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LAJOS MARKUSOVSZKY

(25 April 1815—21 April 1893)

MEMORIAL ADDRESS TO THE HUNGARIAN ACADEMY OF SCIENCES ON THE 1ST OCTOBER, 1965, THE 150TH ANNIVERSARY OF MARKUSOVSZKY'S BIRTH

By

J. Sós

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To draw a portrait true to life is a difficult task. Yet, though not with lines and colours but with words, we must attempt to recall the features of a great man's mind.

Much of what Lajos Markusovszky's contriving genius achieved for Hungarian medicine is still alive after a century, and I shall attempt to trace it back to its very origins, through a closer study of his individuality.

We recognize a great man by his work. His life concerns us only so far as it concerns his work. Lajos Markusovszky was born at Csorba (Strba) in 1815. He began his medical studies at Budapest University in 1834, but had to interrupt them owing to financial difficulties, and to accept the post of a tutor in the Festetich family. This delayed his graduation until 1844. He then went to Vienna to complete his training. There he met such friends as Ignatz Semmelweis and János Balassa. After his return to Budapest he worked as a house surgeon under Balassa who used ether anaesthesia as early as 1847.

During Hungary's War of Independence in 1848—1849, Markusovszky was a field-surgeon. This cost him his position and brought police surveillance upon him in the years of the Habsburg absolutism that followed the country's defeat. But repressed national fealing and the desire to promote Hungarian medicine united the few leading medical men of the nation very closely. Their association was referred to in contemporary records as the "medical faculty on horseback". With social assembly and even professional meetings prohibited as potential centres of conspiracy, Markusovszky, Balassa, Lumniczer, and Kovács, used to ride out in order to meet and to discuss their plans. Later they were joined by Hirschler, Bókay, and Wagner and, after his return from his exile in the provinces, by Frigyes Korányi. This was the birth of the Markusovszky-society, officially non-existent but none the less alive, so much so that it was to remain the driving force of Hungarian medicine through nearly half a century — between 1850 and 1893.

It was not until the political atmosphere began to clear that Markusovszky could resume his work. He published more than 200 papers which is a great

deal for a medical man of the last century. He had success in his life but that was the fruit of hard work and ceaseless struggle.

Let us now recall to our minds what Hungarian medicine owes to Lajos Markusovszky. We must begin, in the order of time, with his merits in medical publishing. The founding in 1857 of Orvosi Hetilap (Medical Weekly), still the most widely read Hungarian medical journal, an efficient vehicle of scientific and practical medicine, was the first stage of this activity. The final stage of it was the founding of the Hungarian Medical Publishing Society, in 1864.

With the start of Orvosi Hetilap the Hungarian physicians were again able to publish papers in their mother tongue and to cultivate Hungarian medical language sadly neglected during the years of oppression, thus adding their share to national revival.

Markusovszky advocated the necessity of a periodical concerned with all the aspects of medicine. He furthermore pointed out in a reply to some discouraging objection that a periodical fails to be the stimulant to medical life if it appears at monthly intervals. It must be a weekly journal though the enterprise may be too hazardous.

In his introduction to the first issue he stressed that the founding of Orvosi Hetilap had been a duty to Science, to our suffering fellowmen, and to the Hungarian nation. — "The collective spirit belongs to the most fertile ideas of our time, and the Press is its natural channel of communication. Action demands unity!" Those are the conclusive words of his address. Orvosi Hetilap became an essential tool in postgraduate training, by Markusovszky and Balassa's initiating interchanges on a high level, extending to problems of health policy. These possibilities were brought now into the orbit of every Hungarian physician.

We find in the first volumes of Orvosi Hetilap both clinical and theoretical papers on diagnostics, therapy, surgical techniques, general surgery, clinical medicine, paediatrics, gynaecology and obstetrics, and a variety of other subjects. Other branches of medicine, which later became independent, such as urology, oto-rhino-laryngology, etc., found room in its pages, as well as pharmacology, public health, school medicine, first-aid, ophthalmology, balneology and dietetics. Hospital building, city planning, health policy, problems regarding ancillary staff, book reviews, medical news, and reports of journeys helped to enlarge the scope of the weekly paper which thus had all the requisites to impart a comprehensive view of the various theoretical, practical and social aspects of medicine and to inspire medical life in Hungary. During the century which has gone by since, there was but little occasion to improve this comprehensive editing.

The editorial tasks Markusovszky had to cope with were fraught with difficulties. His journal was attacked at home and from abroad. In Budapest, a rival paper was launched but Orvosi Hetilap came off victorious, not only by

its scientific standard but also through its impact on professional life. In Vienna, the other capital of the Austro-Hungarian Monarchy, the Wiener Medizinische Wochenschrift decried his organ's value by stating arrogantly "Die Kultur kommt nicht von Osten". This was meant as a final blow to Orvosi Hetilap.

Many times, Orvosi Hetilap was the bearer of sad news. In 1865 the death of Bugát and of Semmelweis were announced in close succession. Markusovszky summed up the meaning of Bugát's work in a few but impressive words: "One of the pioneers of Hungarian medical writing has left us. The era of his activity belongs to the past, the aims he pursued have been reached, but what he has done to improve Hungarian medical writing is of abiding value". Seen from the distance of a century, this tribute which Markusovszky paid to the memory of his friend, applies with the same reason to himself.

Orvosi Hetilap having gained a safe footing, Markusovszky, in 1864, established the Hungarian Medical Publishing Society to promote and disseminate medical knowledge and cultivate the Hungarian professional language. It remained in existence until 1944. The monographs which were issued by this Society on an annual subscription basis, were valuable contributions to Hungarian medicine, enabling the practitioner to keep abreast with the progress of medical science. Many of these volumes serve as historical sources today.

The founding of the National Health Council (1868) whose activity was to result in the statutory regulation of public health in Hungary (1876), was also due to Markusovszky's initiative. Not only did it mark a substantial progress in Hungarian health policy but its original scheme as conceived by Markusovszky has preserved its validity to the present day. It still is an advisory body co-ordinated to the Minister of Health, with members appointed from the leading medical ranks for periods of a few years. The Council keeps an eye on all that is concerned with public health, and not only to give advice on request but to make suggestions freely whenever necessary. As laid down expressedly by one of the members on the 25th anniversary of the Board in 1893, "we intend to make use of our right to initiate and put forward motions whenever we deem it necessary in the interest of the public".

The core of the National Health Council had been a standing committee, first organized in 1860, and its chairmen were Balassa, Wagner, Csatáry (Grosz) and Schwartzer. On the 10th of March, 1868, the Minister for Home Affairs accepted from three drafts submitted to him the one prepared by Balassa, Markusovszky, Jendrassik and Frigyes Korányi. As soon as it had the King's consent, a chairman, a vice-chairman, 10 ordinary and 28 substitute members were appointed, and official representatives of four ministries (Home Affairs, Education, Transport, Trade) were delegated. In this manner the full Council included 44, and the exclusive Committee 16, members. In the deed of foundation the newly found body is registered as "Medical Research Council". It still bears this name, with a slight alteration.

The first Board of the new Council was constituted as follows. Chairman: János Balassa. Vice-chairman: Endre Kovács Sebestyén. Members: Lajos Csatáry-Grosz, Jenő Jendrassik, István Kajdacsy, Frigyes Korányi, Sándor Lumniczer, Mór Moskovitz, János Nepomuk Rupp, Károly Than, Károly Tormay, and János Wagner. Official delegates: Adolf Hollán (Home Affairs), Lajos Markusovszky (Education), Vilmos Zlamál (Trade and Industry), Ferenc Reitter (Transport). The first session was held on the 5th of July, 1865.

The weight of the Council's work is best illustrated by the scope of its activities, such as the reorganization of medical services, hospital building and administration, questions of midwifery (1891), asylums, dentistry and prosthetic dentistry, the reorganization of public health services, training of medical officers, competence of police-surgeons (1881), prison hospitals (1873), the appointment of sanitary inspectors (1884), organization of emergency services, transport of dead bodies (1873), health codes concerned with passenger and goods traffic (1885), matter of pharmaceutics and chemists' shops, food hygiene, meat inspection (1871, 1881, 1882), public-house control, etc. In addition, the Council was concerned with curricula of medical training and the setting-up of new university departments; for instance, they made a plea for a university chair of public health, and also for an "Observation Centre" which a generation later was to take life as the National Institute of Hygiene of today. The training of forensic physicians and of school teachers in hygiene, school health reform (1885), drafting Bills concerning veterinary hygiene (1886), spas (1869), and the affairs of medical societies were likewise on the agenda. In the field of environmental sanitation, river control, flood prevention, and the draining of marshlands were discussed. When the city of Szeged was rebuilt after a great flood, the sanitary supervision of the whole scheme of construction devolved on the experts of the Council.

The Medico-legal Council was a separate body within the framework of the Council of Medical Research, only the degree of its independence varied in the course of a century.

A comprehensive Public Health Act was an issue first raised by Bugát in 1845, at a meeting of Hungarian physicians and naturalists held at Pécs, but in the years of oppression following upon the defeat of the War of Independence the plan was dropped. In 1863 and 1867 the question was resumed but its final solution had to wait until Markusovszky, with full support of the Council, brought it to a successful issue.

The basic principles to underlie the Bill for the Act were being worked out in 1869. Expert committees were appointed to elaborate the details as concerns medical practice, health institutions, and legal questions. A year later the Bill was in final draft. Yet years of exacting negotiations, resulting in various amendments, had had to pass before it was laid before the King by the Prime

Minister for consent and then submitted to Parliament. The Bill was voted on the 3rd and enacted on the 8th of April, 1876.

The original drafters of the Bill were Balassa, Lumniczer, Frigyes Korányi, and Markusovszky, but obviously, Markusovszky, a first class expert in public health organization, must have had a larger share in this work than his associates who were busy clinicians. Thus Markusovszky was in one person the originator, the promoter, and essentially, the builder of the frame-work of this important statute.

The third leading idea inspiring Markusovszky's activities concerns medical teaching and training in all its aspects, including new university departments and building of premises. As early as 1859 he entered upon a campaign for the establishment of a medical centre in Budapest, and also pressed a plea for it in Orvosi Hetilap. His efforts remained fruitless until he had come into office in the Ministry of Education, where he remained until his retirement in 1892. His position enabled him to carry out his plans. When he came into office the University Medical School had 13 chairs; on his retirement, a quarter of a century later, there were 22, and almost all housed in suitable premises.

During his first year in the Ministry of Education he found the funds for the equipment of eight new medical chairs, recruited their permanent staffs, and provided for their annual budgets. The following spring he organized a Government Council discussion with a view to furthering the construction of premises for the University Medical School. This he followed up by pressure on the Budapest Municipal Council for the long-promised donation of suitable building sites. He designed teaching hospitals, institutes of general, applied, and forensic anatomy, as well as of physiology, pathology, and chemistry on both sides of an arterial road in the south of the Capital. The designs took much time to be translated from blue-print into fact. The first building completed was that for the Chair of Chemistry (1870). In the same year the Medico-physical Chair could be housed and the old Institute of Physiology extended. In 1871 the Institute of Pathology and the Institute of Pharmacology were given independent premises. In 1873 an embittered Markusovszky complained that the building operations for the Medical Faculty were "like the mariners' fabulous seaserpent which has no end" and that "the generation that first planned and fought for the scheme has grown grey in harness or died". Still, the very same year the erection of a new home for the Institute of Physiology began, though the building was not completed until 1875.

A special merit of Markusovszky was to have at that time organized, after Moscow and Leipzig, the third Chair in the world for Public Health. József Fodor was appointed its first director.

The years between 1878 and 1881 saw the completion of new and fully equipped premises for the first and second Departments of Sur-

gery, an Institute of Anatomy, and the first and second Departments of Medicine.

The Departments of Obstetrics took a good twenty years to be organized and housed. The first Department was complete by the autumn of 1879. The second Department was erected after the death of Markusovszky but on a site marked out by him.

The Central Building of the University Medical School was opened towards the end of 1884, with premises for all the administrative offices and the Central Library. Large parts of it housed transiently homeless departments and institutes, such as the Institute of Pathology and Therapy, the Institute of Biochemistry, and the Institute of Pharmacology, of which the last-mentioned is still in the building. The fact that the premises could be used for a variety of purposes changing from decade to decade shows Markusovszky's ingenuity in designing with an eye not only on immediate needs but also on future demands.

The square facing the Central Building of the Budapest University Medical School has now been named after Markusovszky, and there it is that we will now unveil his statue.

Owing to the steadily growing number of students, ever widening curricula, and the corresponding increase and diversity in practical and technical training (laboratories, etc.), many departments and institutes have outgrown the buildings originally meant to house them. But never had a building to be abandoned for lack of foresight in planning or incompetent design. Markusovszky grasped from the start that the chief characteristic of knowledge is its many-sidedness and that soon the University Medical School will have to spread its interests and yet preserve studies in depth — and he planned, designed, and organized building construction accordingly.

Markusovszky also projected and drew up plans for Government measures to improve the economic situation of medical university teachers and research workers, but age and illness thwarted his purposes.

