acid phosphatase activity was present in the ganglion cells of the supraoptic and paraventricular nuclei. The vivid red reaction was restricted to the cytoplasm (Fig. 3). It was invariably increased in dehydrated animals (Fig. 4). Activity in the neurohypophysis was negligible and hardly localizable.

Alkaline phosphatase is supposed to play a significant part in the metabolism between capillaries and neural tissue. Since the vessels of the neurohypophysis are known to be permeable to large molecules [5], it was assumed that the enzyme was also involved in transporting the neurosecretion into the blood stream. KOBAYASHI et al. [16, 17] found that treatments which enhance the mobilization of neurohypophyseal hormones, failed to affect the alkaline phosphatase contents of the neurohypophysis. We, too, saw no change of alkaline phosphatase activity in the neurohypophysis of dehydrated and thus antidiuretically hyperactive animals. Alkaline phosphatase activity in the nuclei of the central nervous system is, according to ARVY [2], restricted to the veins, while only ATPase is present in the arterioles; LEGAIT et al. [19], experimenting with dehydrated animals, observed no change in respect of ATPase either.

ERÄNKÖ [6] attributed acid phosphatase activity in the perikaryon of supraoptic and paraventricular neurones to the intensive protein synthesis occurring in this area. Enzymatic activity in the hyperactive supraoptic and paraventricular nuclei of our dehydrated animals was considerably more vigorous than in the controls, in agreement with earlier reports [19, 27].

Until recent years numerous workers observed phosphatase activity in various neuronal constituents owing to the formation of diffusion artefacts. It is now known that alkaline phosphatase in the antidiuretic centres is restricted to the capillaries and acid phosphatase mainly to the perikaryon. The electron-microscopic histochemical observations of OSINCHAK [24] allow the conclusion that acid phosphatase is situated in the lysosomes of the body of ganglion cells and in certain Golgi cisterns. Burstone's azo method, as employed by us, appears to be eminently suitable for the light-microscopic localization of the examined phosphomonoesterases.

Succinic dehydrogenase was found in a pale, T-shaped area in the anterior hypothalamus at the plane of the supraoptic and paraventricular nuclei. A

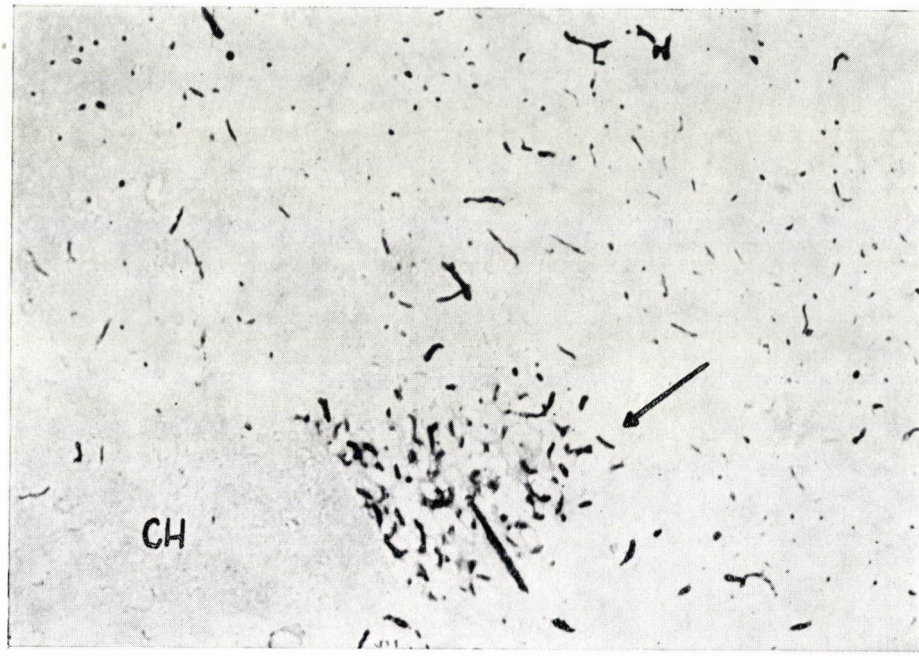


Fig. 1. Alkaline phosphatase reaction in rat brain. Only the wall of capillaries reacts positively. Arrow points to supraoptic nucleus which, more richly vascularized than its surroundings, is well outlined. Ch = optic chiasma. 70 ×

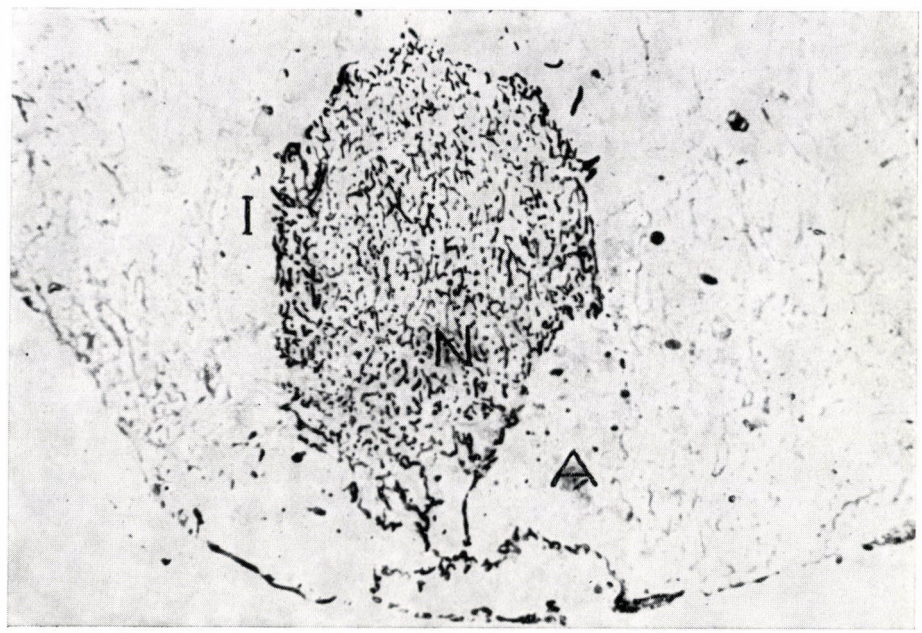


Fig. 2. Alkaline phosphatase activity of the pituitary is likewise restricted to the capillaries. Activity is stronger in the neurohypophysis (N) than in the anterior (A) or the intermediate lobe (I). 43 ×

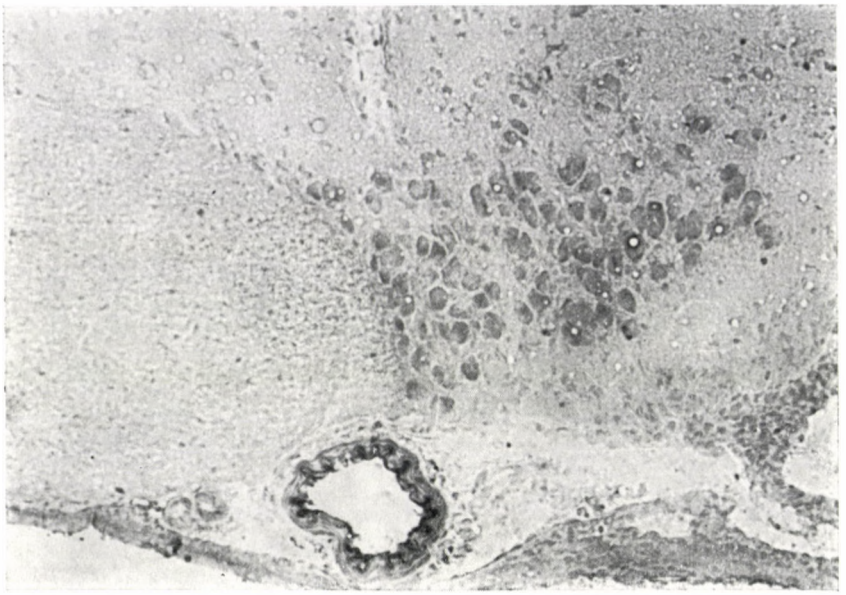


Fig. 3. Acid phosphatase reaction in the supraoptic nucleus of control rat. 140×

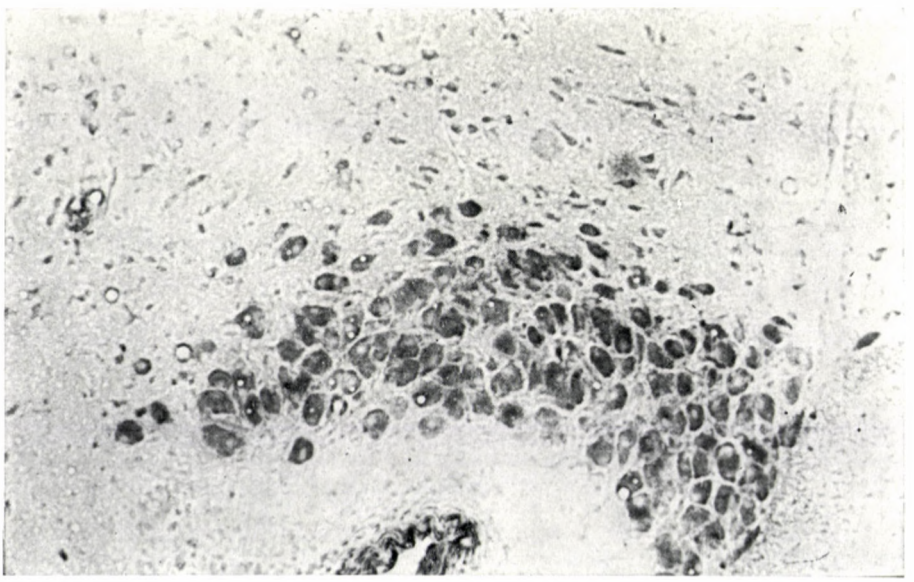


Fig. 4. Increased acid phosphatase activity in supraoptic ganglion cells of rat kept on salt water for 13 days. 140×

moderate amount of formazan was seen to have been produced in the cytoplasms of the supraoptic nucleus: the cells and the contours of the entire nucleus were blurred. Activity in the magnocellular nuclei was less marked than in their surroundings, owing to the number of nonreacting cell nuclei. On the other hand, formazan production was more intensive in the neurohypophysis than in the intermediate and anterior lobes, presumably because the neuhypophysis is more richly provided with positively reacting plasm than the other two lobes. Granular-guttate artefacts occur frequently in the neurohypophysis: they can be removed from the sections by preliminary treatment with acetone. Dehydration, i.e. neuronal hyperfunction, had no effect on the succinic dehydrogenase activity of ganglion cells; hypertrophic, bright ganglion cells bring, however, the nuclear group into sharper relief than in the controls (Fig. 5).

Succinic dehydrogenase oxidizes succinic acid to fumaric acid in the Szent-Györgyi—Krebs cycle, giving a hydrogen to the cytochrome system. In the brain, succinic dehydrogenase activity is displayed by the grey matter only [18, 23], the white substance is negative. The magnocellular nuclei of the hypothalamus are exceptions in this respect inasmuch as they show a weak succinic dehydrogenase activity. IFFT et al. [13] have shown, and our results have confirmed their finding, that dehydration (hyperfunction) does not enhance succinic dehydrogenase activity. (We cannot agree with the finding of KIVALO et al. (15) that enzymatic activity becomes more vigorous in such cases.) It was suggested by SHIMIZU et al. [29, 30] as also by ARVY [2] that anaerobic glycolysis is prevailing in neurosecretory nuclei. This suggestion was confirmed by FRIEDE and FLEMING [10] in rhesus monkeys for lactic acid dehydrogenase at least in respect of resting ganglion cells.

Lactic acid dehydrogenase activity was marked in the cytoplasm of the ganglion cells of the supraoptic and paraventricular nuclei in control rats (Fig. 6). The area of the suprachiasmatic nucleus was slightly outlined. Certain ganglion cells around the lateral area of the hypothalamus also exhibited marked positivity. The neuropil gave a variably strong diffuse, the white substance a weak reaction. The neurohypophysis displayed maplike patches. In dehydrated animals the activity decreased in the body of the hypertrophied ganglion cells (Fig. 7), while the neurohypophysis showed no change.

Alpha-glycerophosphate dehydrogenase activity was weak in the ganglion cells of the supraoptic nucleus: formazan production was like, or hardly more intensive than, in the surrounding tissues. The area of the paraventricular nucleus appeared to be paler than that of the surrounding tissues, and its cells could in most cases not be distinguished separately. Activity was likewise weak in the neurohypophysis. Dehydration had no effect on α-glycerophosphate dehydrogenase in the examined area.

These findings suggested that under physiological conditions anaerobic glycolysis is predominant in the perikaryon of the supraoptic and paraventricu-

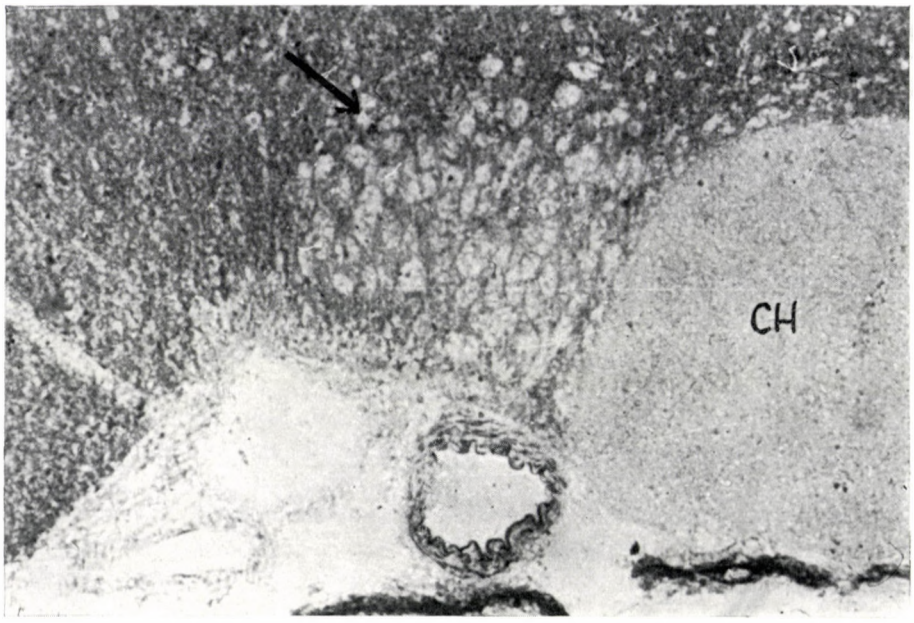


Fig. 5. Succinic dehydrogenase activity in the area of the supraoptic nucleus (arrow) is weaker than in the adjacent tissues of both control and dehydrated animals. Rat kept on salt water for 4 days. Ch = optic chiasma. 113×

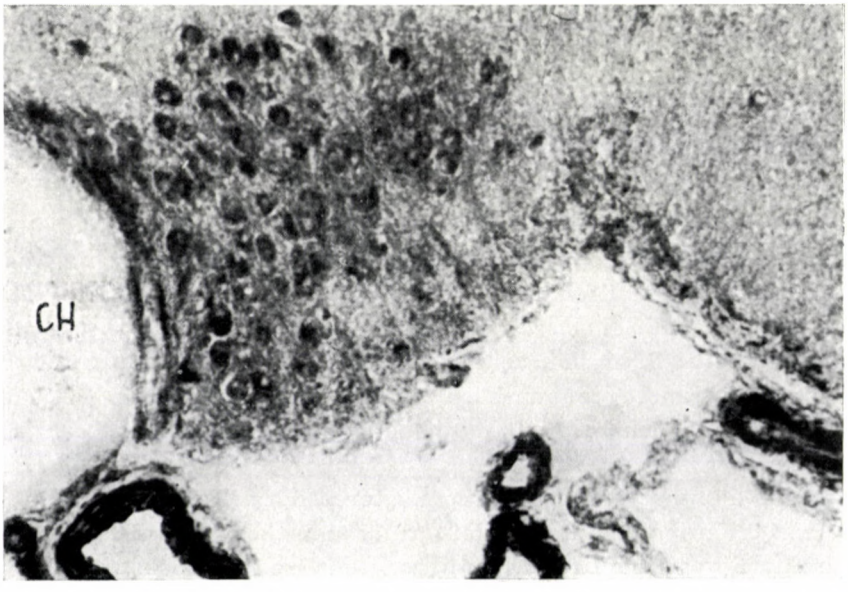


Fig. 6. Intensive lactic acid dehydrogenase reaction in the supraoptic ganglion cells of control animal. Ch = optic chiasma. 113×

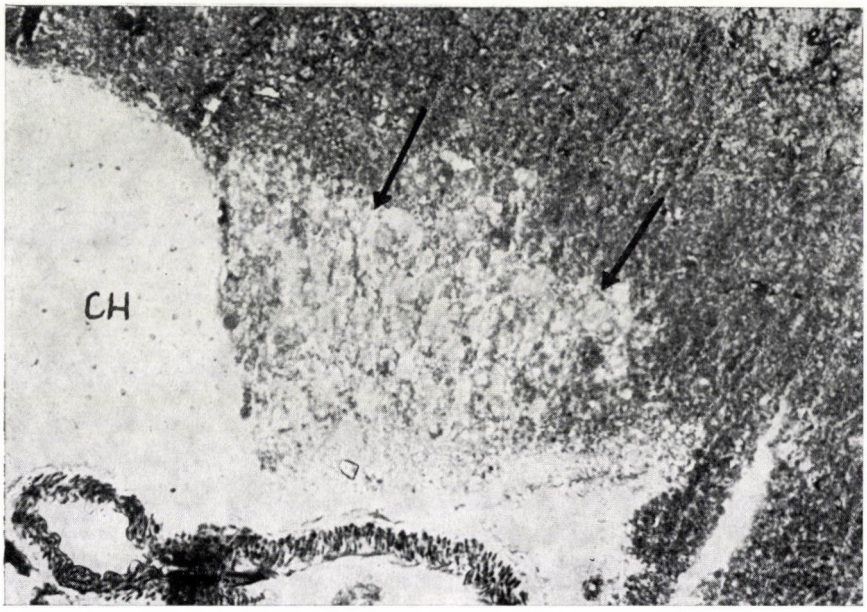


Fig. 7. Lactic dehydrogenase activity decreased in the hypertrophic ganglion cells of the supraoptic nucleus (arrow) of rat kept on salt water for 9 days, although incubation was unusually long (which explains the dark background of the surroundings). Ch = optic chiasma. 113 ×

lar neurones, while their oxidative metabolism (citrate cycle) is low. Glucose appears to be the chief source of fermentation since the activity of α-glycerophosphate dehydrogenase, indicative of glycerol fermentation, was insignificant. The nature of the process by which energy is produced in hyperactive ganglion cells is still obscure; they showed no fermentation, and activity of the enzymes involved in the Szent-Györgyi – Krebs cycle was negligible in them. Some information about the metabolic processes of hyperfunctioning ganglion cells was offered by the behaviour of TPN-diaphorase.

DPN- and TPN-diaphorase. The cerebral distribution of DPN-diaphorase was similar to that of succinic acid dehydrogenase [7], while formazan production seemed to be more pronounced. The magnocellular nuclei of the hypothalamus stood out of their surroundings. The cytoplasm of ganglion cells showed diffuse, finely granular activity (Fig. 8). While the distribution of TPN-diaphorase was like that of DPN-diaphorase, its activity was in most cases considerably less intensive. The amount of formazan in the supraoptic and paraventricular nuclei was negligible, the outlines of this group of nuclei were indistinct. The cells of the intermediate lobe displayed a more intensive activity than did those of the anterior lobe and the neurohypophysis.

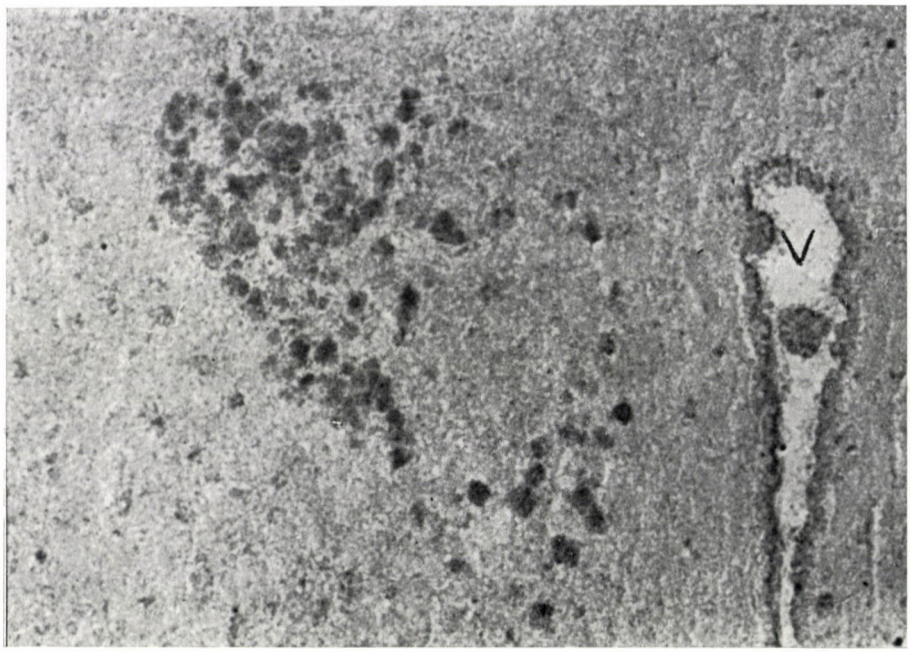


Fig. 8. DPN-diaphorase activity in the paraventricular nucleus of control animal. The reaction is more marked than in the adjacent tissues. In the ependymal cells of the third ventricle (V) activity is most pronounced in the layer of cells facing the ventricular cavity. 140 ×

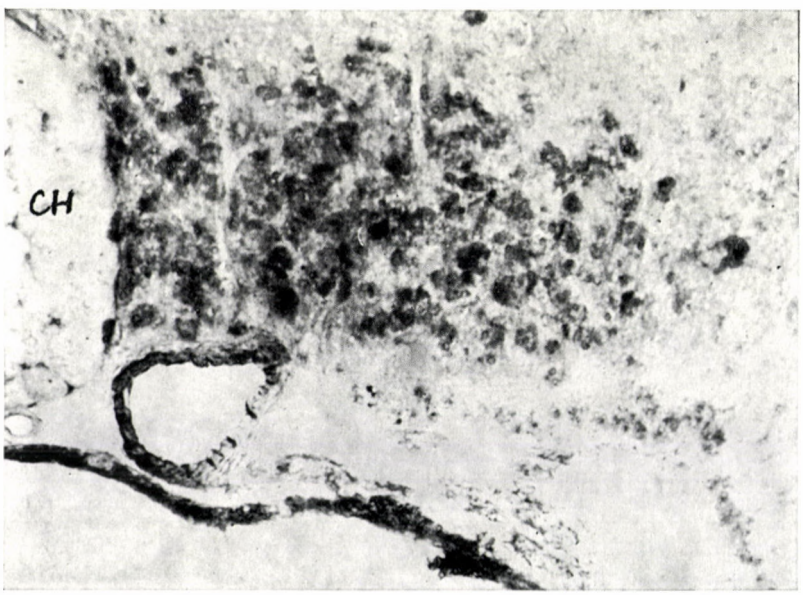


Fig. 9. Marked TPN-diaphorase activity in supraoptic nucleus of rat kept on salt water for 11 days. Hardly any formazan production at resting state. Ch = optic chiasma. 110 ×

Neuronal hyperfunction failed to affect the activity of DPN-diaphorase in the ganglion cells: like lactic acid dehydrogenase, it even decreased in some cases, whereas TPN-diaphorase activity became pronounced (Fig. 9). After dehydration, neurohypophyseal activity was sometimes restricted to the perivascular pituicytes.