Death overtook Lajos Markusovszky at the age of 78, one year after his retirement. For 25 years he was Administrator of Universities in the Ministry of Education. Though he suffered transient setbacks, his was a career rich in success. In recognition of his merits he was elected corresponding member of the Hungarian Academy of Sciences in 1863 and honorary member in 1890. A great organizer, he lived a full life spent in creative work. He could not always realize his ambition, but his accomplishments are not only imposingly grand but in many respects still guiding us.

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AUSCULTATORY PHENOMENA IN PULMONARY INSUFFICIENCY WITHOUT PULMONARY HYPERTENSION

By

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(Received March 10, 1965)

On the basis of its onset, character, timing, and frequency the murmur of pulmonary insufficiency without pulmonary hypertension can be distinguished from that of aortic insufficiency and that of pulmonary functional incompetence (Graham Steell murmur). According to the observation made by the authors, certain types of acquired pulmonary insufficiency may increase in intensity after some time. In connection with the dilatation of pulmonary artery, the so-called right-sided Austin Flint phenomenon may develop. Amyl nitrite is usually suitable for distinguishing the murmur, but an increase in intensity does not occur in every case. On the other hand, the murmur becomes louder in every case after repeated deep inspirations. By way of conclusion the authors state that the mechanograms are a valuable help in the identification of pulmonary insufficiency murmurs.

Organic pulmonary insufficiency may be congenital or acquired (RUNCO and ROOTH, 1963). Congenital pulmonary insufficiency usually develops in consequence of an abnormal form or supernumerous pulmonary valves, bicuspid pulmonary valves, or the regurgitation is associated with pulmonary stenosis. Acquired pulmonary incompetence usually arises in connection with a dilatation of the pulmonary artery or as a consequence of the surgical correction of pulmonary stenosis. Pulmonary insufficiency developing as a consequence of bacterial endocarditis, rheumatic fever, syphilis, fenestration of the pulmonary valves, trauma, or of the carcinoid type, is much less frequent.

The difference between the murmur of organic pulmonary insufficiency and aortic regurgitation has been discussed previously (BODROGI *et al.*, 1963). The murmur of organic pulmonary insufficiency is rough, it is loudest in the left 2nd—3rd intercostal space; the frequency is low, that can be attributed to the low-velocity regurgitation. It starts with a short period after the second heart sound with crescendo-decrescendo character. The murmur reaches its peak at the time when the pressure gradient between the pulmonary artery and right ventricle is the greatest and ends in mid-diastole with the disappearance of the gradient. In pulmonary insufficiency the right ventricular ejection time is prolonged by the regurgitating blood volume, causing an exaggeration of physiologically asynchronous onset of left and right ventricular diastole. In this way produced retardation of right ventricular relaxation added to the absent pulmonic component of the 2nd sound, in most cases result the silent interval after the aortic component. We must remark, however, according to

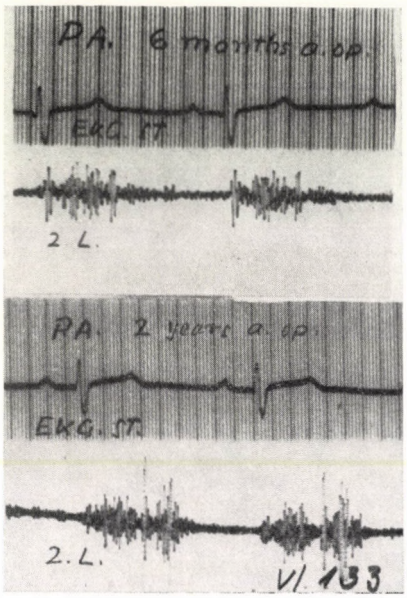

Fig. 1. P. A., 17 years of age operated upon for pulmonary stenosis. The diastolic murmur of pulmonary regurgitation after half and after two years of the surgery

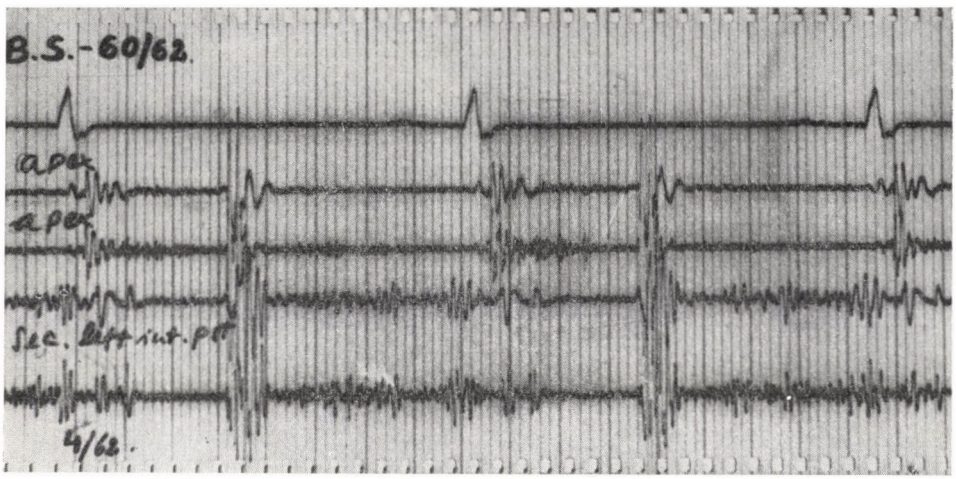

Fig. 2. B. S., 15 years of age. Pulmonary regurgitation had arisen in consequence of pulmonary hypertension associated with patent ductus arteriosus, and persisted after the successful operation even after the pressure in the pulmonary artery had returned to normal. The presystolic crescendo appears after the characteristic murmur of pulmonary incompetence

other authors (LEVIN *et al.*, 1964), that in patients whose regurgitant flow is small, this delay may be absent, so that the murmur begins immediately after the second sound. The murmur is transmitted along the edge of the sternum

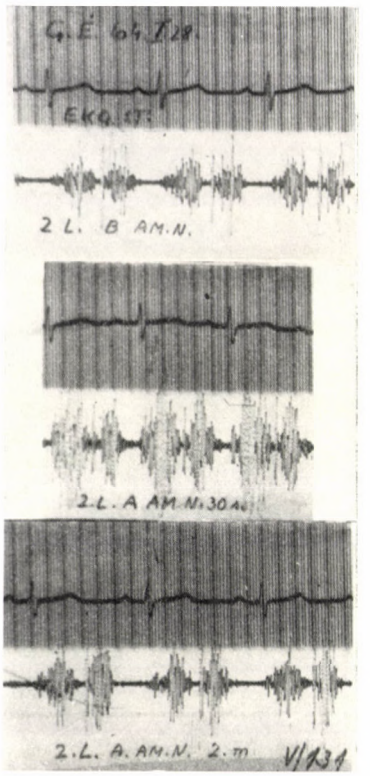

Fig. 3a. Increased intensity of the regurgitation murmur after the effect of amyl nitrite

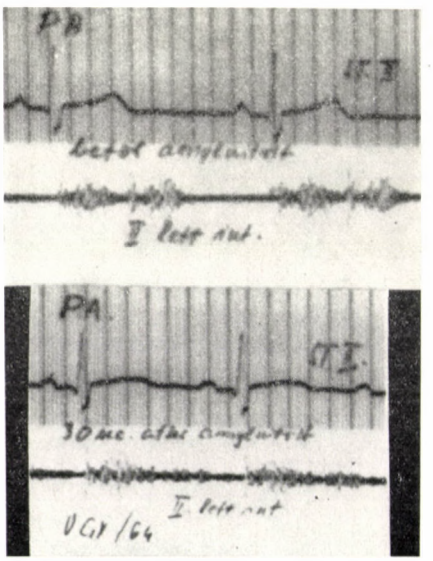

Fig. 3b. Reduction of the amplitude of postsurgical regurgitation murmur after administration of amyl nitrite

down to the 4th—5th intercostal space, occasionally to the right side of the sternum. In the present paper we shall report on two years observation of pulmonary regurgitation in 12 cases.

It is well known, that some regurgitation always occurs after the surgical correction of pulmonary stenosis (TALBERT *et al.*, 1963) and in our material six such patients were observed. In three of our cases, the insufficiency was due to dilatation of the pulmonary artery, the pulmonary insufficiency was congenital

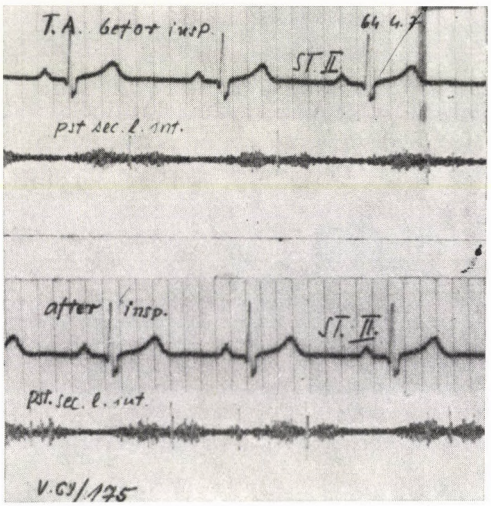

Fig. 4. Increased vibrations of the murmur after repeated inspirations

in two cases, and in one case regurgitation was due to pulmonary hypertension associated with a patent ductus arteriosus; this regurgitation persisted after successful surgery, when the pressure in the pulmonary artery had become almost normal.

In the cases of congenital pulmonary insufficiency diagnosis was confirmed by cineangio-cardiography, and in the rest of the cases by catheterization. At cardiac catheterization the pulmonary artery pressure curve is characterized by a dicrotic limb steeply descending to the diastolic level, which in end-diastole is equal to right ventricular pressure (PRICE 1961, KOHOUT *et al.*, 1955). The pulmonary origin of the regurgitation in the patient with a patent ductus arteriosus was confirmed by palpation too during operation.

Our observations concerning pulmonary regurgitation were as follows: (i) The murmur's intensity increased with time. (ii) An Austin Flint murmur on the right side was associated with pulmonary arterial dilatation. (iii) A positive response to amyl nitrite. (iv) Increase of the regurgitation's intensity on deep inspiration. (v) Its connection with mechanograms.

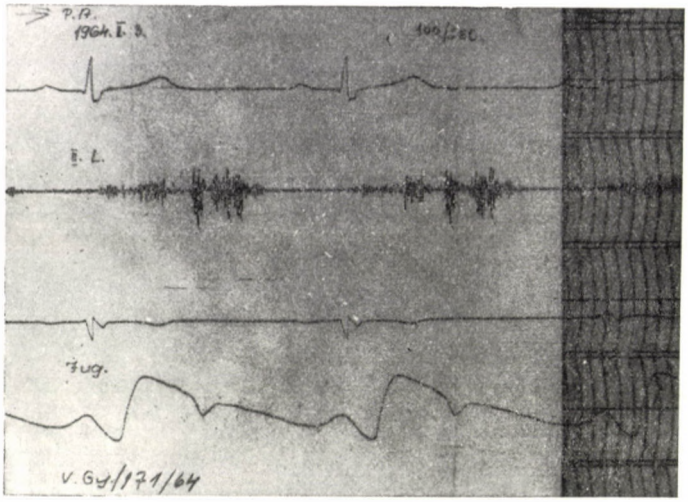

Fig. 5. Murmur associated with pulmonary insufficiency. Its onset occurs a short period after aortic closure

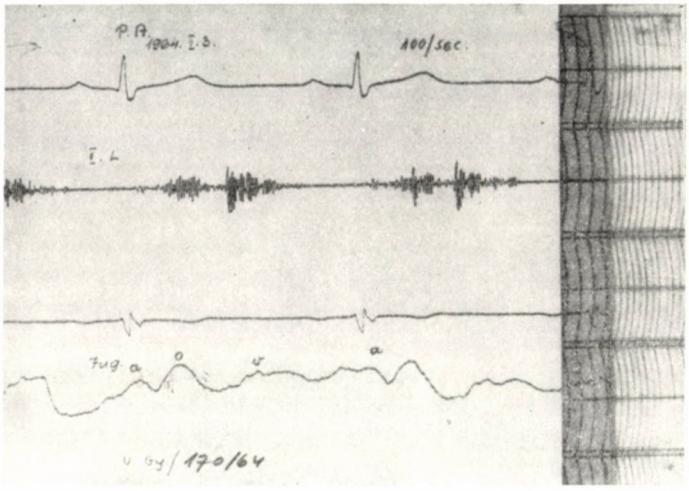

Fig. 6. End of the crescendo-decrescendo type murmur coincides with the onset of the (a) wave of the phlebogram

In two cases where the insufficiency had developed after the correction of pulmonary stenosis two years after the operation the murmur became considerably louder both on auscultation and on the PCG record, although the clinical state of these patients did not deteriorate (Fig. 1). We agree with LUISADA (LUISADA and SATKOWSKY, 1961) in that in such cases the increasing intensity of the murmur is due to fibrosis developing after some time at the

margin of the valves, but it is also possible that the change in intensity is caused by a slight dilatation of the right ventricle (FOWLER and DUCHESNE, 1958).