DPN- and TPN-diaphorase, both of them oxidoreductases, are known to be involved in cellular respiration; they reoxidize reduced di- and triphosphopyridine nucleotides and release hydrogen to the cytochrome system. DPN-diaphorase activity was studied by FELGENHAUER and STAMMLER [7] in the brain of guinea pigs, and by FRIEDE and FLEMING [9] in that of humans. In the guinea pig hypothalamus, ganglion cells are well circumscribed in the magnocellular nuclei since their activity is more vigorous than in the neuropil, whereas in human subjects the cells of these nuclei cannot be distinguished from the surroundings because their activity is weak. Activity seemed to be pronounced in the antidiuretic nuclei of our rats.

Glucose-6-phosphate dehydrogenase. TPN-diaphorase activity was increased in the supraoptic and paraventricular nuclei of dehydrated animals. Since TPN-diaphorase transfers hydrogen from TPNH to the cytochrome system, it was logical to assume the presence of a considerable amount of TPNH. The latter is presumably involved in the primary oxidative degradation of glucose, the so-called hexose-monophosphate shunt. This was supported by our observation that, in contrast to the controls, the activity of glucose-6-phosphate dehydrogenase was marked in the ganglion cells of the magnocellular nuclei of dehydrated rats (Figs 10, 11). Direct oxidation of glucose-6-phosphate occurs in several endocrine glands; it is a principal source of TPNH [8]. Intensification of the hexose-monophosphate shunt (pentose cycle) in hyperactive ganglion cells is evidently in connection with the increased production of pentose nucleotides (ribonucleoproteins). In addition, the pentose cycle might have some role in the energy supply of the supraoptic and paraventricular nuclei, for the citrate cycle is insignificant in the ganglion cells of these nuclei even in cases of hyperfunction.

Our findings in respect of glucose-6-phosphate dehydrogenase are in some contrast to those of FRIEDE et al. [10, 11] inasmuch as they found a pronounced activity even under normal conditions. The discrepancy may be due to differences in the animal species and the methods used.

Cytochrome oxidase. As in the case of succinic dehydrogenase, it was the paleness of the supraoptic and paraventricular nuclei which distinguished them from the surrounding structures (Fig. 12). Activity was strongest in the neurohypophysis, which is rich in neuroplasm and comparatively poor in nuclei. Dehydration caused no significant change. It is nevertheless conceivable that the number of granules was somewhat increased in the cytoplasm, considering that the ganglion cells undergo hypertrophy under the effect of dehydration.

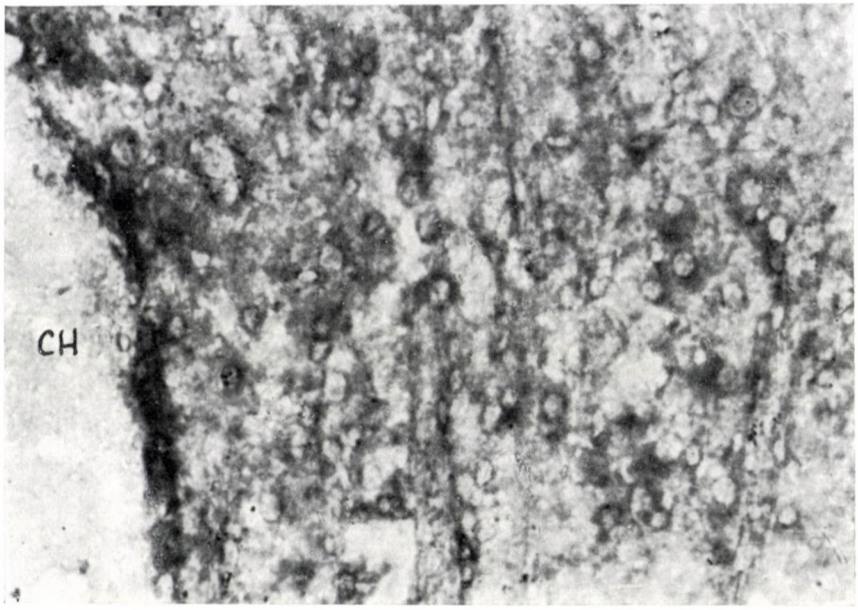


Fig. 10. Low glucose-6-phosphate dehydrogenase activity in the ganglion cells of the supraoptic nucleus of control animal. Ch = optic chiasma. 358 ×

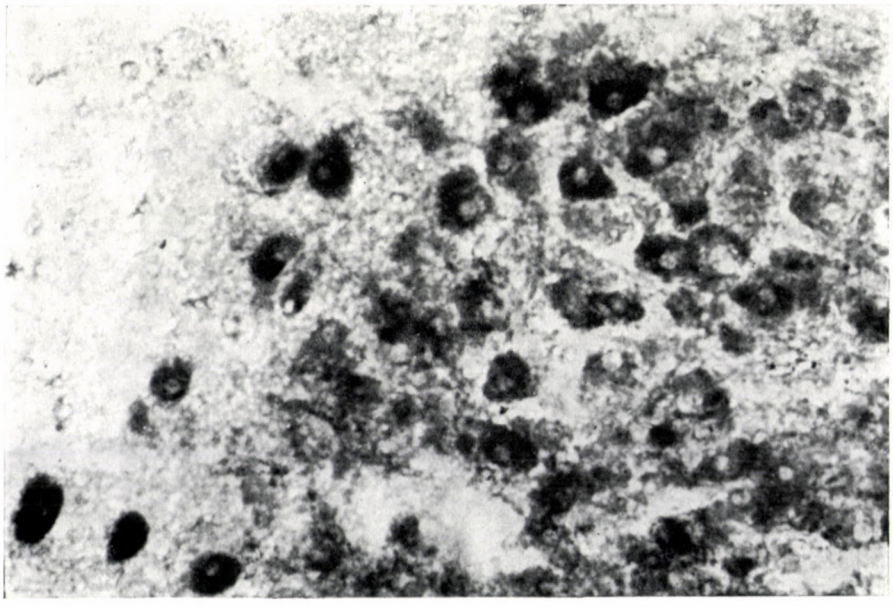


Fig. 11. Glucose-6-phosphate dehydrogenase activity in the supraoptic ganglion cells is significantly increased in the dehydrated (9 days on salt water) than in the control animal. 224 ×

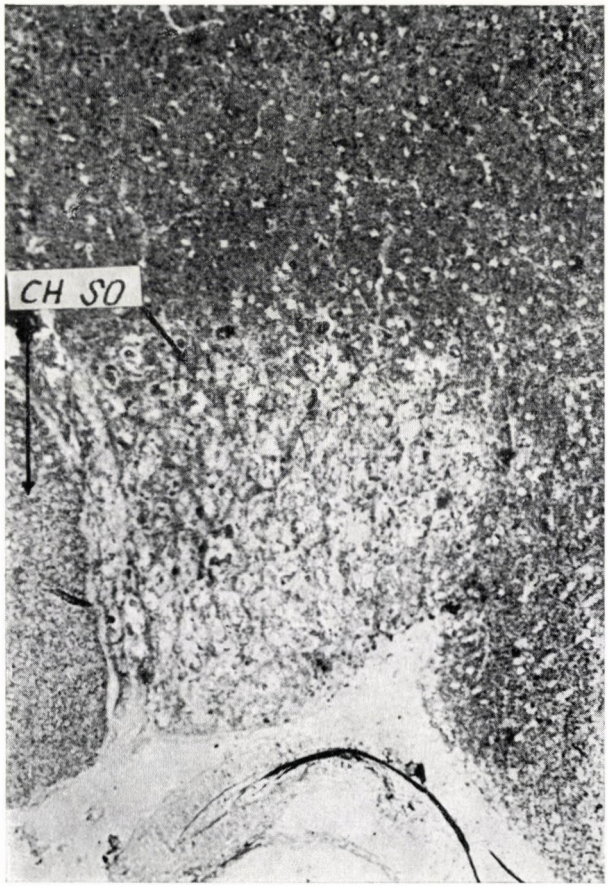


Fig. 12. Low cytochrome oxidase activity in the supraoptic region. SO = supraoptic nucleus. Ch = optic chiasma. 88 ×

The neurohypophysis of animals kept on salt water showed no appreciable change.

SHIMIZU et al. [30] found cytochrome oxidase activity likewise insignificant in the magnocellular nuclei of the rabbit. ARVY [2] obtained the same result in several species including rats. This phenomenon is in accord with the low degree of succinic dehydrogenase activity, i.e. with the low rate of oxidative metabolism, while it is also possible that the supraoptic and paraventricular ganglion cells contain enzymes which do not require the function of the cytochrome system.

Glutamic acid dehydrogenase was present in small amounts in the neurones of the anterior hypothalamus, and its activity was not enhanced by dehydration.

No reaction products were observed after inactivation or without substrate. Acid phosphatase and TPN-diaphorase activity increased in antidiuretic hyperfunction irrespective of the duration of salt treatment (3—16 or 1—13 days). The activity of lactic acid dehydrogenase after dehydration for 9, 11 and 13 days, and that of glucose-6-phosphate dehydrogenase after dehydration for 6, 7 and 9 days displayed identical changes. With the exception of glutamic acid dehydrogenase, all employed reactions brought the choroid plexus into sharp relief. Enzymatic activity in the ependymal cells lining the third ventricle was mostly restricted to the surface facing the ventricular cavity. Reactions occurring outside the magnocellular hypothalamic nuclei were beyond the scope of the present investigations.

REFERENCES

1. Abrahams, V. C., Koelle, G. B., Smart, P.: (1957) Histochemical Demonstration of Cholinesterases in the Hypothalamus of the Dog. J. Physiol. (Lond.) **139,** 137—144. — 2. Arvy, L.: (1962) Histochemical Demonstration of Enzymatic Activities in Neurosecretory Centres of Some Homoisothermic Animals. 3rd International Symposium on Neurosecretion. Bristol. 1961. Mem. Soc. Endocr. **12,** 215—225. — 3. Bargmann, W., Hild, W., Ortmann, R., Schiebler, T. H.: (1950) Morphologische und experimentelle Untersuchungen über das hypothalamisch-hypophysäre System. Acta neuroveg. (Wien) **1,** 233—275. — 4. Barka, T., Anderson, P. J.: (1963) Histochemistry: Theory, Practice and Bibliography. Hoeber, New York. — 5. Barrnett, R. J.: (1954) Histochemical Demonstration of Disulfide Groups in the Neurohypophysis under Normal and Experimental Conditions. Endocrinology **55,** 484—501. — 6. Eränkö, O.: (1951) Histochemical Evidence of Intense Phosphatase Activity in the Hypothalamic Magnocellular Nuclei of the Rat. Acta physiol. scand. **24,** 1—6. — 7. Felgenhauer, K., Stammler, A.: (1962) Das Verteilungsmuster der Dehydrogenasen und Diaphorasen im Zentralnervensystem des Meerschweinchens. Z. Zellforsch. **58,** 219—233. — 8. Field, J. B., Pastan, I., Herring, B., Johnson, P.: (1960) Studies of Pathways of Glucose Metabolism of Endocrine Tissues. Endocrinology **67,** 801—806. — 9. Friede, R. L., Fleming, L. M.: (1962) A Mapping of Oxidative Enzymes in the Human Brain. J. Neurochem. **9,** 179—198. — 10. Friede, R. L., Fleming, L. M.: (1963) A Mapping of the Distribution of Lactic Dehydrogenase in the Brain of the Rhesus Monkey. Amer. J. Anat. **113,** 215—234. — 11. Friede, R. L., Fleming, L. M., Knoller, M.: (1963) A Comparative Mapping of Enzymes Involved in Hexosemonophosphate Shunt and Citric Acid Cycle in the Brain. J. Neurochem. **10,** 263—277. — 12. Holmes, R. L.: (1961) Phosphatase and Cholinesterase in the Hypothalamo-hypophyseal System of the Monkey. J. Endocr. **23,** 63—67. — 13. Ifft, J. D., McNary, W. F., jr., Simoneit, L.: (1964) Succinic Dehydrogenase and RNA in the Supraoptic Nucleus and Hypothalamic Anterior Area in Dehydrated Rats. Proc. Soc. exp. Biol. (N. Y.) **117,** 170—171. — 14. Imoto, T.: (1957) Histochemical Studies on the Hypothalamo-hypophyseal Neurosecretory System. III. Vitamin C, Phosphatase, Peroxydase and Zinc. Arch. hist. (Jap.) **13,** 491—502. — 15. Kivalo, E., Rinne, U. K., Mäkelä, S.: (1959) Activity of Some Enzymes in the Hypothalamo-neurohypophyseal System in Experimental Dehydration and Rehydration. A histological study. Ann. Med. exp. Fenn. **37,** 28—32. — 16. Kobayashi, H., Farner, D. S.: (1960) The Effect of Photoperiodic Stimulation on Phosphatase Activity in the Hypothalamo-hypophyseal System of the Whitecrowned Sparrow, Zonotrichia leucophryis gambelii. Z. Zellforsch. **53,** 1—24. — 17. Kobayashi, H., Kambara, S.: (1959) Alkaline Phosphatase Activity in the Pituitary Body of the Rat. Endocrinology **64,** 615—618. — 18. Leduc, E. H., Wislocki, G. B.: (1952) The Histochemical Localization of Acid and Alkaline Phosphatases, Non-specific Esterase and Succinic Dehydrogenase in the Structures Comprising Hematoencephalic Barrier of the Rat. J. comp. Neurol. **97,** 241—280. — 19. Legait, E., Legait, H., Mercier, J. M. (1964): Contribution à l'étude histoenzymologique du lobe intermédiaire de l'hypophyse. C. R. Soc. Biol. (Paris) **158,** 851—854. — 20. Matsui, T., Kobayashi, H.: (1965) Histochemical Demonstration of Monoamine Oxidase in the Hypothalamohypophyseal System of the Tree Sparrow and the Rat. Z. Zellforsch. **68,** 172—182. — 21. McManus, J. F. A., Lupton, C. H., jr., Gardener Harden, B. A.: (1952) Histochemical Studies of 5-nucleotidase. Lab. Invest.

1, 480—487. — 22. OKINAKA, S., YOSHIKAWA, M., UONO, M., MOZAI, T., TOYOTA, M., MURO, T., IGATA, T., TANABE, H., UEDA, T.: (1962) Studies on the Neurohumoral Control of the Human Body and its Clinical Applications: Histochemical Study on Cholinesterase of the Human Hypothalamus. Acta neuroveg. (Wien) **22,** 53—62.— 23. ORTMANN, R., (1957) Histochemische Untersuchungen auf Succinodehydrogenase am Gehirn bei verschiedenen Vertebraten. Acta histochem. (Jena) **4,** 158—165. — 24. OSINCHAK, J.: (1964) Electron Microscopic Localization of Acid Phosphatase and Thiamine Pyrophosphatase Activity in Hypothalamic Neurosecretory Cells of the Rat. J. Cell Biol. **21,** 35—47. — 25. PEARSE, A. G. E.: (1958) Esterases of the Hypothalamus and Neurohypophysis and their Functional Significance. In: Pathophysiologia Diencephalica. Eds. S. B. Curri, L. Martini. Springer, Wien. Pp. 329—335. — 26. PICKFORD, M.: (1939) The Inhibitory Effect of Acetylcholine on Water Diuresis in the Dog, and its Pituitary Transmission. J. Physiol. (Lond.) **95,** 226—238. — 27. RINNE, U. K., KIVALO, E.: (1958) Effect of Dehydration and Rehydration on the Acid Phosphatase Activity of the Hypothalamic Magnocellular Nuclei. Ann. Med. exp. Fenn. **36,** 350—355. — 28. SHIMIZU, N. (1950) Histochemical Studies on the Phosphatase of the Nervous System. J. comp. Neurol. **93,** 201—217. — 29. SHIMIZU, N., MORIKAWA, N.: (1957) Histochemical Studies of Succinic Dehydrogenase in the Brain of Mice, Rats, Guinea Pigs and Rabbits. J. Histochem. Cytochem. **5,** 334—345. — 30. SHIMIZU, N., MORIKAWA, N., ISHII, Y.: (1957) Histochemical Studies of Succinic Dehydrogenase and Cytochrome Oxidase of the Rabbit Brain, with Special Reference to the Results in the Paraventricular Structures. J. comp. Neurol. **108,** 1—21. — 31. SHIMIZU, N., MORIKAWA, N., OKADA, M.: (1959) Histochemical Studies of Monoamine Oxidase of the Brain of Rodents. Z. Zellforsch. **49,** 389—400. — 32. SLOPER, J. C.: (1955) Hypothalamic Neurosecretion in the Dog and Rat, with Particular Reference to the Identification of Neurosecretory Material with Posterior Lobe Hormone. J. Anat. (Lond.) **89,** 301—316.

HISTOENZYMOLOGIE DER ANTIDIURETISCHEN ZENTREN

D. BARA und J. SKALICZKI

In den antidiuretischen Zentren normaler und dehydrierter Ratten wurde die Aktivität mehrerer Enzyme histochemisch untersucht.

Im Plasma der supraoptischen und paraventrikulären Ganglienzellen läßt sich im Ruhezustand eine erhebliche Lactat-dehydrogenaseaktivität beobachten. Die Aktivität der Sukzino-dehydrogenase, Glukose-6-phosphatdehydrogenase und Cytochromoxydase ist indessen gering. Diese Beobachtungen deuten darauf, daß in den Hydroregulatorkernen normaler Ratten die anaerobe Glykolyse vorherrscht, der oxydative Stoffwechsel hingegen unbedeutend ist.

In den hyperfunktionierenden, hypertrophierten Ganglienzellen dehydrierter Tiere ist die Aktivität der Enzyme des Krebs-Zyklus unbedeutend. In der hyperfunktionierenden Ganglienzelle zeigt der primäre, oxydative Glukose-Abbau (Hexosemonophosphat-Shunt, Pentose-Zyklus) einen Anstieg. Bei neuronaler Hyperfunktion ist nämlich die TPN-Diaphorase- sowie die Glukose-6-Phosphat-Dehydrogenaseaktivität ausgeprägter als in den Kontroll-Ganglienzellen.

Im vorderen hypothalamo-neurohypophysären System zeigt nur das Gefäßnetz eine alkalische Phosphatase-Reaktion. Nach Dehydratation erfolgt keine Aktivitätsveränderung. Die im Plasma der magnozellulärer Kerne nachweisbare lebhafte saure Phosphatase wird bei neuronaler Hyperfunktion intensiver. Diese Erscheinung dürfte mit der gesteigerten Proteinsynthese im Zusammenhang stehen.

ГИСТОЭНЗИМОЛОГИЯ АНТИДИУРЕТИЧЕСКИХ ЦЕНТРОВ

Д. БАРА и Й. ШКАЛИЦКИ

Активность нескольких энзимов, играющих важную роль в клеточном обмене веществ, была изучена — с помощью гистохимических методов исследования — в антидиуретических центрах у нормальных и дегидрированных крыс.

В плазме супраоптических и паравентрикулярных ганглионарных клеток в состоянии покоя наблюдается значительная реакция дегидрогеназы молочной кислоты. В то же время активности сукцинодегидрогеназы, глюкозо-6-фосфат-дегидрогеназы и

цитохромоксидазы — незначительны. Эти наблюдения указывают на то, что в гидрорегуляторных ядрах нормальных крыс доминирует анаэробный гликолиз, причем окислительный обмен веществ низок.

В гиперфункционирующих, гипертрофированных ганглионарных клетках дегидрированных животных активность энзимов, указывающих на ферментацию, и энзимов цикла Сент-Дьёрдьи–Кребса — невелика. В гиперфункционирующей ганглионарной клетке усиливается первичное окислительное расщепление глюкозы [так наз. гексозомонофосфатный шунт, цикл пентозы]. Дело в том, что при нейрональной гиперфункции активность ТПН-азы и глюкозо-6-фосфат-дегидрогеназы более выражена, чем в норме.

В системе: передний гипотамус — нейрогипофиз реакцию щелочной фосфатазы показывает лишь сосудистая сеть. При дегидратации животных активность не изменяется. Реакция кислой фосфатазы, выявляемая в плазме ганглионарных клеток магноцеллюлярных ядер, которая и в норме очень интенсивна, в случае нейрональной гиперфункции еще более усиливается. Это явление, предположительно, связано с повышением синтеза белков.

Dr. Dénes Bara
Dr. József Skaliczki
Szeged, Kórbonctani Intézet, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(4), pp. 455—462. 1968*

Department of General Zoology and Comparative Anatomy (Director: G. MÖDLINGER), Eötvös Loránd University of Sciences, Budapest

RESPONSE OF THE ADRENAL GLAND TO THE ACTIVATION OF THE SUBCOMMISSURAL ORGAN IN THE PIGEON

P. PÉCZELY and T. MURAY

(Received January 30, 1968)

The peripheral interrenal matter of the adrenal gland and the subcommissural organ have been studied by histologic methods. In the peripheral zone treatment with prednisolone, or with prednisolone and subsequently ACTH produced no changes, while insulin treatment and insulin loading following prednisolone administration resulted in hypertrophy. The zone lying deeper showed atrophy following prednisolone treatment and hypertrophy in response to insulin treatment, in the course of treatment with prednisolone and ACTH the atrophy which had developed in response to prednisolone administration disappeared. The subcommissural organ responded to treatment with insulin and with prednisolone and insulin by release of Gomori-positive fluid, and in the case of insulin treatment an increase of nuclear volume. The treatment caused no substantial increase of nuclear volume in the aqueduct or in the mural ependyma of the third ventricle. The results were suggestive of a functional correlation between the subcommissural organ and the peripheral zone of the adrenal gland.