In the patient with pulmonary dilatation at the 2nd—4th intercostal space parasternally the murmur could be divided into two parts, one low pitched of crescendo-decrescendo character, starting with a discrete interval after the 2nd heart sound, and one of crescendo character falling in the presystole (Fig. 2). As far as we know, this phenomenon has been reported in the literature in 3 cases only, in connection with pulmonary arterial dilatation

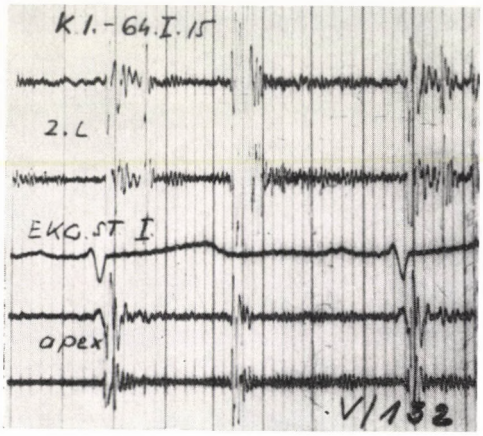

Fig. 7. Patient with primary pulmonary hypertension. The double sound tracing clearly shows the murmur of functional insufficiency well transmitted to the apex

(BRAYSHAW and PERLOFF, 1962, ONGLEY *et al.*, 1960, GREENE *et al.*, 1949). According to these authors, in such cases the murmur may be divided into two parts: a medium frequency murmur in the 2nd intercostal space and a murmur of lower frequency in the 4th—5th intercostal space parasternally. The phenomenon has been termed right sided Austin Flint murmur. There is no explanation of the phenomenon; the comparison is entirely clinical with the Austin Flint murmur heard in aortic incompetence.

According to the literature, pulmonary regurgitation murmurs usually become louder or show no change in intensity after administration of amyl nitrite (SUH SOON KYU, 1960, ENDRYS and BARTOVA, 1962). In 8 of our patients amyl nitrite made the murmur to grow in intensity, while in 2 cases it remained the same and in further two cases it was even reduced. In one of these, the insufficiency had developed after surgery for pulmonary stenosis, in the other the insufficiency was a consequence of pulmonary dilatation (Fig. 3 a—b).

Under the effect of repeated deep inspirations (CRISCITIELLO, and HARVEY, 1963) an increase in the murmur's intensity was noted (Fig. 4). Such an increase in intensity was found in every case where the patients expired

slightly after a few deep inspirations. This in our opinion is a simple and practical test, and its mechanism agrees that of Rivero—Carvallo test.

The simultaneous recording of the murmur with other mechanograms is a valuable help (Bousvaros and Deuchar, 1961). The murmur never starts synchronously with the incisure of the carotid curve and ends with the onset of the (a) wave of the phlebogram and apexcardiogram (Figs 5, 6).

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PROTEIN-BOUND CARBOHYDRATES IN SERUM AND LYMPH IN EXPERIMENTAL INFLAMMATION

By

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The serum glycoprotein level has been found to rise in experimental turpentine inflammations. A parallel rise was noted in thoracic duct lymph whereas in the cervical trunk lymph, derived from the affected area, the values at 24 and 72 hours were significantly lower than those in blood and the thoracic duct lymph. It is concluded that the glycoproteins are retained at the site of inflammation for tissue repair.

Increased protein-bound carbohydrate levels have been found in turpentine-induced inflammation [6, 23]. The cause of this increase is still controversial. Certain authors ascribe it to tissue breakdown involved by the injury [8, 22, 26], others believe it to be the consequence of enhanced protein synthesis by the liver [4, 5, 14, 15, 16]. In support of the first assumption it is alleged that the concentration of protein-bound carbohydrates in the venous blood of the effected area is higher than in the arterial blood [26]. No account has been, however, taken of the fact established by STARLING, namely that the interstitial colloids, therefore the proteins too, are carried into the blood stream through the lymph vessels. In order to gain a closer insight into the problem, the concentrations of protein-bound carbohydrates have been investigated in the lymph and in the venous blood collected from the affected area, as well as in the arterial blood supplying it.

Material and methods

55 dogs of both sexes weighing between 12 and 16 kg were used in the studies. Blood samples were taken at the beginning of the experiment, then 1 ml of rectified turpentine oil was injected subcutaneously into the right ear of each animal. The animals were divided into three groups, to be operated at 24, 48 and 72 hours, respectively, under chloralose anaesthesia (0.10 g per kg body weight). The thoracic duct and the right cervical trunk were isolated from a median cervical approach, and a polyethylene catheter was inserted into them. The lymph was collected into marked centrifuge tubes, while the head of the animals was moved to and fro at the rate of 15/min. At the mid-point of collection, blood samples were taken from the femoral vein. From the animals to be operated upon 48 and 72 hours after the turpentine injury, blood samples were taken daily.

In a separate group of animals, 48 hours after the injury blood was collected from the external carotid artery supplying, and from the external jugular vein draining the area of inflammation.

Serum total protein content was estimated by the biuret test, the level of protein-bound hexose by the method of STARY *et al.* [24] using orcin as a reagent, the serum glucosamine level

by the method of SÜDHOF and PETROVIĆ [25] with EHRLICH's reagent. The serum diphenylamine reaction was evaluated by the method of NIAZI and STATE [17] as modified by AYALA et al. [1], with DISCHE's reagent (DPA being the extinction value read on the scale of the drum). Neuraminic acid was estimated by the method of BÖHM, DAUBER and BAUMEISTER [3] using BIAL's orcin reagent.

For colour estimation a Pulfrich type Zeiss photometer was used; layer thickness for total protein determination was 2 cm, otherwise 1 cm. For neuraminic acid determination a S_{57}, otherwise a S_{53} filter was used.

The results were evaluated by SUDENT's "t" test.

Results

The turpentine injections failed to elicit a change in the serum total protein level as compared to the initial values.

A significant increase in the serum DPA values was found as early as 24 hours after the injury, a further increase was noted at 48 hours and a falling tendency at 72 hours. The serum level of protein-bound hexose showed a similar behaviour. No significant rise in the serum neuraminic acid and glucosamine levels was demonstrated in the first 48 hours of inflammation (Table I).

Table I

Serum total protein and glycoprotein levels in dogs with turpentine inflammation in relation to normal values

Time	Serum						
	No. of animals	Total protein g per 100 ml ± SE	DPA Ext ± SE	Protein-bound hexose mg per 100 ml ± SE	Neuraminic acid mg per 100 ml ± SE	No. of animals	Glucosamine mg per 100 ml ± SE
Normal	25	5.82 ± 0.14	254.6 ± 8.8	103.8 ± 6.5	61.9 ± 3.68	23	98.4 ± 8.57
24 hrs	18	5.82 ± 0.17	288.9 ± 8.7	127.3 ± 4.0	71.0 ± 4.1	16	107.0 ± 6.9
48 hrs	12	6.00 ± 0.16	382.9 ± 10.0	166.9 ± 9.9	92.83 ± 6.1	10	128.0 ± 9.6
72 hrs	6	5.63 ± 0.20	330.0 ± 10.5	155.0 ± 14.6	80.2 ± 10.5	5	124.4 ± 14.7

Fortyeight hours after the first signs of inflammation, in the thoracic duct lymph of the total proteins significantly exceeded the normal values. DPA and protein-bound hexose values showed a significant rise at 48 hours and this was still demonstrable at 72 hours; the glucosamine level was essentially unchanged (Table II).

In the cervical trunk lymph, total protein values were significantly increased at 24 hours. DPA and glucosamine values, too, were significantly increased up to 72 hours. A significant reduction in the protein-bound hexose level occurred after the first 48 hours, and in that of neuraminic acid after the first 24 hours (Table III).

The serum values for carbohydrate per g protein underwent a significant rise during the first 48 hours following injury, high values being still present at

Table II

Serum total protein and glycoprotein levels in thoracic duct lymph in dogs with turpentine inflammation in relation to normal values

Time	Thoracic duct lymph					
	No. of animals	Total protein g per 100 ml ± SE	DPA Ext ± SE	Protein-bound hexose mg per 100 ml ± SE	Neuraminic acid mg per 100 ml ± SE	Glucosamine mg per 100 ml ± SE
Norm.	6	3.55 + 0.49	180 ± 9.04	65.5 + 8.34	57.2 ± 7.69	79.5 ± 18.9
24 hrs	8	4.21 ± 0.46	165 ± 4.53	92.5 ± 8.54	40.4 ± 3.24	87.4 ± 15.4
48 hrs	5	5.20 ± 0.62	268 ± 19.1	146.2 ± 14.7	78.0 ± 13.9	121.0 ± 12.2
72 hrs	5	4.70 ± 0.36	276 ± 29.3	125.2 ± 14.9	69.2 ± 8.52	107.0 ± 14.1

Table III

Serum total protein and glycoprotein levels in cervical trunk lymph in turpentine inflammation, in relation to normal values

Time	Cervical trunk lymph								
	No. of animals	Total protein g per 100 ml ± SE	No. of animals	DPA Ext ± SE	Protein-bound hexose mg per 100 ml ± SE	No. of animals	Neuraminic acid mg per 100 ml ± SE	No. of animals	Glucosamine mg per m ± SE
Norm.	6	2.45 ± 0.55	6	93.3 ± 6.15	50.2 ± 8.80	6	50.0 ± 5.09	5	39.2 ± 3.38
24 hrs	4	4.10 ± 0.17	7	145.0 ± 7.95	61.4 ± 6.36	5	28.0 ± 2.28	4	57.0 ± 6.56
48 hrs	6	4.02 ± 0.42	6	203.3 ± 18.4	81.7 ± 7.28	6	56.3 ± 9.90	5	71.2 ± 6.47
72 hrs	5	3.30 ± 0.37	5	189.0 ± 26.2	68.8 ± 13.9	5	59.0 ± 8.50	4	63.5 ± 10.5

Table IV

Carbohydrate concentration per g protein in turpentine inflammation, in relation to normal values

Time	Serum				
	No. of animals	Protein-bound hexose mg per 100 ml ± SE	Neuraminic acid mg per 100 ml ± SE	No. of animals	Glucosamine mg per 100 ml ± SE
Norm.	25	18.5 ± 0.57	10.7 ± 0.65	20	17.0 ± 1.06
24 hrs	18	22.2 ± 0.93 $P < 0.0005$	12.4 ± 0.74 $P < 0.05$	16	12.7 ± 1.52 $P < 0.0125$
48 hrs	12	28.1 ± 1.86 $P < 0.0005$	15.7 ± 1.16 $P < 0.0005$	10	21.7 ± 1.68 $P < 0.0125$
72 hrs	6	27.5 ± 2.36 $P < 0.0005$	14.1 ± 1.62 $P < 0.025$	5	21.7 ± 2.94 $P < 0.05$

72 hours. Neuraminic acid behaved similarly. A fall of the glucosamine level was registered during the first 24 hours, in contrast to the significant increase at 48 and 72 hours (Table IV).

In thoracic duct lymph, it was only after the first 48 hours of inflammation that a significant elevation of the values for protein-bound hexose per g protein could be noted. On the other hand, neuraminic acid was significantly reduced only after the first 24 hours, and glucosamine exhibited no distinct change (Table V).

Table V

Carbohydrate concentration per g protein in thoracic duct lymph in turpentine inflammation, in relation to normal values

Time	Thoracic duct lymph			
	No. of animals	Protein-bound hexose mg per 100 ml ± SE	Neuraminic acid mg per 100 ml ± SE	Glucosamine mg per 100 ml ± SE
Norm.	6	19.21 ± 1.97	16.8 ± 2.28	22.26 ± 3.86
24 hrs	8	21.9 ± 1.60 P < 0.20	10.04 ± 1.02 P < 0.01	20.27 ± 1.31 P < 0.30
48 hrs	5	28.36 ± 0.65 P < 0.0025	14.88 ± 2.16 P < 0.30	23.56 ± 1.37 P < 0.40
72 hrs	5	26.48 ± 2.14 P < 0.025	14.80 ± 1.53 P < 0.30	22.44 ± 1.92 P < 0.49

Table VI

Carbohydrate concentration per g protein in cervical trunk lymph in turpentine inflammation in relation to normal values

Time	Cervical trunk lymph				
	No. of animals	Protein-bound hexose mg per 100 ml ± SE	Neuraminic acid mg per 100 ml ± SE	No. of animals	Glucosamine mg per 100 ml ± SE
Norm.	6	23.26 ± 3.28	24.54 ± 3.82	5	19.07 ± 5.84
24 hrs	4	12.36 ± 1.25 P < 0.025	6.47 ± 0.53 P < 0.005	4	13.99 ± 1.67 P < 0.25
48 hrs	6	21.12 ± 1.96 P < 0.30	14.28 ± 2.45 P < 0.025	5	16.62 ± 1.90 P < 0.35
72 hrs	5	20.97 ± 3.21 P < 0.35	19.0 ± 3.54 P < 0.20	4	20.52 ± 5.09 P < 0.45

Table VII

Concentration of protein-bound hexose per g protein in thoracic duct and cervical trunk lymph in turpentine inflammation in relation to the serum levels

	Protein-bound hexose per g protein							
	No. of animals	Normal	No. of animals	24 hours	No. of animals	48 hrs	No. of animals	72 hrs
Serum	25	18.5 ± 0.6	18	22.2 ± 0.93	12	28.1 ± 1.86	6	27.5 ± 2.36
Thoracic duct lymph	6	19.2 ± 2.0 $P < 0.35$	8	21.9 ± 1.60 $P < 0.45$	5	28.36 ± 0.63 $P < 0.475$	5	26.48 ± 2.14 $P < 0.40$
Cervical trunk lymph	6	23.3 ± 3.3 $P < 0.01$	4	12.36 ± 1.25 $P < 0.0005$	6	21.12 ± 1.96 $P < 0.025$	5	20.97 ± 3.21 $P < 0.010$

In the right cervical trunk lymph the protein-bound hexose and neuraminic acid levels reckoned for 1 g of protein showed a significant fall at 24 hours; at 48 hours the values of the former approximated the initial levels and those

Table VIII

Concentration of neuraminic acid per g protein in thoracic duct and cervical trunk lymph in induced inflammation, in relation to the serum levels

	Neuraminic acid per g protein							
	No. of animals	Normal	No. of animals	24 hrs	No. of animals	48 hrs	No. of animals	72 hrs
Serum	25	10.7 ± 0.7	18	12.4 ± 0.74	12	15.7 ± 1.16	6	14.1 ± 1.62
Thoracic duct lymph	6	16.8 ± 2.3 $P < 0.0025$	8	10.04 ± 1.2 $P < 0.05$	5	14.88 ± 2.15 $P < 0.40$	5	14.8 ± 1.53 $P < 0.04$
Cervical trunk lymph	6	24.5 ± 3.8 $P < 0.0005$	4	6.47 ± 0.53 $P < 0.0025$	6	14.28 ± 2.45 $P < 0.45$	5	19.0 ± 3.54 $P < 0.15$

of the latter were significantly below the normal. Glucosamine did not show any significant change (Table VI).