A study has been made of the correlation between the function of the subcommissural organ and that of the peripheral zone of the adrenal gland. This was based on the one hand upon the results obtained by KONDICS, according to which the avian adrenal gland would not represent a uniform organ, but is divided into a peripheral part producing presumably aldosterone, and a deeper one producing glycocorticoids [5, 6, 7, 8]. On the other hand, our previous investigations suggested that the deeper zone of the interrenal matter of the adrenal gland was controlled by the pituitary hypothalamic system [16, 17, 18], and a functional relationship could be assumed to exist between the subcommissural organ and the peripheral zone of the adrenal gland [19]. The evidence published by PALKOVITS and LUKÁCS [13] suggests that adrenaline may cause a release of subcommissural secretion. In our case insulin treatment seemed to be a more adequate activator, since adrenaline has a weak effect on the avian adrenal [12], while treatment with insulin induces a marked hypertrophy of the interrenal matter [9]. As insulin treatment elicits adrenaline secretion, we have assumed that the elevation of the endogenous adrenaline level would activate the subcommissural organ.

Material and methods

The experiments were conducted in September and October, 1966. The animals were exposed to light 11 hours every day. The following experimental groups were used:

1. Control group (8 pigeons)

2. Prednisolone group (8 pigeons). The birds were given 5 mg of prednisolone daily in two equal doses, one at 10 a.m. the other at 6 p.m., over a period of 14 days.

3. Prednisolone + insulin group (8 pigeons). Over a period of 14 days the birds were given 5 mg of prednisolone daily in two doses, one at 10 a.m. and the other at 6 p.m. On the 14th day of treatment 4 units of protein zinc insulin were injected subcutaneously at 10 a.m. and a same dose at 6 p.m. The birds were killed 24 hours after the first injection.

4. Prednisolone + ACTH group (8 pigeons). Like in group 3, on the 14th day of prednisolone treatment each bird received 8 I.U. of ACTH at 10 a.m. and a same dose at 6 p.m., intramuscularly. The birds were killed 24 hours after the first injection.

5. Insulin group (9 pigeons). At 0 and at 8 hours the birds received subcutaneously 8 I.U. of protein zinc insulin. The birds were killed 24 hours after the first injection.

The birds were killed by decapitation. The brain and adrenals were fixed in Heidenhain's Susa. After double embedding according to PÉTERFI 5 μ sections were cut. The subcommissural organ or the mural ependyma was stained with paraldehydefuchsin and haematoxylin, the adrenals with haematoxylin-eosin. The sections were evaluated partly on the basis of the histologic pattern, partly by measuring the changes in nuclear volume.

In each case we measured the diameter of 200 nuclei in the subcommissural organ, mural ependyma and the peripheral zone of the adrenal gland, at $\times 2.500$ projection magnification. Values for nuclear volume were computed on the basis of the Fischer-Inke nomogram by means of the formula $\frac{\pi}{6} LB^2$. The frequency distribution of the log values for nuclear volume for the control and treated groups were determined. Significance was estimated by the t test. The difference from the arithmetic means was considered significant when $t \geqq 1.96$.

Results

In the control group, in the ependymal layer the apical secretion was clearly visible, the cytoplasm contained fine granules. At the bottom of the ependymal layer granulation was rough. In some ependymal cells well-defined vacuoles with rough aldehyde-fuchsin positive granulation were apparent. The nuclear volume curve was regular, with the maximum in the 1.6 class (Fig. 1).

Following insulin treatment apical secretion was less than in the controls, the basal granulation completely disappeared, the cytoplasm of the ependymal cells contained large amounts of fine, dust-like secretion. The nuclear volume value was significantly higher than in the controls, the maximum almost levelling out between the 1.7 and 1.8 values ($t = 6.10$) (Fig. 2).

In the prednisolone-treated group the histologic patterns were similar to those in the controls. There was a characteristic intensive basal and apical secretion of great in the subcommissural organ. The fine granulation of the cytoplasm was like in the controls; nuclear volume was smaller, the curve shifted to the left, the maximum was in the 1.5 class ($t = -4.12$).

On treatment with prednisolone and insulin the fine and rough granular secretion was evacuated from the subcommissural organ. Nuclear volume was significantly lower than in the controls, its curve almost coincided with that for the prednisolone-treated group ($t = -5.30$).

In the prednisolone + ACTH group the histologic patterns were the same as in the control and the prednisolone-treated groups. The nuclear volume value was significantly lower than in the former groups ($t = -2.83$) (Fig. 4).

To decide to what extent the treatments applied are specific for the subcommissural organ, we evaluated statistically the nuclear variations in the aqueduct and the mural ependyma of the third ventricle. It was found that the

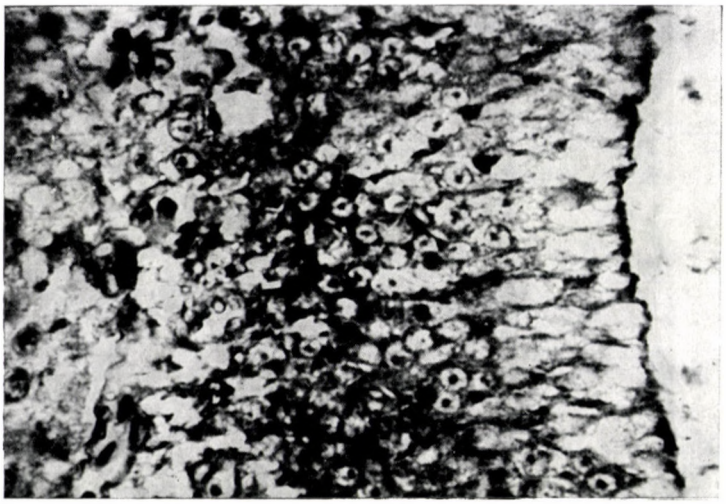


Fig. 1. Control pigeon. Subcommissural organ. Bouin. Aldehyde-fuchsin, ×800

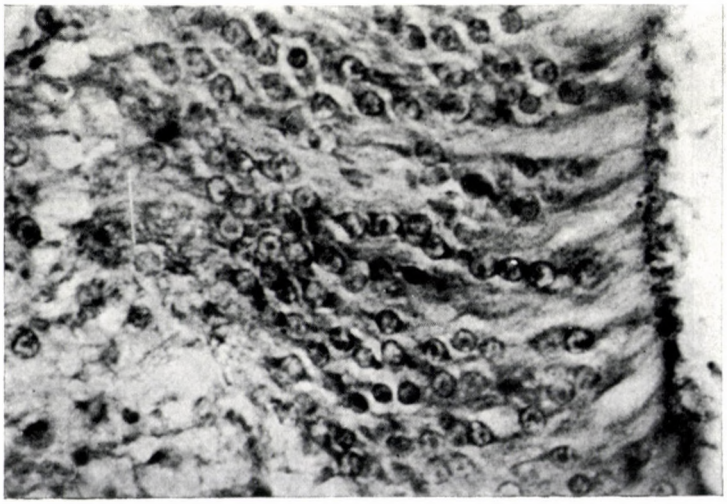


Fig. 2. Insulin-treated pigeon. Subcommissural organ. Bouin. Aldehyde-fuchsin, ×800

nuclear volume of the ependymal cells reacted only slightly to the treatments, with the maximum always in the 1.6 class. The difference from the controls was not significant statistically in any of the cases (Insulin, $t = +1.89$; pred-

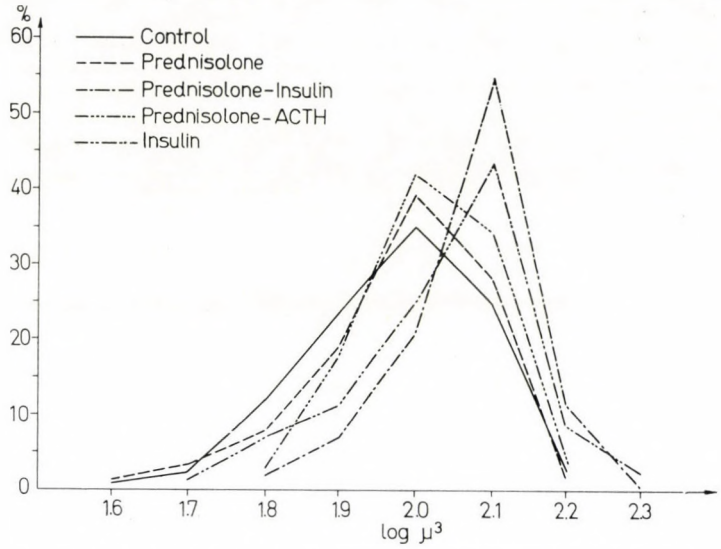


Fig. 3. Nuclear volume curves of the peripheral zone of the adrenal gland. Control and treated pigeons

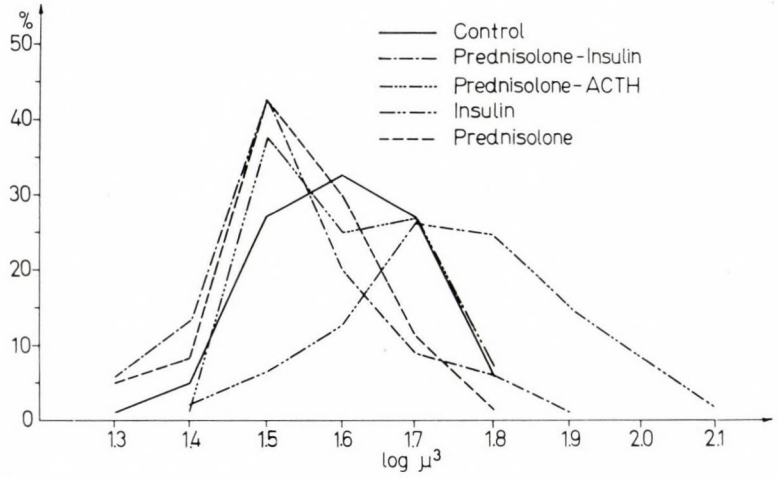


Fig. 4. Nuclear volume curves of the subcommissural organ in control and treated pigeons

nisolone + insulin, $t = -0.32$; prednisolone + ACTH, $t = -1.24$; prednisolone, $t = +1.36$) (Fig. 5).

In the interrenal substance of the adrenal of the control birds, nuclear volume in the peripheral zone was larger than in the deeper zone in agreement with the findings of KONDICS [7]. The maximum of the curve was in the 2.0 class.

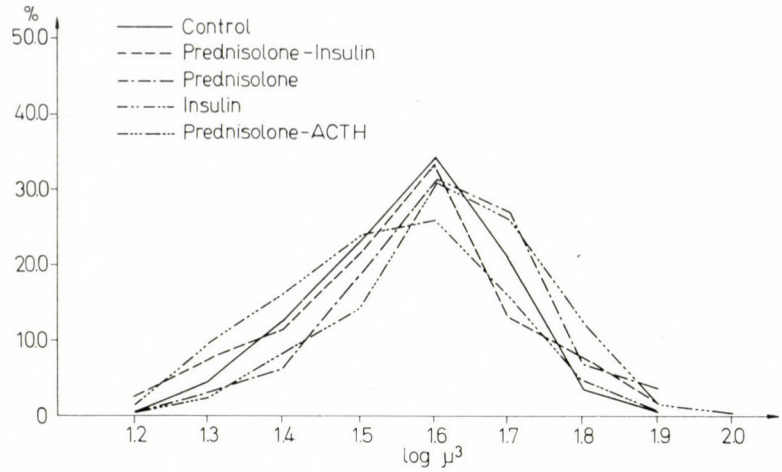


Fig. 5. Nuclear volume curves of the aqueduct and the mural ependyma of the third ventricle in control and treated pigeons

In response to insulin, nuclear volumes increased significantly in both the peripheral and the deeper layers, there were many swollen cell bundles. The maximum shifted one unit to the right ($t = +2.57$).

Prednisolone treatment caused atrophy of the deeper zone, but no change in nuclear volume in the peripheral zone. Histologically, the peripheral zone was similar to the controls ($t = +0.65$).

In the prednisolone + ACTH group, ACTH acted on the deeper zone in the first place and abolished the atrophy caused by prednisolone. In the peripheral zone slight hypertrophy and a small increase of nuclear volume was found, but this was not significant statistically ($t = +1.63$) (Fig. 3).

Discussion

It has been shown that there is a functional zonation in the interrenal matter of the avian adrenals [2, 5—8, 21] and that it undergoes hypertrophy on insulin treatment [9]. Our results have confirmed these findings in that nuclear volume in the interrenal substance always increased on insulin treatment. The hypertrophy of the deeper zone might have been due to an ACTH effect [20]. It remains, however, to be seen, whether the hypertrophy of the peripheral zone was caused by a release of endogenous ACTH or by a non-specific glomerulotropic factor. The subcommissural organ responds to insulin treatment by increasing the synthesis and release of secretion, accompanied by an increase of nuclear volume.

As insulin treatment raises the endogenous adrenaline level, it may be assumed that the experimental changes of the subcommissural organ may be traced back to an adrenaline effect.

To decide whether insulin may exert its action not only through the pituitary-hypothalamic-system on the peripheral zone of the adrenal gland, we treated the birds with prednisolone, prednisolone + insulin and prednisolone + ACTH. Prednisolone blocks ACTH release [20], the deeper zone becomes atrophic, but there is no change in the histology of the peripheral zone. These statements are in agreement with the results obtained by KONDICS [7]. Histologically, the subcommissural organ did not differ from the control, but its nuclear volumes decreased. Treatment with prednisolone and insulin elicited a selective hypertrophy of the peripheral zone of the adrenal. Secretion was released by the subcommissural organ, but nuclear volumes decreased. These results indicate that if ACTH secretion is suppressed by prednisolone, insulin is capable of inducing hypertrophy of the peripheral zone through another route. As in birds the peripheral zone is more sensitive to ACTH than in mammals, though ACTH acts on the deeper zone, in the first place [4], one might argue that on prednisolone and insulin treatment the hypertrophy of the peripheral zone is evoked by the stimulation of ACTH secretion by insulin. It seemed therefore necessary to carry out prednisolone pretreatment followed by ACTH loading. We have shown that this intervention had no effect on the Gomori positive secretion content of the subcommissural organ (although, as every treatment combined with prednisolone, it reduced the nuclear volume values), and that the hypertrophy of the peripheral zone was much less marked than that induced by insulin treatment following prednisolone administration.

The results suggest that the endogenous adrenaline secretion evoked by insulin may activate the subcommissural organ and may give rise at the same time to a hypertrophy of the peripheral interrenal zone of the adrenals. This would confirm our hypothesis that in birds, too, there is a functional correlation between the subcommissural organ and the peripheral zone of the adrenal gland. The results must be evaluated with caution, since in mammals the subcommissural organ extract causes hypertrophy of the zona glomerulosa, but still reduces aldosterone production both *in vivo* and *in vitro* [14]. Thus, the histological pattern alone may be misleading. Furthermore, some authors have failed to demonstrate a functional correlation between the subcommissural organ and the zona glomerulosa, or the changes in aldosterone production [1, 10]. The existence of a functional correlation between the subcommissural organ and adrenal aldosterone production could be confirmed exclusively by unequivocal results of experiments involving a lesion to, and the use of extracts of, the subcommissural organ.

In several experiments we have employed prednisolone. The observed histological patterns suggested that the drug caused no change in the production

of subcommissural secretion. It was, however, remarkable, that whenever prednisolone had been used, the nuclear volume curve shifted to the left. So the subcommissural organ might contain some secretion which does not stain with aldehyde-fuchsin and the release of this substance would be affected by prednisolone, or, else it could be assumed that the glycocorticoids are capable of influencing the activity of the subcommissural organ. This hypothesis may be correlated with the finding by GHIANI [3] of considerable amounts of steroid in the secrete of subcommissural secretion, as also with the statement made by PALKOVITS [15] that in addition to aldosterone and DOCA, hydrocortisone also reduces the nuclear volume values in the subcommissural organ. The role of the epithalamic complex in the control of glycocorticoid secretion has been confirmed by the observation that dexamethasone implanted into the habenules markedly reduced adrenal corticoid production [11].

Thus, our finding of a decrease of nuclear volume in the subcommissural organ in response to prednisolone treatment has confirmed the literature observation that there is a functional correlation between the subcommissural organ and adrenal glycocorticoid production. It appears therefore that a certain kind of functional overlapping exists between the hypothalamic-adrenocorticotropic system and the epithalamic complex. According to our results, the existence of such an overlapping is likely in birds, and on the basis of the less specialized structure and function of their adrenal gland the existence of such a mechanism is more probable in birds than it is in mammals.

REFERENCES

1. BROWN, D. D., AFIFI, A. K.: (1965) Histological and Ablation Studies on the Relation of the SCO and Rostral Midbrain to Sodium and Water Metabolism. Anat. Rec. **153,** 255. — 2. FRANKEL, A., GRABER, J. W., NALBANDOV, A. V.: (1967) The Effect of Hypothalamic Lesions on Adrenal Function in Intact and Adenohypophysectomized Cockerels. Gen. comp. Endocr. **8,** 387. — 3. GHIANI, P.: (1967) Steroids in Epithalamus: Subcommissural Organ Activity. A Histophysiological Approach. Abstracts of IV. European Conference on Comparative Endocrinology. Carlsbad. P. 26. — 4. HOLMES, W. N., PHILLIPS, J. G., CHESTER JONES, I.: (1963) Adrenocortical Factors Associated with Adaptation of Vertebrates to Marine Environments. Rec. Progr. Hormone Res. **19,** 619. — 5. KONDICS, L.: (1963) Über die Wirkung des Kochsalzes auf die Interrenalen Zellen der Nebenniere bei Haustauben. Ann. Univ. Sci. Rolando Eötvös nom. Sect. Biol. **6,** 101. — 6. KONDICS, L.: (1964) Über die Wirkung der Dehydration auf die funktionelle Zonation der Nebenniere bei Haustauben. Ann. Univ. Sci. Rolando Eötvös nom. Sect. Biol. **7,** 115. — 7. KONDICS, L.: (1965) Die Wirkung von ACTH und Prednisolon auf die funktionelle Zonation der Nebenniere bei der Taube. Acta morph. Acad. Sci. hung. **13,** 233. — 8. KONDICS, L., KJAERHEIM, A.: (1966) The Zonation of Interrenal Cells in Fowls. An Electron Microscopical Study. Z. Zellforsch. **70,** 81. — 9. KUMISHIMA, I.: (1960) Histological Observation on the Adrenal Gland of the Chick after Injection of Insulin and Adrenaline. Arch. Histol. Jap. **21,** 129. — 10. LEMYS, R.: (1965) Contribution à l'étude de la structure et du rôle de l'organe sous-commissurale. Thèse, Nancy. — 11. MARTINI, L., FRASCHINI, F., MESS, B., MOTTA, M.: (1967) L'hypothalamus et les structures extrahypothalamiques qui contrôlent l'hypophyse antérieure. Path. et Biol. **15,** 186. — 12. MILLER, R. A., RIDDLE, O.: (1942) The Cytology of the Adrenal Cortex of Normal Pigeons and in Experimentally Induced Atrophy and Hypertrophy. Amer. J. Anat. **71,** 311. — 13. PALKOVITS, M., LUKÁCS, GY.: (1963) Karyometric Examination of the System Subcommissural Organ-Adrenal

Cortex in the Rat. Acta biol. Acad. Sci. hung. **13,** 361. — 14. PALKOVITS, M.: (1965) Morphology and Function of the Subcommissural Organ. Academic Press, Budapest, P.75. — 15. PALKOVITS, M.: (1968) Karyometrische Untersuchungen zur Klärung der osmo- bzw. volumen-regulatorischen Rolle des Subcommissuralorganes und seiner funktionellen Verbindung mit der Nebennierenrinde. Z. Zellforsch. **84,** 59. — 16. PÉCZELY, P.: (1966) Effect of ACTH on the Hypothalamic Neurosecretion in the Pigeon (Columba livia domestica L.). Acta biol. Acad. Sci. hung. **16,** 291. — 17. PÉCZELY, P.: (1966) Effect of Thirst and Water Load on the Hypothalamic Regulation of the Adrenal in the Pigeon (Columba livia domestica L.). Acta morph. Acad. Sci. hung. **14,** 227. — 18. PÉCZELY, P.: (1967) Az eminentia mediana szerepe a madarak mellékveséjének központi szabályozásában. Állattani Közl. **54,** 121. — 19. PÉCZELY, P., MURAY, T.: (1967) The Effect of KCl, NaCl, Hydration and Dehydration on the Subcommissural Organ of the Domestic Pigeon. Acta biol. Acad. Sci. hung. **18,** 115. — 20. PÉCZELY, P.: (1967) A hypothalamo-hypophysealis rendszer szerepe a házigalamb mellékveséjének szabályozásában. Magyar Endokrinológiai és Anyagcsere Társaság Vándorgyűlése. Pécs, P. 36. — 21. DE ROOS, R.: (1963) The Physiology of the Avian Interrenal Gland: A Review. Proc. 13th int. ornithol. Congr. P. 1041.

EINFLUSS DER AKTIVIERUNG DES SUBKOMMISSURALEN ORGANS AUF DIE NEBENNIERE DER HAUSTAUBE

P. PÉCZELY und T. MURAY

Die periphere interrenale Substanz der Nebenniere und das subkommissurale Organ wurden histochemisch untersucht. In der peripheren Zone führt die Verabfolgung von Prednisolon bzw. ACTH nach Prednisolon keine Veränderung herbei, während Insulin sowie Insulinbelastung nach Prednisolon eine Hypertrophie verursacht. Die tiefer gelegene Zone atrophisiert nach Prednisolonverabfolgung, auf Insulinwirkung zeigt sie indessen Hypertrophie. Bei Prednisolon + ACTH-Belastung hört die Prednisolonbedingte Atrophie auf. Das subkommissurale Organ reagiert auf Insulin- bzw. auf Prednisolon + Insulin mit der Ausscheidung eines feinkörnigen, Gomori-positiven Sekrets. Bei Insulinbehandlung allein wird die Sekretausscheidung von einer Vergrößerung des Kernvolumens begleitet. Die vorgenommenen experimentellen Eingriffe verursachten im Aquaeductus bzw. im Ependym der dritten Hirnkammer keine erhebliche Veränderung des Kernvolumens. Die Ergebnisse deuten auf einen funktionellen Zusammenhang zwischen dem subkommissuralen Organ und der peripheren Zone der Nebennieren hin.

ДЕЙСТВИЕ АКТИВАЦИИ СУБКОММИСУРАЛЬНОГО ОРГАНА НА НАДПОЧЕЧНИКИ ДОМАШНЕГО ГОЛУБЯ

П. ПЕЦЕЙИ и Т. МУРАИ

Авторы с помощью гистологических методов исследования изучали периферическое межпочечное (интерренальное) вещество надпочечников и субкоммисуральный орган. В периферической зоне введение преднизолона или введение АКТГ после дачи преднизолона не вызывают изменений, тогда как введение инсулина и нагрузка инсулином после дачи преднизолона обусловливают гипертрофию. Более глубоко расположенная зона после введения преднизолона атрофируется, под влиянием инсулина показывает гипертрофию, а при введении АКТГ после преднизолона атрофия, наблюдаемая под влиянием преднизолона, прекращается. Субкоммисуральный орган реагирует на введение инсулина или преднизолона + инсулина выделением тонкозернистого, Гомориположительного секрета, что́ при введении одного инсулина сопровождается увеличением объема ядер. Примененные авторами экспериментальные вмешательства в эпендиме водопровода (aquaeductus) или третьего желудочка мозга не вызывают значительных изменений объема ядер. Результаты указывают на то, что между субкоммисуральным органом и периферической зоной надпочечников существует функциональная связь.