The mutual relationship of carbohydrate per g protein in serum, thoracic duct and cervical trunk lymph, respectively, has also been studied. In this respect there was no significant difference between thoracic duct lymph and blood serum. In contrast, the relative values in the cervical trunk lymph were significantly lower than those in serum at 24 and 48 hours (Table VII).

Neuraminic acid levels in thoracic duct and cervical trunk lymph in relation to its serum values showed a significant decline in the first 24 hours only (Table VIII).

The concentration of glucosamine per g protein in relation to the serum values showed a significant decline only after the first 24 hours in the thoracic duct lymph and after 48 hours in the cervical trunk lymph (Table IX).

Further comparative studies dealt with the concentrations of protein-bound carbohydrates in the blood of the external carotid artery and of the external jugular vein. At 24 hours there was a hardly evaluable elevation in the arterial as well as in the venous blood whereas at 48 hours a significant rise in both values was noted. Serum total protein was below its initial value in both arterial and venous blood at 24 and 48 hours (Table X).

Table IX

Concentration of glucosamine per g protein in thoracic duct and cervical trunk lymph in induced inflammation, in relation to the serum levels

	Glucosamine per g protein							
	No. of animals	Normal	No. of animals	24 hrs	No. of animals	48 hrs	No. of animals	72 hrs
Serum	20	17.0 ± 1.1	16	12.7 ± 1.52	10	21.7 ± 1.68	5	21.7 ± 2.94
Thoracic duct lymph	6	22.3 ± 3.9 $P < 0.3$	8	0.27 ± 1.31 $P < 0.0025$	5	23.56 ± 1.37 $P < 0.25$	5	22.44 ± 1.92 $P < 0.45$
Cervical trunk lymph	5	19.1 ± 5.8 $P < 0.30$	4	13.99 ± 1.67 $P < 0.35$	5	16.52 ± 1.90 $P < 0.05$	4	20.52 ± 5.09 $P < 0.045$

Discussion

In evaluating the data obtained, it must be taken into account that the lymph of the thoracic duct is largely hepatic whereas that of the cervical trunk is peripheral in origin. Presumably, the protein-bound carbohydrates in the lymph of the thoracic duct are mostly derived from the liver, whereas those of the cervical trunk from transcapillary protein transfer. The lymph of both vessels carries, moreover, those protein-bound carbohydrates which leak from the connective tissue ground substance across the interstitial spaces into the blood stream. This is supported by the fact that under normal conditions the concentration of protein-bound carbohydrates per g protein is higher in thoracic duct and cervical trunk lymph than in blood serum. When the inflammation has become manifest, the carbohydrate level in the lymph draining the affected area is significantly reduced whereas the values found in the lymph of the thoracic duct remain identical with those of the blood serum.

BERENCSI and KROMPECHER [2] concluded from histochemical studies that the polysaccharides originating from tuberculous lesions pass into the blood stream through the lymphatic pathways of the tissue. They based their view on the assumption of GERSH and CATCHPOLE [8] and other authors that depoly-

Table X

Serum total protein and protein-bound hexose in external carotid arterial and jugular venous blood

		Normal		24 hours			48 hours		
		No. of animals	mg per 100 ml ± SE	No. of animals	mg per 100 ml ± SE		No. of animals	mg per 100 ml ± SE	
Total protein	Art. carot. ext.	8	5.54 ± 0.05	5	4.80 ± 0.34 P < 0.01	P < 0.25	4	4.58 ± 0.35 P < 0.0025	P < 0.45
	Vena jug. ext.	8	5.46 ± 0.08	5	4.46 ± 0.22 P < 0.005		4	4.73 ± 0.43 P < 0.025	
Protein-bound hexose	Art. carot. ext.	8	113.1 ± 3.3	5	123.2 ± 6.6 P < 0.10	P < 0.40	4	163.1 ± 6.1 P < 0.0005	P < 0.49
	Vena jug. ext.	8	111.6 ± 4.4	5	120.8 ± 2.8 P < 0.05		4	162.6 ± 4.8 P < 0.0005	

merization of connective tissue ground substance results in increased blood concentrations of water-soluble and diffusible glycoproteins. Local accumulation of protein-bound carbohydrates due to inflammations or injuries of small extent would, however, be hardly so serious as to account for the high serum values.

It is a further question, to what extent the protein-bound carbohydrates originating from the liver are represented in the thoracic duct lymph and how far they are mobilized in order to meet the organism's demands. HOUCK [9] found cortisol to reduce in rats the amount of subcutaneous connective tissue in the first 24 hours, but to cause no further tissue loss upon continued administration.

It is therefore thought that mobilization of glycoproteins does not exceed the demands. SCHMIDT [21] has shown in animal experiments that the hexosamine level is definitely lower in linear wounds than in autografts. According to our observations, in cases of malignant disease associated with increased glycoprotein values no further increase occurs in the level of these substances if the tumour has successfully been removed. On the other hand, when normal values had been present prior to surgery, they have been found to rise invariably subsequent to it [18]. This favours the view that even when the primary cause of enhanced glycoprotein mobilization has been eliminated (*e.g.* a tumour by surgery), sufficient amounts required for tissue repair are still available in the blood.

The reduced glycoprotein levels in the lymph of the cervical trunk involved by turpentine-induced inflammation are in conformity with the increased amounts of these substances found after various injuries in the affected area by JACKSON [13], FISHKIN *et al.* [7], WHITE *et al.* [28] and HOUCK [10, 12]. In other words, our observations lend support to the theory that the glycoproteins leaving the capillaries are retained within the damaged area for the purpose of tissue repair. On the evidence of further studies by HOUCK and JACOB [11], the concentration of hydroxyproline in the wound decreases while that of hexosamine increases, then, as healing of the wound proceeds, a quantitative increase in its collagen and acid mucopolysaccharide contents takes place at the expense of glycoproteins.

According to the results of ROSENLUND [20], MIETTIENEN [14], and others, the concentration of protein-bound carbohydrates fails to rise after hepatic injury. It has been confirmed by our earlier experiments that the level of glycoproteins correlates well with the extent of liver damage [19]. Though there is a certain rise in glycoprotein concentrations 24 hours following carbon tetrachloride poisoning, nevertheless, this increase only amounts to 50 per cent of that occurring in healthy animals. After partial hepatectomy, the level persists at the initial value. This would seem to account for the poor wound healing in patients with liver disease.

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STEROIDS IN HUMAN SKIN AND HAIRS

I. ZIMMERMANN CHROMOGENS IN THE SKIN OF NORMAL AND OF HIRSUTE WOMEN

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Specimens of the hairless abdominal and the hairy pubic skin excised from endocrinologically healthy women were found to contain Zimmermann chromogens. The chromogen content was considerably higher in the hairy skin.

Zimmermann chromogens were detected in much greater amounts in the hairy abdominal and the pubic skin of hirsute than non-hirsute women.

Skin extracts were studied by means of paper and thin-layer chromatography. Thin-layer chromatography with Kieselgel G as adsorbent, revealed substantial quantities of cholesterol and several kinds of steroids in moderate amounts; with aluminium oxide as the adsorbent, a few 17-ketosteroids were detected.

Several disturbances of steroid metabolism have been brought to light by urinary and blood steroid studies aimed at clarifying the pathogenesis of hirsutism, yet we are still ignorant of the extent to which they may be essential in the causation of the condition (BROOKSBANK, 1961; DORFMAN, 1963; JULESZ et al. 1963; FAREDIN et al., 1964). This lack of knowledge is a source of much uncertainty in the diagnosis and treatment of hirsutism and this is why many cases reported in the literature, and even one third of our own cases, had to be diagnosed as "idiopathic hirsutism".

The working hypothesis underlying the present investigations is, that the causative factor of hirsutism must be sought for in the skin, respectively in the enzyme systems which are active in the skin and its secondary structures. Hirsutism develops in women in whose skin, or around whose hair follicles, the androgen to oestrogen ratio is high. Theoretically it may therefore be put forward that, on the cellular level, the woman becomes hirsute whose hair follicles are reached by much androgen from the blood, or whose follicular cells, due to some enzyme defect, bind more than the normal amount of androgen or break down oestrogens at an excessive rate. It is equally possible that steroids are produced from precursors by the skin itself. A detailed study of these questions might lead to a better understanding of what is termed idiopathic hirsutism and, perhaps, to the clarification of its Seabright bantam syndrome-like form.

Little is known of the processes of steroid metabolism occurring in the skin. Their study rests on the detection and estimation of the steroids in the skin. The present paper will give an account of investigations into substances detected in the skin of normal and hirsute women by application of the Zimmermann reaction.

Methods

Solubilization of skin tissue

From normal and hirsute women, subjected to lower median laparotomy for various gynaecological conditions, 1 to 3 g specimens of abdominal skin (hairless in normals) and of hairy skin from the pubes, were excised, carefully cleansed of fat and blood, frozen and, after removal of the horny layer, cut into sections 20 to 25 μ thick. The sections were then collected, weighed, suspended in 40 ml of a 2.5 per cent solution of NaOH, and allowed to stand at room temperature for 24 hours.

Enzymatic hydrolysis and extraction

The lysed tissue suspension was adjuncted to near pH 6 with about 2.5 ml of 11 n HCl, and then acidified to pH 4.5 with 5 ml of acetate buffer. After addition of 0.5 ml of a solution of crystalline penicillin (25,000 IU) and 50,000 U of β-glucuronidase, the tissue suspension was incubated at 43 °C for 18 hours.

Following hydrolysis, the tissue suspension was centrifuged, and the separated aqueous phase extracted with 4×20 ml of chloroform. The chloroform was separated from the aqueous phase by centrifugation. The collected chloroformic extract was shaken out first with 5 ml of cold 1 n NaOH and then with 5 ml of cold 0.1 NaOH, lastly with 5 ml of cold distilled water, thereafter dehydrated with 2 to 4 g of anhydrous Na_2SO_4, and evaporated to dryness in vacuo at 45 °C. The dry residue was dissolved in 2 ml of absolute ethanol, and 0.2 ml portions of this solution were used for the Zimmermann reaction (FAREDIN et al., 1956).

Hydrochloric-acid hydrolysis and extraction

The aqueous phase left over after chloroformic extraction was measured and added to the remains of the tissue that had been separated by centrifugation following enzymatic hydrolysis. To this, 3 ml of concentrated HCl was added for every 20 ml of the aqueous phase and the mixture was boiled with glass beads under a reflux condenser for 15 minutes.

The tissue hydrolysate was centrifuged and the separated aqueous phase extracted with the same 20 ml of ether with which the tissue fragments had previously been shaken out. The aqueous phase was then extracted with additional 3×20 ml of ether.

The collected ethereal extract was washed with 2×20 ml of 2 n NaOH and 2×10 ml of distilled water, then dehydrated with 2 to 3 g of anhydrous Na_2SO_4 and evaporated to dryness. The dry residue was dissolved in 2 ml of absolute ethanol, and 2 ml portions of this solution were used to estimate the Zimmermann chromogens.

Total Zimmermann chromogens

The data obtained with enzymatic and hydrochloric-acid hydrolysis were computed for 1 g of wet skin tissue and added up.

Ketonic Zimmermann chromogens

The ketonic Zimmermann chromogens were separated by treating the total Zimmermann chromogens obtained after enzymatic and hydrochloric-acid hydrolysis with the Girard-T reagent, following the method of PINCUS and PEARLMAN (1941), and purified on Nymco florisil adsorbent column (FAREDIN and TÓTH, 1964). The dry substance of the resulting eluate was dissolved in 1 ml of absolute ethanol, and 0.2 ml portions of the solution were used for the quantitative determination of the ketonic Zimmermann chromogens. The values were computed for 1 g of skin tissue, and added up.

Identification studies

Paper chromatography. Schleicher-Schüll No. 2043 b. M.G.I. filter-paper was impregnated with a methanol—propylene glycol mixture 1 : 1, according to KOCHAKIAN and STIDWORTHY (1952). A 1 : 1 mixture of benzene and cyclohexane saturated with propylene glycol was used as the mobile phase. On completion of the run, which took 3 to 4 hours, the Zimmermann colour reaction was developed.

Thin-layer chromatography was done in a Desaga apparatus, using a type AK-01-AK-02 analytical quartz lamp.

With Merck Kieselgel G as the adsorbent, a 0.25 mm layer was made on a glass plate measuring 10 cm by 20 cm. After running in 4 per cent ethanol benzene the plate was thoroughly soaked with 50 per cent H_3PO_4 and it was dried at 80 °C for half an hour to develop the colour reaction.

With aluminium oxide a 0.25 mm layer was spread on a glass plate measuring 10 cm by 35 cm. The Zimmermann chromogens were applied onto a layer activated at 120 °C, and run in a mixture of n-hexane, ethyl acetate, glacial acetic acid and absolute ethanol (120 : : 120 : 2 : 1) for 3 to 4 hours at 30 °C. The separated steroids were developed with the Zimmermann reaction.

Results

Samples of abdominal hairless skin excised from 19 endocrinologically healthy women, operated on for various gynaecological diseases (retroflexion, Douglas' abscess, etc.), were studied for total Zimmermann chromogens. In five of these women, determinations were also carried out in samples of hairy skin excised from the pubic region. All results are expressed in micrograms of androsterone referred to 1 g of wet skin.

Table I shows that in the hairless abdominal skin of 19 non-hirsute women the amount of the total Zimmermann chromogens ranged between 53 and 170 μg/g (mean, 107 μg/g), and in the hairy skin from the pubes of 5 women between 104 and 274 μg/g (mean, 193 μg/g). Though it varied over wide ranges, the amount of Zimmermann chromogens was twice as much in the pubic than in abdominal skin. The difference was significant statistically ($t_{(22)} = 5.251$; $p < 0.001$).

The Zimmermann reaction is not specific for 17-ketosteroids; several other steroids, and even contaminants accompanying them, react with alkaline *m*-dinitrobenzene. Therefore, the ketonic fraction was separated from the total Zimmermann chromogens and purified on Nymco florisil. We succeeded in determining this fraction in 12 of the abdominal and 4 of the pubic skins. Table I shows that ketonic Zimmermann chromogens varied in hairless abdominal skin from 11 to 44 μg/g (mean, 24 μg/g), and in pubic skin from 24 to 82 μg/g (mean, 52 μg/g).

The finding thus revealed that only a small proportion of the total Zimmermann chromogens is ketonic (21 and 23 per cent, respectively); further, that the hairy skin tissue of healthy women contains more ketonic Zimmermann chromogens.

So far we could study the Zimmermann chromogen content of abdominal and pubic skin in two women with ovarian hirsutism. The diagnosis, verified by histology, was in both cases ovarian microcystic degeneration. The total Zimmermann chromogen content in the abdominal skin of one woman was 147 μg/g, and in that of the other 240 μg/g (mean, 193 μg/g). These abdominal skins were as hairy as were the pubic skins from the same two women.

Table I

Zimmermann chromogen contents of skin in non-hirsute women

Initials	Age, years	Diagnosis	Hairless abdominal skin			Hairy pubic skin		
			Total Z. chr.	Ketonic Z. chr.		Total Z.chr.	Ketonic Z. chr.	
			μg/g	μg/g	%	μg/g	μg/g	%
Sz. I.	19	Douglas abscess	136	25	18.5	—	—	—
Zs. I.	35	Ovarial tumour	121	19	15.7	—	—	—
T. J.	36	Ovarial cyst	107	16	15.1	—	—	—
Sz. L.	37	Perineal rupture	109	28	26.0	274	82	29.8
N. I.	38	Perineal rupture	85	22	26.0	—	—	—
M. Gy.	38	Hypertrophic uterus	65	16	25.3	—	—	—
Sz. P.	39	Ovarial cyst	133	—	—	—	—	—
S. S.	39	Perineal rupture	170	36	21.4	262	55	21.0
K. Gy.	41	St. p. lapar.	117	30	25.6	—	—	—
K. J.	42	Myoma	58	—	—	104	—	—
R. J.	42	Perineal plast. oper.	96	16	16.8	147	24	15.9
F. L.	43	Explor. laparotomy	149	21	13.8	180	47	26.0
F. J.	55	Amput. of the uter. cerv.	59	—	—	—	—	—
Z. L.	56	Myoma	53	—	—	—	—	—
Cs. L.	65	Salpingitis	129	11	8.7	—	—	—
Sz. S.	33	Perineal rupture	119	44	36.6	—	—	—
B. I.	28	Perineal rupture	125	—	—	—	—	—
H. G.	48	Myomatosis	56	—	—	—	—	—
Cs. G.	53	Myoma	161	—	—	—	—	—
		Mean	107±11	23.7±2.7		193±33	52±12	
		Limits	53—170	11—44		104—274	24—82	

Statistical analysis:

Total Zimmermann chromogens: $t_{(22)} = 5.251$, $p < 0.001$
Ketonic Zimmermann chromogens: $t_{(14)} = 4.710$, $p < 0.001$

The ketonic Zimmermann chromogens amounted to 31 and 52 μg/g, respectively (mean, 42 μg/g) (Table II).

The amount of total Zimmermann chromogens in the pubic skins was 206 and 450 μg/g (mean, 328 μg/g). After purification, the ketonic Zimmermann chromogens were 73 and 75 μg/g, respectively (mean, 74 μg/g). These findings hardly permit conclusions; all one may say is that the hirsute abdominal skin

Table II

Zimmermann chromogen contents of skin in hirsute women

Initials	Age, years	Diagnosis	Hairy abdominal skin μg/g		Hairy pubic skin μg/g	
			Total	Ketonic	Total	Ketonic
Sz. I.	23	Ovarial hirsutism	147	52	206	73
L. D.	24	Ovarial hirsutism	240	31	450	75
		Means	193	42	328	74
		After treatment with ACTH				
Á. K.	21	Ovarial hirsutism	225	95	—	—
B. E.	27	Ovarial hirsutism	148	34	91	41
M. E.	18	Stein—Leventhal syndrome	220	79	307	148
		Means	198	69	199	95

most probably contains more Zimmermann chromogens than does the non-hirsute skin.

The question arose whether the skin of hirsute women might not respond to ACTH treatment with a rise in the amount of Zimmermann chromogens. Three patients with ovarian hirsutism were each given 40 IU of ACTH for the three last preoperative days. To control the effect of this treatment, the total 17-ketosteroids and 17-hydroxy-corticosteroids were studied in the urine. The results of the experiments on the skin are presented in Table II. In the hairy abdominal skin the total Zimmermann chromogen content varied between 148 and 225 μg/g (mean, 198 μg/g), and the ketonic one between 34 and 95 μg/g (mean, 69 μg/g). Though no appreciable difference was found between the untreated and ACTH treated groups, the results confirmed our former finding that hirsute abdominal skin contains much more Zimmermann chromogens than does the non-hirsute one. In two of these three women in pubic skin the total Zimmermann chromogens amounted to 91 and 307 μg/g (mean, 198 μg/g), and the ketonic to 41 and 148 μg/g (mean value of 95 μg/g). Despite the fact that these values appear to be higher than those obtained for hirsute patients not treated with ACTH, no significance can be attributed to them in view of the limited number of cases and the wide scatter.

In order to determine whether the Zimmermann chromogens found were steroids, the following studies were undertaken.

Paper chromatography. The total and the ketonic Zimmermann chromogens in hairy and hairless skins were studied. At the places corresponding to androsterone, etiocholanolone, dehydroepiandrosterone and epiandrosterone,

used for control purposes, it was not possible to demonstrate these steroids with the Zimmermann reaction either from the hairy or the hairless skin samples. Only an elongated spot was detected whose R_f values varied between 0.88 and 0.92. Such high R_f values were obtained only with 3-chlorodehydroepiandrosterone and the acetylated derivatives of androsterone, etiocholanolone, dehydroepiandrosterone and epiandrosterone. The colour of the spots of the control steroids was much more stable than those of the Zimmermann chromogens separated from the skins.

Thin-layer chromatography. Proceeding from the assumption that skin extracts obtained by enzymatic or hydrochloric acid hydrolysis may contain steroids not demonstrable with the Zimmermann reaction, attempts were made to separate the total Zimmermann chromogens by thin-layer chromatography with Kieselgel G as adsorbent, and to demonstrate them by fluorescence and the phosphoric acid colour reaction methods allowing to detect minute quantities of steroids.

Fig. 1 presents the thin-layer chromatogram of the total Zimmermann chromogens obtained with enzymatic (E) and hydrochloric acid (S) hydrolysis, as viewed under ultraviolet light. The controls were 20 g of dehydroepiandrosterone, 40 g of androsterone, and 40 g of cholesterol. As seen, none of these three control steroids was demonstrable under ultra-violet light. In the skin extracts a few intensely fluorescent spots are visible; in addition, the spot with a high R_f value also appeared.

Fig. 2 presents the same chromatoplate with colours developed by phosphoric acid. It shows, in addition to the control spots of dehydroepiandrosterone and cholesterol, a considerable amount of cholesterol (violet-red spot) and a high R_f russet spot, in both skin extracts.

Fig. 3 shows the same chromatoplate under ultra-violet light. It distinctly reveals intensely fluorescent spots of the control steroids and of the skin extracts obtained by enzymatic and hydrochloric acid hydrolysis.

With Kieselgel G the skin extracts revealed, in addition to cholesterol, the same spot of rapid mobility, which could be shown to be present by paper chromatography. Besides, below and above the cholesterol spot, several other substances were detected in minute amounts, which most probably were steroids.

Next it was studied whether some of the Zimmermann chromogens determined in substantial amounts were 17-ketosteroids. In our experience, the total Zimmermann chromogens contain considerable quantities of foreign chromogens, fat, etc. To reduce the interfering effect of such substances, we applied the ketonic fraction of the Zimmermann chromogens obtained from a 4.3 g sample of hairless abdominal skin excised from two non-hirsute women, using thin-layer chromatography with aluminium oxide which was found the best adsorbent for the separation of 17-ketosteroids.

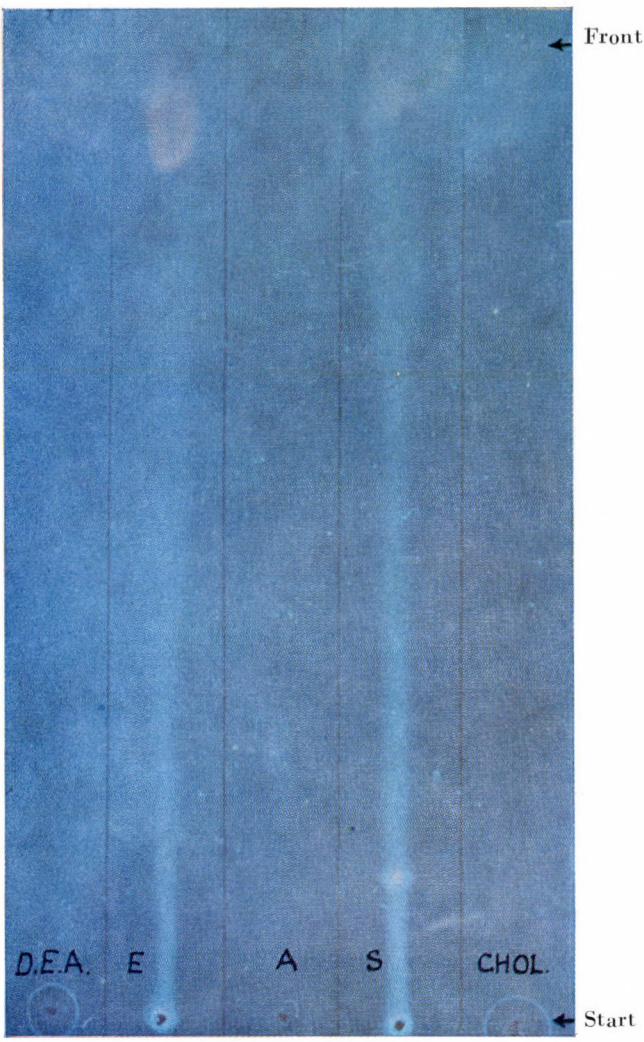

Fig. 1. Chromatogram of skin extracts on Kieselgel G layer under ultra-violet light