Dr. Péter PÉCZELY
Dr. Tibor MURAY
Budapest VIII. Puskin u. 3, Hungary

Acta Morphologica Acad. Sci. hung. **16** *(4), pp. 463—468, 1968*

Second Department of Paediatrics (Director: E. KERPEL-FRONIUS), University Medical School, Budapest

EFFECT OF 1,2,5,6-TETRAMETHANESULFONYL-D-MANNITOL(R-52) ON THE CHROMOSOMES OF HUMAN LEUCOCYTE CULTURES

D. SCHULER and G. GÁCS

(Received February 6, 1968)

The effect of 1,2,5,6-tetramethanesulfonyl-D-mannitol has been studied on 72-hour cultures of human peripheral blood leucocytes. In normal individuals the compound completely inhibited mitosis at concentrations of 1.0 μg/ml and higher. Applied at a concentration (0.75 μg/ml) causing 50% inhibition of mitosis it gave rise to numerous chromosomal breaks and recombinations. The former concentration increased almost fourfold the number of spontaneous breaks and increased also the number of translocations in cultures of leucocytes obtained from individuals with Fanconi's anaemia.

A significant percentage of congenital disorders and tumours is due to mutation. This is indicated also by the frequency of chromosomal aberrations in such conditions. It was only 49 years ago that experimental studies of the causes of mutations were started with *Drosophila melanogaster* [15]. Among the known causative factors (thermic effects [17, 18], X-rays and ionising rays [23, 3], viruses [16], etc.) the chemical agents occupy an important position. According to the data obtained so far, most of the mutagens produce chromosomal aberrations in the higher organisms which may be examined cytologically [14]. In the present investigation we have studied the effect of R-52, an alkylating cytostatic, on human cell cultures.

Material and methods

The studies involved normal children, children with acute lymphoblastic leukaemia, and children with Fanconi's anaemia. Venous blood was obtained and heparinized (25 mg crystalline heparin per 10 ml of blood). They made the samples stand at room temperature and after the sedimentation of the erythrocytes 0.5 ml of the leucocyte-rich layer between plasma and erythrocytes was transferred to the culture medium which had the following composition: TC-199 (Difco), 7.5 ml; AB Rh positive human serum, 1.5 ml; penicillin, 5 mg; streptomycin, 5 mg; phytohaemagglutinin "P", 0.08 ml. The drug R-52 was dissolved in distilled water and the required quantity in 0.1 ml volume was added to the culture at the onset of incubation. The cultures were incubated at 37 °C for 72 hours. After 70 hours colchicine was added to the culture to an end concentration of 3.2×10^{-4} mg per 100 ml. After incubation the cells were re-suspended, washed once with 2 ml of TC-199, then hypotonized for 10 minutes with 2 ml of distilled water (added to 0.5 ml of the sediment). After centrifugation the sediment was fixed for 30 minutes in 2 ml of methanol-acetic acid (3 : 1), then washed with several changes of fixative until discolorization. The cell suspension was dropped on a slide, dried and stained with Giemsa's dye. The chromosomes were examined under $\times 1000$ magnification.

Results

To study the toxic, anti-mitotic effect, the lowest drug concentration at which there was no cell division was determined. Chromosomal aberrations were examined at a drug concentration, at which cell division was inhibited to 50%, i.e. the mitotic index (the number of cell divisions per 1000 cells) was reduced to that extent. In some experiments instead of colchicine R-52 was added to the leucocyte culture after 70 hours. The chromosomes were examined in 100 cells at each drug concentration, repeating every experiment once, except in the case of Fanconi's anaemia, in which only 50 cells were tested for the R-52 effect.

Table I

No.	Diagnosis	R-52 μg/ml	Inhibition of mitosis, per cent	Number of cells examined	Modal chromosome number	Break, per cent	Translocation, per cent
1—2	Normal	0.1 —0.5	0	100	46	8	—
3—4	Normal	0.5 —1.0	50	100	46	19	8
5	Leucosis	0.1	100	—	—	—	—
6—7	Fanconi's anaemia	—	0	100	46	28	—
8	Fanconi's anaemia	0.5	50	50	46	84	14

R-52 inhibited mitosis completely at concentration above 1.0 μg/ml, while 0.5 to 1.0 μg/ml caused 50% inhibition. R-52 added to the cultures 2 hours before the termination of cultivation did not influence the mitotic index. In leukaemic patients, R-52 at 0.1 μg/ml concentration completely inhibited mitosis. In the lymphocytes from normal subjects, R-52 caused no appreciable change in ploidy. On the other hand, structural changes were marked in every experiment. At concentrations which did not yet reduce the mitotic index (0.1 to 0.5 μg/ml) breaks could be demonstrated in 7 to 9%, at concentrations causing an 50% inhibition of mitosis, in 19 per cent (Fig. 1 and Table I). The concentration causing 50% inhibition of mitosis induced in 8% of the cases not only breaks, but also chromosomal changes indicative of translocation (Fig. 2). The above percentages mean the number of dividing cells in which one or more breaks or translocations occurred, and not the number of chromosomes showing breaks, since in the same cell often more than one chromosome displayed the above changes.

The spontaneous fragility of chromosomes in the 72-hour leucocyte cultures is characteristic of Fanconi's anaemia. In our experiment this occurred in 28%. The R-52 concentration causing 50% inhibition of mitosis (0.5 μg/ml) induced breaks in 84% and translocations in 14%; much more often than in the normal cells.

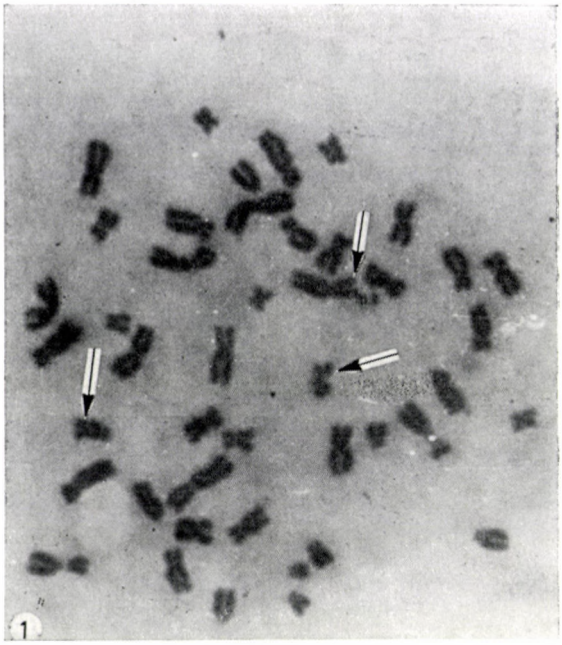


Fig. 1. Chromatid breaks in human lymphocyte culture in response to R-52 treatment in vitro (75 μg/ml concentration)

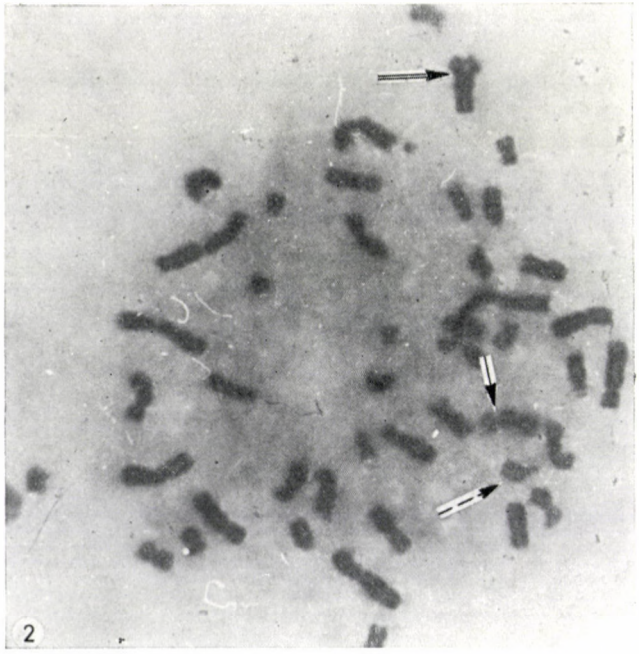


Fig. 2. Translocation figure in human peripheral lymphocyte culture treated with R-52 in vitro (double arrow). Also visible are a chromatid break (arrow) and an acentric fragment (arrow with broken shaft), which resulted from chromosome break

Discussion

Relatively scarce evidence has been published concerning the mutagenic effects of chemicals on human tissue cultures. Chromosomal breaks have been described in response to treatment with caffeine [19] and 8-ethoxycaffeine [9], thalidomide [12], lysergic acid [27], aminopterin [21], cyclophosphamide [2], pyrimethamine [4], azathiopirine [10], base analogues (6-mercaptopurine, cytosine arabinoside, 5-bromodeoxyuridine [20, 5, 7]. Breaks and translocations are caused by acridine dyes bound to DNA [19] and by certain antibiotics of cytostatic action (mitomycin, daunomycin, streptonigrin) [6, 19, 22].

The noxious effect of alkylating agents on the chromosomes has been known for long [19, 26, 1]; we have shown busulfan and mannomustine to cause chromosomal breaks [11].

Like the mutagenic alkylating agents reacting with DNA, R-52 induced numerous chromosomal breaks, and translocations. In certain cases the number of chromosomal breaks exceeded those found in our experiments with mannomustine [25].

The great number of chromosomal translocations occurring in response to R-52 resembled those induced by mitomycin, ethyleneimine and proflavin [19]. These translocations are due to a defective "healing" of breaks and as a permanent change they can be inherited from cell to cell.

R-52 exerts its inhibitory action not in the metaphase, because no colchicine-like antimetaphase effect could be observed.

The chromatid breaks due to R-52 occurred in larger numbers in the vicinity of the centromere. They were not simply added to the breaks found in Fanconi's anaemia, but their number was the double of what could have been expected on the basis of the values obtained in the normal children. This may have been due either to a genetic constitution [8, 13] or to a peculiar structure of chromosomes in Fanconi's anaemia.

Data in the literature seem to indicate that chemical agents reacting with, or bound to, DNA induce chromosome aberrations in tissue cultures. In experiments on plants and animals these compounds have been proved to be mutagenic [19]. Though results *in vitro* obviously cannot be applied without restriction to conditions *in vivo*, dependent upon a variety of extrinsic and intrinsic factors [24], this method of testing may supply useful information as to the mutagenicity of drugs and chemicals. It may, however, not supply information as to teratogenicity, in which many other factors different from those involved in the mutagenic effect have a role.