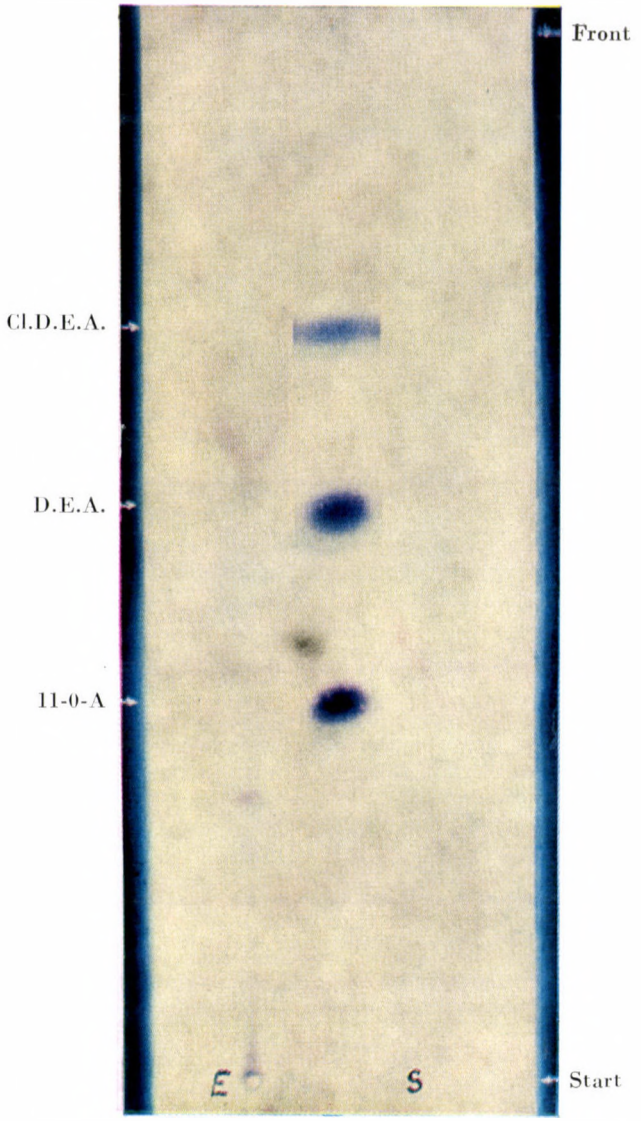

Fig. 4. Chromatogram of skin extracts on aluminium oxide layer, developed with the Zimmermann reaction

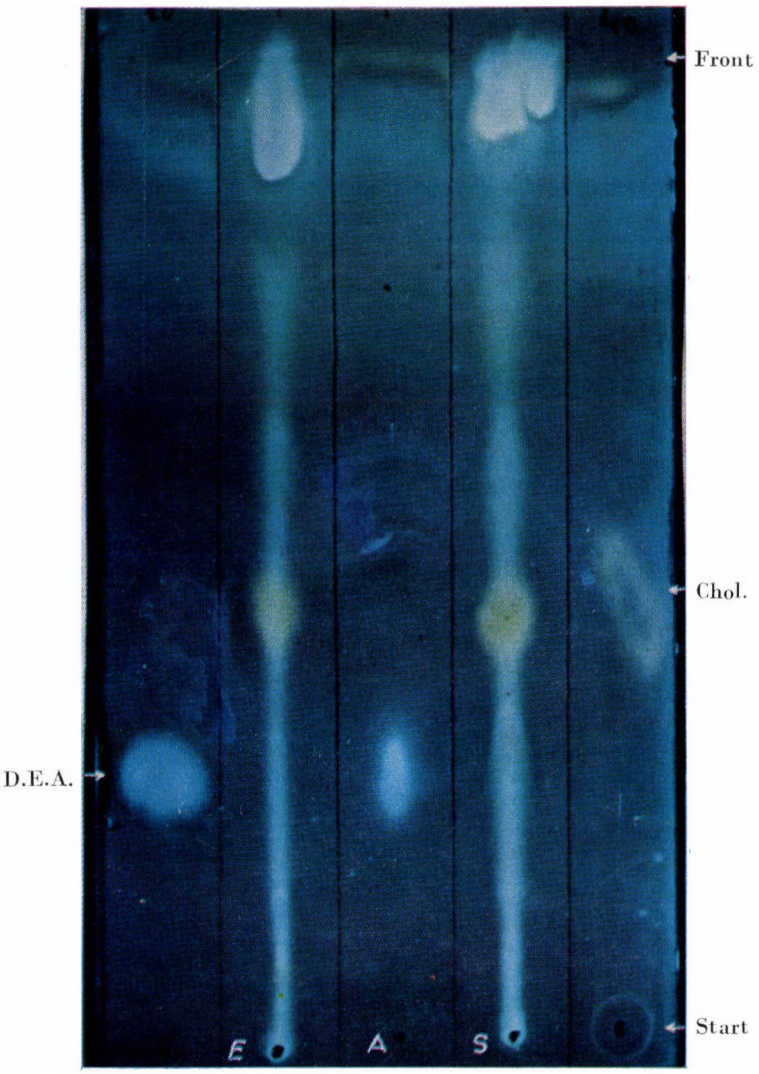

Fig. 3. The same chromatogram as on Fig. 2 under ultra-violet light

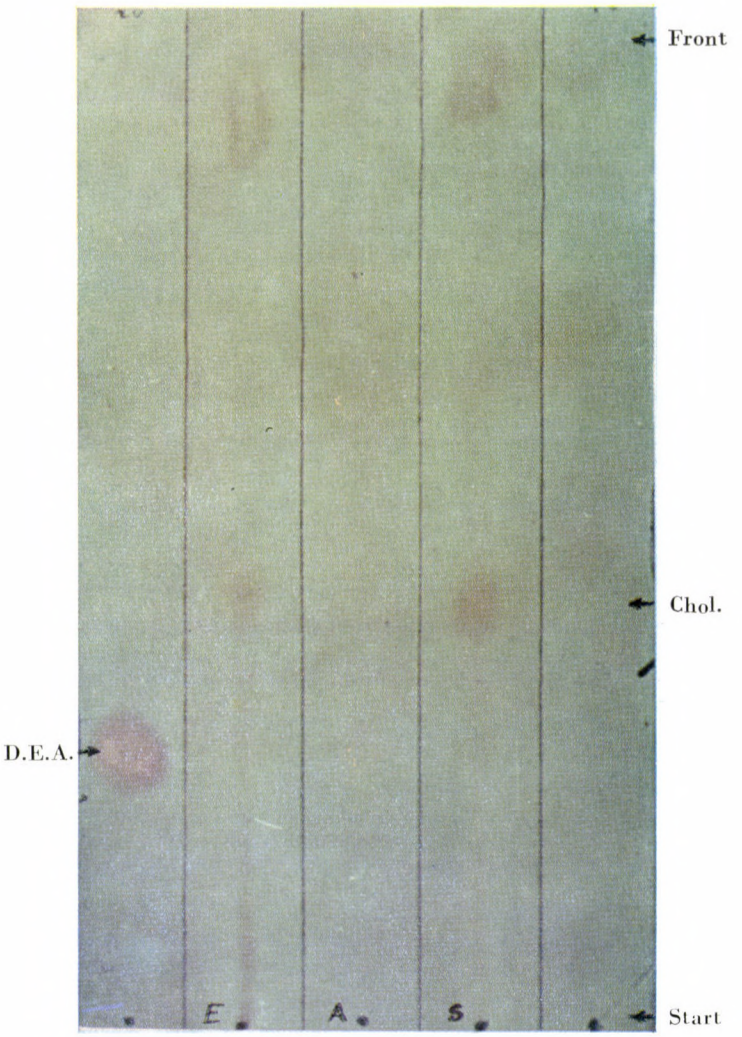

Fig. 2. Chromatogram of skin extracts on Kieselgel G layer developed with 50 per cent phosphoric acid in normal light

Fig. 4 presents the resulting picture. On the first strip (from left to right), to which the extract obtained by enzymatic hydrolysis had been applied, development by the Zimmermann reaction produced four violet-blue spots and a large brownish-blue one of high mobility, indicating different 17-ketosteroids. To the second strip were applied the standard steroids (from above downwards): 10 μg of 3-chlorodehydroepiandrosterone, 20 μg of dehydroepiandrosterone, and 11 μg of 11-ketoandrosterone. On the third strip the extract obtained by hydrochloric acid hydrolysis was run. On this, there appeared three faint spots. Because of the interference by impurities (mostly fat), this chromatogram could not be used for identification purposes but demonstrated the presence of 17-ketosteroids.

Discussion

The skin is no longer thought to be the passive organ, for which it had been taken formerly. Its lifelong restitution and the cyclic process of hair-growth require vigorous metabolism. A wide variety of enzymes has been described from the skin and its secondary structures: cytochromoxydase, mucindehydrogenase, monoamino oxydase, dermopeptidase, phosphorylase, etc. (MONTAGNA, 1962).

MEYER (1931) detected substantial amounts of cholesterol in the human skin. Several authors demonstrated that various animal tissues, including human skin, are capable of synthesizing cholesterol from acetate (SRERE et al., 1950; POPJAK and BEECKMAN, 1950; KRITCHEVSKY, 1958). However, from the point of view of our working hypothesis, greater importance attaches to two questions: can the human skin be shown to contain steroid hormones? and if so, is the cholesterol in it capable of conversion into a steroid hormone? In his monograph, ROTHMAN definitely stated in 1955: "up to this time, no biologically active steroid hormones have been demonstrated to be present in the skin." The situation has not changed since (MONTAGNA, 1962). An increasing number of data has accumulated on the relationship of hair-growth to hormones, but none seem to be available as yet on the demonstration of steroid hormones in the skin. MALKINSON *et al.* (1959) suggested a role for the skin in glycocorticoid metabolism, but the published data refer only to the steroid contents of the sebum and sweat (COOK and LÖRINCZ, 1963; BOSSE and PASCHER, 1964; BOSSE *et al.*, 1964). FAZEKAS (1961) suggested a role for the skin in glyco- and mineralocorticoid metabolism, too.

The part played by the androgens and oestrogens in the development of the secondary sexual structures, has long been recognized. The difference in hair growth between the human male and female is commonly attributed to these steroids. Our working hypothesis assigns to them also a role as aetiological agents in hirsutism. This is a difficult problem, for very little is known of the

biochemical, physiological and pathophysiological conditions prevailing in the hair follicles. Nor have we any knowledge of the mechanism through which the androgens and oestrogens would participate in the causation of hirsutism.

To detect and quantitatively determine androsterone, dehydroepiandrosterone, epiandrosterone, and certain other androgens, the Zimmermann reaction is most widely used. This reaction is not, however, suitable for the detection of testosterone and it is of limited specificity; more reliable are the values obtained for the ketonic fraction separated by the use of the Girard's reagent T.

The present results have shown less Zimmermann chromogens in the hairless abdominal than the hairy pubic skin of non-hirsute women. The quantity of Zimmermann chromogens is practically the same in the hairy abdominal skin of hirsute women as in the hairy pubic skin of non-hirsute women. Treatment with ACTH leaves unaffected both the abdominal and the pubic skin of hirsute women.

Our paper chromatographic studies have shown the presence of a spot of high R_f value. Thin-layer chromatography with Kieselgel G revealed in the total Zimmermann chromogens a substantial quantity of cholesterol and lesser amounts of substances fluorescing with phosphoric acid; these are presumably steroids. On aluminium oxide layer, the ketonic Zimmermann chromogens are separable and the Zimmermann reaction yields four steroid spots. Their identification is in progress.

Further investigations are required to explain the differences found in the amount of Zimmermann chromogens and to identify the steroids present in the skin extracts.

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STEROIDS IN HUMAN SKIN AND HAIRS

II. NEW METHODS OF CHOLESTEROL ASSAY IN HUMAN SKIN AND HAIRS

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Thin-layer and column chromatography revealed considerable amounts of cholesterol in extracts of human skin and hairs. Thin-layer chromatograms showed, in addition, the occurrence of other steroids.

A procedure has been elaborated to estimate minute quantities of total cholesterol in human skin and hairs. The maximum difference between parallel determinations amounted to 12.5 per cent. The mean recovery rate was 92.2 per cent.

It has been known since MEYER's work (1931) that the human skin contains cholesterol in substantial quantities. MOORE and BAUMANN (1952) showed that most human organs are rich in it. Its biosynthesis in the skin, especially in the rat, has been studied by several investigators (MILLER and BAUMANN, 1954; NEIDERHISER and WELLS, 1959; GAYLOR, 1960; HORLICK and AVIGAN, 1963; CLAYTON *et al.*, 1963, etc.).

Opinions now agree that cholesterol is one of the principal steroid precursors. Incubating adrenal, testicular, ovarian, and placental homogenates, several authors have shown the formation of intermediate products during the conversion of cholesterol to pregnenolone (SOLOMON *et al.*, 1956; SHIMIZU *et al.*, 1960; DORFMAN, 1961, etc.). A question still open is whether the cholesterol present in the skin is capable of conversion into biologically active steroid hormones. Nor are any data available on the quantity of cholesterol in human hairs.

In the hope that a detailed study of the latter point will lead to a better understanding of the genesis of hirsutism, we first had to elaborate a rapid and simple method for the quantitative determination of the total cholesterol contents in human skin and hairs.

Methods

1. *Solubilization and exgtaction of hhman skin and hairs*

Hairs obtained from different areas (head, pubic region, armpit, etc.) was defattened with a 5 per cent solution of a commercial detergent, washed several times with distilled water, and dried. Amounts of 30 to 50 mg were used in the experiments.

Samples of skin, excised during an operation, were cleansed of fat and surface iodine, cut into slices, and used in quantities of 80 to 100 mg.

Skin and hairs alike were solubilized by boiling under a reflux condenser for 20 minutes in 20 ml of 2.5 per cent sodium hydroxide solution. After cooling, the homogeneous solution was acidified with 11 n hydrochloric acid to pH 2—3, and shaken out with 4×15 ml of ether. The ethereal extract was washed with 3×20 ml of 2 n sodium hydroxide and 2×20 ml of distilled water, then treated with 1—3 g of anhydrous sodium monosulphate, and evaporated to dryness.

2. *Thin-layer chromatography of skin and hair extracts*

A 20 sq. cm Desaga glass plate was used, with 250 μ Merck Kieselgel G layer (after Stahl). The skin and hair extracts, and cholesterol as the standard, were applied in benzene to strips 2 cm in width. During their application, a stream of hot air was directed to the starting points. Running time was 60—70 minutes.

The dried plate was sprayed with 50 per cent phosphoric acid and kept at 80 °C for 30 minutes, whereupon the characteristic spots of cholesterol appeared.

3. *Purification of skin and hair extracts on Nymco florisil column*

The crude extracts of skin and hairs treated according to ZLATKIS *et al.* (1953), in addition to the violet red colour characteristic of cholesterol, often revealed a reaction brownish in colour. As this was presumably due to impurities, the crude extracts were purified on Nymco florisil (FAREDIN and TÓTH, 1964). The cholesterol extract was applied onto a 2 g column suspended in benzene, and washed first with 15 ml of benzene and then with 10 ml of 0.5 per cent ethanol in benzene. Thereafter, the pure cholesterol was eluted from the column with 12 ml of 0.5 per cent ethanol in benzene. The eluate was evaporated to dryness and the colour reaction of ZLATKIS *et al.* (1953) was performed. This yielded the same colour as did the cholesterol standard.

4. *Identification of the purified cholesterol fraction*

Apart from the sulphuric acid-ferric chloride method of ZLATKIS *et al.* (1953), to identify the purified cholesterol obtained from human skin and hairs the sulphosalicylic acid method (RAPPAPORT and EICHHORN, 1960), the Liebermann-Burchard reaction (SCHOENHEIMER and SPERRY, 1934), the perchloric acid (TAUBER, 1952), and the phosphomolybdic acid (KRITCHEVSKY and KIRK, 1952) colour reactions were performed.

5. *Technique*

To the dry residue obtained from the florisil column 4.5 ml of glacial acetic acid are added and the content of the flask is dissolved under mild heating. The solution is mixed with 2.5 ml of ferric chloride under vigorous shaking. After cooling for 5 minutes, the intensity of the developing violet red colour is measured against the blank in a Havemann photometer, using a V.G. 9 colour filter and 0.5 cm cuvettes.

Results

For the qualitative study of the cholesterol contents, skin and hairs were obtained from different sites on the body of a patient with ovarian hirsutism who had been treated with ACTH preoperatively for three days. The results are shown in three chromatograms, in each of which the sequence of the substances studied is as follows (from left to the right): 1 = extract of hair from the head; 2 = extract of axillary hairs; 3 = 40 μg of control cholesterol; 4 = extract of pubic hairs; 5 = extract of hairs from the thigh; 6 = hairy abdominal skin extract obtained by enzymatic hydrolysis; 7 = 40 μg of control dehydroepi-

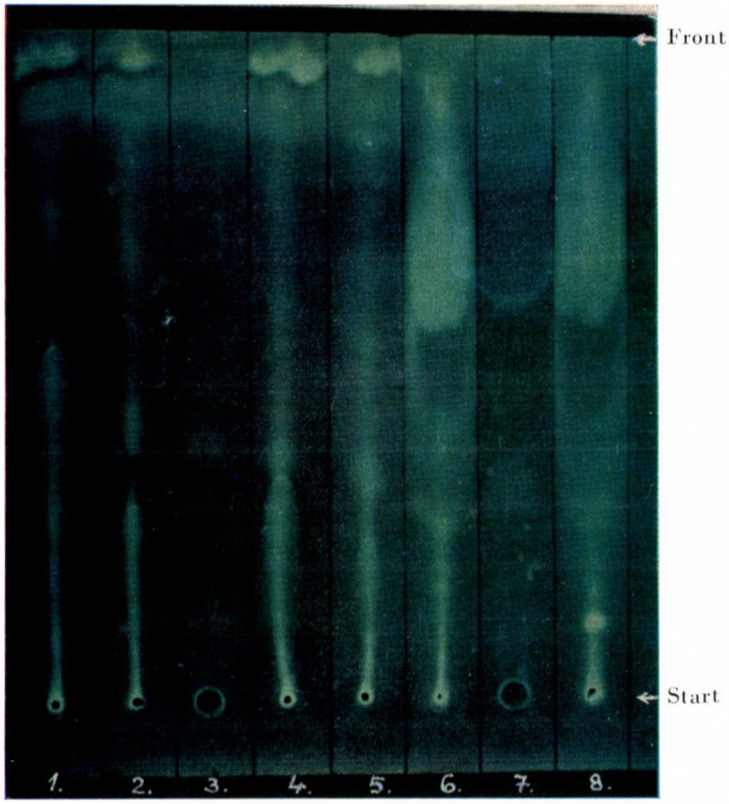

Fig. 1. Chromatogram of extracts of skin and hairs under ultra-violet light

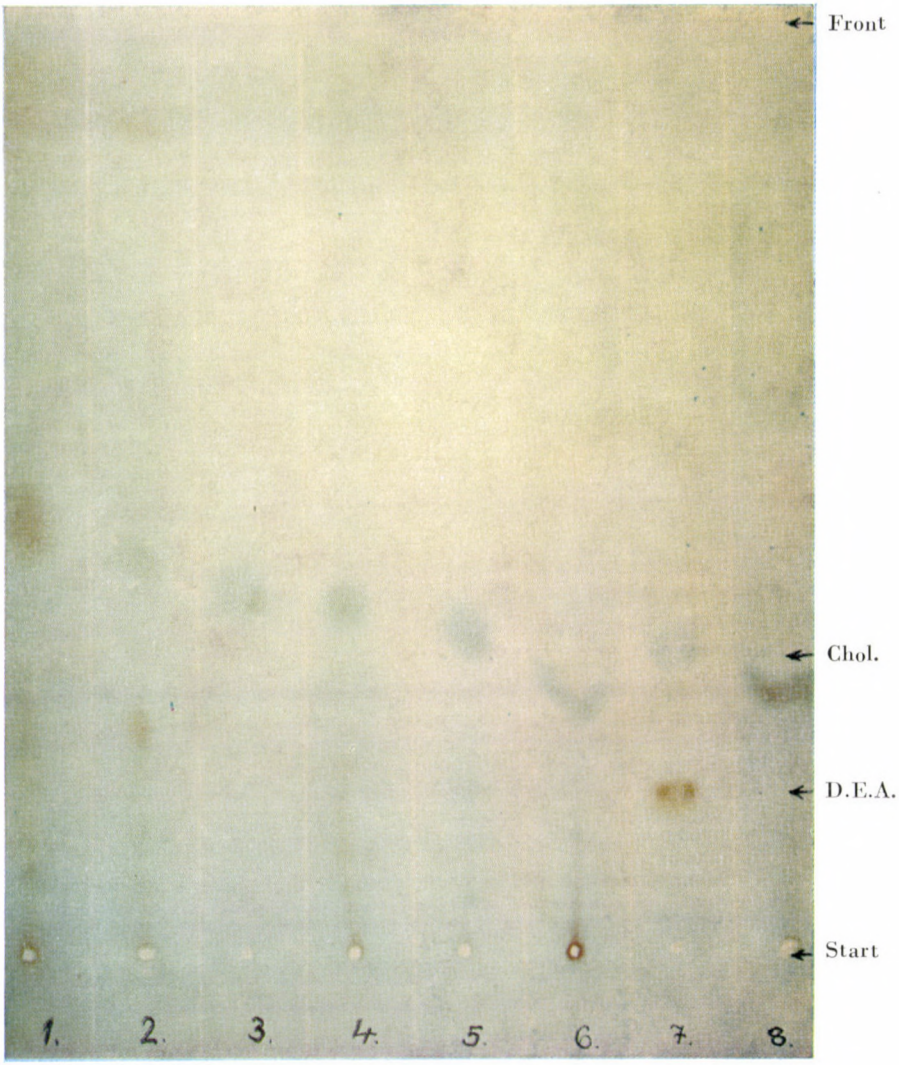

Fig. 2. Chromatogram of extracts of skin and hairs detected with 50 per cent phosphoric acid in daylight

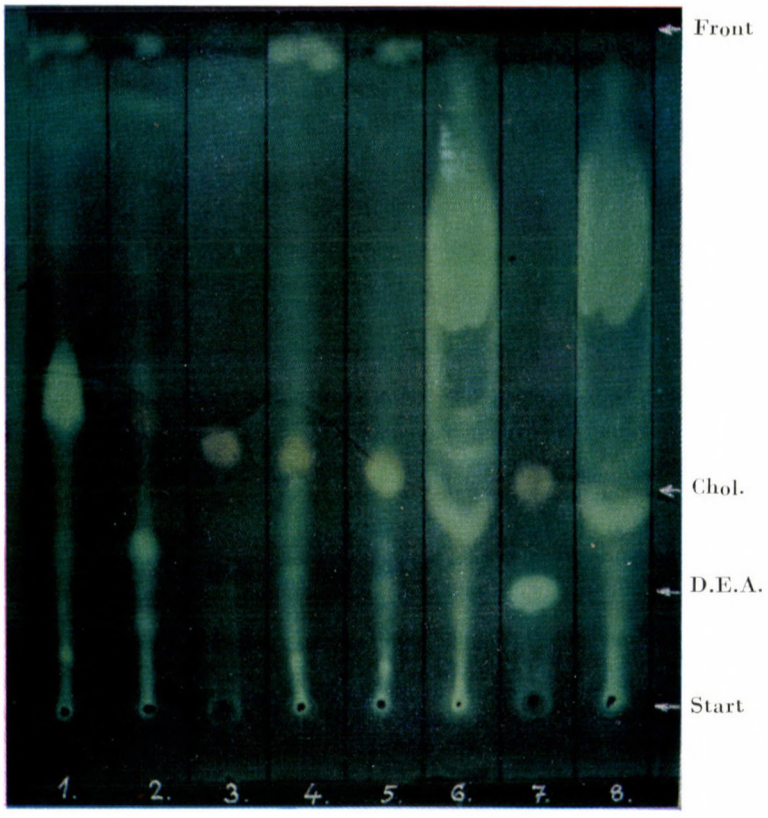

Fig. 3. Chromatogram of extracts of skin and hairs detected with 50 per cent phosphoric acid under ultra-violet light

androsterone and 40 μg of cholesterol; 8 = abdominal skin extract obtained by hydrochloric acid hydrolysis (JULESZ *et al.*, 1966).

Fig. 1 presents the Kieselgel G thin-layer chromatogram of the extracts of hairs and abdominal skin viewed under ultra-violet light. The control steroids are barely perceptible. The extracts of hairy abdominal skin show the picture known from earlier work (JULESZ *et al.*, 1966). The extracts of hairs from different areas reveal a few fluorescent spots.

Fig. 2 presents the phosphoric acid colour reaction on the same chromatoplate as seen in daylight. The control cholesterol and dehydroepiandrosterone spots are distinct. At the level of the cholesterol standard, there appear the cholesterol spots of the hairs and the abdominal skin. At and below the level of the dehydroepiandrosterone standard russet-coloured spots are demonstrable in the axillary and pubic hairs.

Fig 3. shows the same chromatoplate viewed under ultra-violet light. This is the most distinctive and informative of the three chromatograms. It displays the spots of the control steroids and the fluorescence of the abdominal skin extracts. Remarkably, in each type of hairs at least three intensely fluorescent spots are visible. Most intense is the spot appearing at the level of the cholesterol standard. In the extract of axillary hairs the spot appearing at the level of the standard dehydroepiandrosterone, with one or two smaller fluorescent spots above and below it, compels attention. The spot detected at the same level in the extract of pubic and thigh hairs respectively likewise attracts notice. The different extracts show the fluorescent spots of apolar substances already described from skin extracts.

Table I

Cholesterol contents of human hairs before and after purification on florisil column

Hairs studied	Cholesterol found, mg/g		Difference, per cents
	without purification	after purification	
Of the head	2.22	1.66	−25.2
	2.37	1.94	−18.1
	2.30	1.56	−31.8
	3.40	3.04	−10.6
	2.42	1.82	−24.8
	3.11	2.60	−16.4
Pubic	3.39	3.43	+ 1.2
	3.36	3.26	− 3.0
	3.21	3.20	− 0.3
	3.45	3.27	− 5.2

From the findings it appears that the crude cholesterol extracts of skin and hairs contain foreign substances in considerable quantities. This is definitely supported by ZLATKIS' colour reaction, which along with the violet red colour characteristic of cholesterol shows a brownish tint, which is particularly marked in extracts of hairs.