REFERENCES

1. ABAD, R.: (1965) Study of Chromosomal Alterations Produced by Cytostatic Agents. Acta oncol. (Madrid) **4,** 57. — 2. ARRIGHI, F. E., HSU, T. S., BERGSAGEL, D. E.: (1962) Chromosomal Damage in Murine and Human Cells Following Cytoxan Therapy. Tex. Rep. Biol. Med. **20,** 545. — 3. BLOOM, A. D., NERIISHI, S., AWA, A., HONDA, T., ARCHER, P. P.: (1967) Chromosome Aberrations in Leukocytes of Older Survivors of the Atomic Bombing in Hiroshima and Nagasaki. Lancet **2,** 802. — 4. BOTTURA, C., COUTINHO, V. (1965) Pyrimethamine (Daraprime) Induced Chromosome Abnormalities in Man. Hum. Chrom. Newsl. **15,** 4. — 5. BREWEN, J. G.: (1965) The Induction of Chromatid Lesions by Cytosine Arabinoside in Post-DNA-Synthetic Human Leukocytes. Cytogenetics **4,** 28. — 6. COHEN, M. M.: (1963) The Specific Effects of Streptonigrin Activity on Human Chromosomes in Culture. Cytogenetics **2,** 271. — 7. HSU, T. C., SOMERS, C. E.: (1961) Effect of 5-bromodeoxyuridine on Mammalian Chromosomes. Proc. nat. Acad. Sci. (Wash.) **47,** 396. — 8. HSU, T. C., MANNA, G. K.: (1959) High Frequency of Chromatid Breaks in Two in vitro Cell Populations. Amer. Naturalist **207,** 111. — 9. JACKSON, J. F.: (1964) Chromosome Aberrations in Cultured Human Leukocytes Treated with 8-ethoxycaffeine. J. Cell Biol. **22,** 291. — 10. JENSEN, M. K., SØBORG, M.: (1966) Chromosome Aberrations in Human Cells Following Treatment with Imuran. Acta med. scand. **179,** 249. — 11. KAUFMANN, B. N., SCHULER, D.: Genetic and Chromosome Changes Produced by Drugs in: SIEGLER, P. E., MOYER, J. H. (eds.): (1967) Pharmacological Techniques in Drug Evaluation. Year Book Publ. Chicago p. 113. — 12. KROGH, J. M.: (1965) Chromosome Aberrations in Human Cells Induced by Thalidomide in vitro. Acta med. scand. **177,** 783. — 13. MULLER, H. J.: (1928) The Measurement of Gene Mutation Rate in Drosophila, its High Variability, and its Dependence upon Temperature. Genetics **13,** 279. — 14. MULLER, H. J.: (1953) The Nature of the Genetic Effects Produced by Radiation. Radiat. Biol. **1,** 351. — 15. MULLER, H. J., ALTENBURG, E.: (1919) The Rate of Change of Hereditary Factors in Drosophilia. Proc. Soc. exp. Biol. (N. Y.) **17,** 10. — 16. NICHOLS, W. W.: (1966) The Role of Viruses in the Etiology of Chromosomal Abnormalities. Amer. J. hum. Genet. **18,** 81. — 17. OSTERTAG, W.: (1964) The Change of Ploidy of Human Cells in Culture after Temperature Shock. Exp. Cell Res. **34,** 194. — 18. OSTERTAG, W., KRÜGER, H.: (1963) The Change in Chromosome Number of Human Cells in Culture after Heat Shock. Proc. XI. Internat. Congr. Genet. **1,** 310. — 19. OSTERTAG, W.: (1966) Chemische Mutagenese an menschlichen Zellen in Kultur. Abh. Math. Naturwiss. (Mainz) **1,** 3. — 20. PEDERSEN, B.: (1964) Chromosome Aberrations in Blood, Bone Marrow and Skin from a Patient with Acute Leukemia Treated with 6-mercaptopurine. Acta path. microbiol. scand. **61,** 261. — 21. RYAN, T. J., BODDINGTON, M. M., SPRIGGS, A. J.: (1965) Chromosomal Abnormalities Produced by Folic Acid Antagonists. Brit. J. Dermat. **77,** 541. — 22. SHAW, M. W., COHEN, M. M.: (1965) Chromosome Exchanges in Human Leukocytes Induced by Mitomycin C. Genetics **51,** 181. — 23. SCHMICKEL, R.: (1967) Chromosome Aberrations in Leukocytes Exposed in vitro to Diagnostic Levels of X-rays. Amer. J. hum. Genet. **19,** 1. — 24. SCHÖNEICH, J.: (1967) Spontane und induzierte Chromosomenaberrationen in Gewebekulturen. Biol. Zbl. **86,** (Suppl.) 69. — 25. SCHULER, D.: Unpublished Observations. — 26. WOODLIFF, H. J.: (1964) The Effects of Cytotoxic Therapeutic Agents on Cell Cultures. In: Blood and Bone Marrow Cell Culture. Lippincott, Philadelphia, P. 80. — 27. ZELLWEGER, H., MCDONALD, J. S., ABBO, G.: (1967) Is Lysergic Acid Diethylamide a Teratogen? Lancet **2,** 1066.

DIE WIRKUNG DES 1,2,5,6-TETRAMETHANSULPHONYL-D-MANNITS (R-52) AUF DIE CHROMOSOMEN IN MENSCHLICHEN LEUKOZYTENKULTUREN

D. SCHULER und G. GÁCS

Die Wirkung von 1,2,5,6-Tetramethansulphonyl-D-mannit (R-52) auf 72 stündige Kulturen von menschlichen peripheren Leukozyten wurde untersucht. In der Leukozytenkulturen Gesunder ruft R-52 in einer Konzentration von 1,0 μg/ml oder mehr vollkommene Hemmung der Zellteilung hervor. In 50%iger Mitosehemmender Konzentration (0,75 μg/ml) verursacht R-52 zahlreiche Chromosomenbrüche und neue Rekombinationen. In den Leukozytenkulturen von Patienten mit Fanconi-Anämie wird durch R-52 in obiger Konzentration die Anzahl der spontanen Chromosomenbrüche nahezu auf das Vierfache erhöht und auch eine größere Zahl von Translokationen hervorgerufen.

ДЕЙСТВИЕ 1,2,5,6-ТЕТРАМЕТАНСУЛЬФОНИЛ-D-МАННИТА (R-52) НА ХРОМОСОМЫ В КУЛЬТУРАХ ЧЕЛОВЕЧЕСКИХ ЛЕЙКОЦИТОВ

Д. ШУЛЕР и Г. ГАЧ

Авторы изучали действие 1,2,5,6-тетраметансульфонид-D-маннита на 72-часовые культуры лейкоцитов, взятых из периферической крови человека. В концентрации 1,0 μг на мл или выше, R-52 в культурах лейкоцитов здоровых лиц совершенно прекращает митозы. В 50%-ной тормозящей митозы концентрации (0,75 μг на мл) R-52 вызывает многочисленные расщепления хромосом и новые рекомбинации. В культурах лейкоцитов больных анемией Фанкони, R-52 при вышеуказанной концентрации повышает число самопроизвольных распадов хромосом почти в четыре раза, и вызывает также больше транслокаций.

Dr. Dezső SCHULER
Dr. Gábor GÁCS
Budapest IX. Tűzoltó u. 7/9, Hungary

INDEX

Morphologia Normalis et Experimentalis

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Farkas Sándor

A kézirat nyomdába érkezett: 1968. XI. 29. — Terjedelem: 8.25 (A/5) ív, 67 ábra (2 szines), 1 melléklet

69.66719 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

The Acta Morphologica publish papers on experimental medical subjects in English, German, French and Russian.

The Acta Morphologica appear in parts of varying size, making up volumes.

Manuscripts should be addressed to:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Correspondence with the editors and publishers should be sent to the same address.

The rate of subscription to the Acta Morphologica is 165 forints a volume. Orders may be placed with "Kultúra" Foreign Trade Company for Books and Newspapers (Budapest I., Fő utca 32. Account No. 43-790-057-181) or with representatives abroad.

Les Acta Morphologica paraissent en français, allemand, anglais et russe et publient des travaux du domaine des sciences médicales expérimentales.

Les Acta Morphologica sont publiés sous forme de fascicules qui seront réunis en volumes.

On est prié d'envoyer les manuscrits destinés à l'adresse suivante:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

Toute correspondence doit être envoyée à cette même adresse.

Le prix de l'abonnement est de 165 forints par volume.

On peut s'abonner à l'Entreprise du Commerce Extérieur de Livres et Journaux «Kultúra» (Budapest I., Fő utca 32. Compte-courant No. 43-790-057-181) ou à l'étranger chez tous les représentants ou dépositaires.

«Acta Morphologica» публикуют трактаты из области экспериментальных медицинских наук на русском, немецком, английском и французском языках.

«Acta Morphologica» выходят отдельными выпусками разного объема. Несколько выпусков составляют один том.

Предназначенные для публикации авторские рукописи следует направлять по адресу:

Acta Morphologica, Budapest IX., Tűzoltó u. 58.

По этому же адресу направлять всякую корреспонденцию для редакции и администрации.

Подписная цена «Acta Morphologica» — 165 форинтов за том. Заказы принимает предприятие по внешней торговле книг и газет «Kultúra» (Budapest I., Fő utca 32. Текущий счет № 43-790-057-181) или его заграничные представительства и уполномоченные.

36,— Ft

Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

ALBANIA
Ndermarja Shtetnore e Botimeve
Tirana

AUSTRALIA
A. Keessing
Box 4886, GPO
Sydney

AUSTRIA
Globus Buchvertrieb
Salzgries 16
Wien I

BELGIUM
Office International de Librairie
30, Avenue Marnix
Bruxelles 5
Du Monde Entier
5, Place St. Jean
Bruxelles

BULGARIA
Raznoiznos
1, Tzar Assen
Sofia

CANADA
Pannonia Books
2, Spadina Road
Toronto 4, Ont.

CHINA
Waiwen Shudian
Peking
P. O. B. 88

CZECHOSLOVAKIA
Artia
Ve Směčkách 30
Praha 2
Poštovná Novinová Služba
Dovoz tisku
Vinohradská 46
Praha 2
Mad'arská Kultura
Václavské nám. 2
Praha I
Poštová Novinová Služba
Dovoz tlače
Leningradská 14
Bratislava

DENMARK
Ejnar Munksgaard
Nörregade 6
Copenhagen

FINLAND
Akateeminen Kirjakauppa
Keskuskatu 2
Helsinki

FRANCE
Office International de Documentation et Librairie
48, rue Gay Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC
Deutscher Buch-Export und Import
Leninstraße 16
Leipzig 701
Zeitungsvertriebsamt
Fruchtstrasse 3—4
1004 Berlin

GERMAN FEDERAL REPUBLIC
Kunst und Wissen
Erich Bieber
Postfach 46
7 Stuttgart S.

GREAT BRITAIN
Collet's Holdings Ltd.
Dennington Estate
London Rd.
Wellingborough. Northants.
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
Oxford

HOLLAND
Swetz and Zeitlinger
Keizersgracht 471—487
Amsterdam C.
Martinus Nijhof
Lange Voorhout 9
The Hague

INDIA
Current Technical Literature
Co. Private Ltd.
India House OPP
GPO Post Box 1374
Bombay I

ITALY
Santo Vanasia
Via M. Macchi 71
Milano
Libreria Commissionaria Sansoni
Via La Marmora 45
Firenze

JAPAN
Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
Tokyo
Maruzen and Co. Ltd.
P. O. Box 605
Tokyo-Central
Far Eastern Booksellers
Kanda P. O. Box 72
Tokyo

KOREA
Chulpanmul
Phenjan

NORWAY
Johan Grundt Tanum
Karl Johansgatan 43
Oslo

POLAND
Ruch
ul. Wronia 23
Warszawa

ROUMANIA
Cartimex
Str. Aristide Briand 14—18
Bucureşti

SOVIET UNION
Mezhdunarodnaya Kniga
Moscow G—200

SWEDEN
Almquist and Wiksell
Gamla Brogatan 26
Stockholm

USA
Stechert Hafner Inc.
31, East 10th Street
New York, N. Y. 10003
Walter J. Johnson
111, Fifth Avenue
New York, N. Y. 10003

VIETNAM
Xunhasaba
19, Tran Quoc Toan
Hanoi

YUGOSLAVIA
Forum
Vojvode Mišičá broj 1
Novi Sad
Jugoslovenska Knjiga
Terazije 27
Beograd

31. III. 1969

Index: 26.017