The data in Table I show differences in the amount of cholesterol found in unpurified and florisil purified hair extracts; these differences ranged from −10.6 to −31.8 per cent for hair from the head and from −5.2 to +1.2 per cent for pubic hairs.

As to the other colour reactions, 12 per cent sulphosalicylic acid in the presence of sulphuric acid gave with cholesterol isolated from skin and hairs a bluish-green colour. The Liebermann-Burchardt reaction with acetic acid anhydride and sulphuric acid, gave a greenish-blue colour. The cholesterol separated from skin and hairs yielded a pink colour with 70 per cent perchloric acid in chloroform at 56°C. This colour disappeared upon the effect of water. Skin and hair extracts dropped on paper and sprayed with 10 per cent phosphomolybdic acid and kept at 110°C, gave a blue spot. These reactions also indicated that the substance separated from skin and hairs and identified by thin-layer and florisil column chromatography, was truly cholesterol.

With a view to assessing the accuracy of our method, parallel examinations were carried out. As the data in Table II show, the difference between the parallels was less than 10 per cent for hairs and maximum 12.5 per cent for skin.

Table II

Parallel cholesterol determinations in human hairs and skin

Material studied	A mg/g	B mg/g	A−B difference, per cent
Hair from the head	1.45	1.38	− 4.8
	2.70	2.74	+ 1.5
	2.42	2.23	− 7.9
	2.33	2.55	+ 9.4
Pubic hairs	3.18	3.38	+ 6.3
	2.51	2.54	+ 1.2
	2.57	2.37	− 7.8
	1.76	1.89	+ 7.4
Skin	1.64	1.84	+12.2
	1.12	1.26	+12.5
	1.16	1.16	0
	1.26	1.27	+ 0.8

As shown in Table III, an average of 92.2 per cent of the cholesterol added to the reaction mixture could be recovered.

Table III

Cholesterol recovered (after alkaline lysis, ethereal extraction and purification with florisil)

Added to reaction mixture, μg	Recovered		Mean recovery, per cent
	g	per cent	
80	73	91.2	
80	71	88.7	92.2
120	114	95.0	
120	108	90.0	

Discussion

It has been shown earlier (JULESZ *et al.*, 1966) that human skin, whether hairy or hairless, contains cholesterol. This is a fact known since MEYER's investigations (1931) but, to our knowledge, the different body hairs themselves have not yet been studied for their cholesterol contents. Most investigators have only concerned themselves with the cholesterol in the sebaceous and lipid layer present on the surface of skin and hairs. We have succeeded in detecting substantial amounts of cholesterol in extracts of skin and different types of hairs by thin-layer and florisil column chromatography as well as various colour reactions.

In the chromatograms of the crude skin and hair extracts in addition to the large cholesterol spots, several small spots were visible. The fluorescent spots seen below it, particularly at the level of the standard dehydroepiandrosterone, were most probably steroids.

Our working hypothesis assigns a role to the cholesterol contents of skin and hairs in the normal growth of hair in humans, and in the development of hirsutism in women. We have therefore worked out a method of cholesterol assay in skin and hairs. The method allowed the recovery of about 92 per cent of the cholesterol added to the reaction mixture. The differences between parallels amounted at most to 12.5 per cent. On these grounds, the procedure is thought to be suitable for the estimation of minute quantities of cholesterol in skin and hairs. The values for cholesterol purified on florisil column and determined with phosphoric acid-ferric chloride as the reagent, are regarded as the "total cholesterol" in skin and hairs; a study of its fractionation will be the subject of a subsequent paper.

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STEROIDS IN HUMAN SKIN AND HAIRS

III. CHOLESTEROL IN THE SKIN AND HAIRS OF NORMAL AND HIRSUTE WOMEN, AND IN THE HAIRS OF NORMAL MEN

By

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The total cholesterol contents of the skin and of the hairs of hirsute and endocrinologically normal women and normal men, were studied. In both hirsute and normal women more cholesterol was found in the pubic than in the abdominal skin. Hairs were found to contain cholesterol in considerable quantities, particularly the axillary hairs of normal men and women. Hairs of hirsute women were rich in cholesterol. ACTH appeared to have no effect on the cholesterol content of hairs.

It has been shown that human skin and hairs contain considerable amounts of cholesterol (JULESZ *et al.*, 1966), and a method has been elaborated for its quantitative determination (TÓTH *et al.*, 1966).

It has long been known that in the peripheral endocrine glands (adrenal cortex, testis, ovary, placenta) a wide variety of steroids are formed from cholesterol. According to DORFMAN (1961), the way cholesterol breaks down in these glands is probably the following: 20 α-hydroxycholesterol $\rightarrow$ 20, 22 (?) dihydroxycholesterol ($\rightarrow$ 20α-hydroxy, 22-ketocholesterol) $\rightarrow$ pregnenolone + isocapronic acid. There are, however, no data in the literature available to us on the role of the cholesterol present in skin and hairs. Still, a study of the biochemical processes taking place in the skin might supply the key to the pathogenesis of hirsutism. Disturbances of steroid metabolism can only be studied if the metabolites are reliably estimated. It was on these considerations that we undertook the quantitative determination of cholesterol in the skin and hairs of normal males and females and hirsute women.

Methods

Specimens of hairy and hairless abdominal skin and pubic skin were excised from patients subjected to surgical or gynaecological operations. Patients not affected by endocrinological disease formed the normal group. The patients in the hirsute group were afflicted with the ovarian type of the condition.

Hairs were taken from different sites (head, armpits, pubic region, etc.) of normal males and females, and from women with ovarian, adrenal or idiopathic hirsutism. The hirsute women were subjected to treatment with 80 I.U. of ACTH (Exacthin; Richter, Budapest) daily for 3 days before sampling.

Statistical analyses were carried out with Student's t test.

Results

In the hairless abdominal skin from 8 non-hirsute women, the cholesterol contents varied between 0.71 and 1.39 mg/g, averaging 1.09 mg/g. In pubic skin

of 6 of the same 8 women, the cholesterol contents ranged from 1.12 to 1.94 mg/g, with an average of 1.41 mg/g. With the exception of one patient, more cholesterol was found in the pubic than in the abdominal skin (Table I). The values refer to 1 g of wet skin.

Table I

Cholesterol contents of the skin of non-hirsute women

Patient's initials	Age, years	Cholesterol in skin, mg/g	
		Abdominal (hairless)	Pubic (hairy)
H. G.	48	1.22	—
B. I.	28	1.16	1.27
V. J.	32	1.36	1.94
Z. I.	39	0.88	1.21
K. K.	35	0.71	1.12
F. J.	55	1.39	—
A. S.	41	1.14	1.19
Sz. S.	33	0.88	1.72
Mean		1.09 ± 0.09	1.41 ± 0.13
Extremes		$0.71 - 1.39$	$1.12 - 1.94$

Significance $t_{(12)} = 2.052$
$p > 0.05$

In 5 women with ovarian hirsutism, cholesterol in abdominal skin varied between 0.62 and 1.09 mg/g (mean, 0.81 mg/g), and in pubic skin between 0.87 and 1.56 mg/g (mean, 1.27 mg/g) (Table II).

Table II

Cholesterol contents of the skin of women with ovarian hirsutism

Patient's initials	Age, years	Cholesterol in skin, mg/g	
		Abdominal (hairy)	Pubic (hairy)
Á. K.	21	0.97	1.56
B. É.	27	0.74	1.42
L. D.	24	1.09	1.45
M. E.	18	0.62	1.04
S. I.	19	0.63	0.87
Mean		0.81 ± 0.11	1.27 ± 0.17
Extremes		$0.62 - 1.09$	$0.87 - 1.56$

Significance $t_{(8)} = 2.251$
$p > 0.05$

Table III

Cholesterol contents of hairs of normal women

Patient's initials	Age, years	Hair, mg/g	Pubic hairs, mg/g	Axillary hairs, mg/g
W. M.	41	1.56	2.53	2.97
K. J.	27	1.66	1.73	3.46
K. M.	32	1.65	2.47	3.84
B. J.	26	1.87	1.42	3.23
K. Zs.	22	1.94	2.11	3.17
Ö. M.	48	1.49	—	—
L. I.	28	1.82	—	—
F. E.	20	2.85	2.31	3.23
F. I.	25	1.40	2.30	2.28
B. J.	26	1.37	2.59	2.47
V. J.	32	1.22	2.53	3.12
Mean		1.71 ± 0.13	2.22 ± 0.13	3.09 ± 0.15
Extremes		1.22 — 2.85	1.42 — 2.59	2.28 — 3.84

Significance in comparison with axillary hairs $t_{(18)} = 7.337$, $p < 0.001$; $t_{(16)} = 4.242$, $p < 0.001$

Table IV

Cholesterol contents of hairs of normal men

Patient's initials	Age, years	Hairs (mg/g)			
		head	pubic	axillary	chest
T. I.	29	2.64	1.89	3.09	1.73
W. M.	40	2.94	1.55	3.52	1.38
F. I.	41	2.43	1.36	3.47	1.96
F. I.	27	2.06	2.11	4.03	2.30
Sz. I.	49	2.11	2.73	2.70	1.77
B. I.	27	2.39	1.85	3.18	1.77
M. F.	54	2.56	2.17	3.40	2.63
B. S.	24	2.96	1.35	4.15	1.30
D. E.	26	2.12	2.25	3.09	—
H. S.	58	1.90	2.19	2.23	1.39
Sz. K.	55	—	1.54	—	1.55
Mean		2.41 ± 0.10	1.90 ± 0.13	3.28 ± 0.18	1.78
Extremes		1.90 — 2.96	1.36 — 2.73	2.23 — 4.15	1.30 — 2.30

Significance in comparison with axillary hairs (without the chest-hairs): $t_{(18)} = 3.780$, $p < 0.01$; $t_{(19)} = 6.293$, $p < 0.001$

Table V

Cholesterol contents of hairs of hirsute women

Patient's initials	Age, years	Hairs (mg/g)							
		head	pubic	axillary	chest	abdominal	thigh	beard	buttocks
G. A.	21	2.97	3.50	—	2.30	—	—	—	—
Gy. A.	49	2.29	2.90	—	3.20	—	—	—	—
L. M.	21	2.00	3.16	—	2.14	—	—	—	—
H. Zs.	18	2.34	2.41	—	3.12	—	—	—	—
L. Zs.	27	3.64	3.59	4.56	—	—	—	—	—
K. A.	28	2.74	3.37	3.98	—	—	—	—	—
N. J.	20	1.64	1.76	2.93	3.11	3.01	2.46	5.59	2.94
N. E.	23	1.91	1.40	2.34	—	2.95	3.04	4.85	2.34
B. É.	27	2.51	—	2.64	—	—	2.70	—	—
L. D.	24	1.64	2.02	2.92	—	4.61	—	—	—
K. G.	21	2.78	1.34	2.64	—	—	3.13	—	—
S. I.	19	1.14	2.62	2.86	—	—	—	3.25	—
K. Zs.	18	1.72	1.47	2.60	—	—	3.43	—	—
M. E.	20	1.42	1.28	3.10	3.24	3.67	—	—	—
Mean		2.19	2.37	3.05	2.85	3.53	2.95	4.56	2.64
Extremes		1.14—2.97	1.28—3.59	2.34—4.56	2.14—3.24	2.95—4.61	2.46—3.43	3.25—5.59	2.34—2.94

The question arose how much of the cholesterol found in the skin originated from hairs left embedded in the epidermis. Thus the investigations were extended to hairs in different areas.

Table III presents the data for normal women. It shows individual variations in the cholesterol contents between 1.22 and 2.85 mg/g for hair (mean of 11 patients, 1.71 mg/g); between 1.42 and 2.59 mg/g for pubic hairs (mean of 9 patients, 2.22 mg/g); and between 2.28 and 3.84 mg/g for axillary hairs (mean for 9 patients 3.09 mg/g).

The corresponding values for normal men were (Table IV) between 1.90 and 2.96 mg/g for hair (mean of 10 patients, 2.41 mg/g); between 1.36 and 2.73 mg/g for pubic hairs (mean of 11 patients, 1.90 mg/g); and between 2.23 and 4.15 mg/g for axillary hairs (mean of 10 patients, 3.28 mg/g). Hairs growing on the male chest were found to contain cholesterol in quantities between 1.30 and 2.30 mg/g (mean of 10 patients, 1.78 mg/g).

Table V shows the values for cholesterol found in various skin areas of hirsute women.

In the hair 1.14 to 2.97 mg/g; mean of 14 cases, 2.19 mg/g; in pubic hairs 1.28 to 3.59 mg/g; mean of 13 cases, 2.37 mg/g; in axillary hairs 2.34 to 4.56 mg/g; mean of 10 cases, 3.05 mg/g; in hairs on the chest 2.14 to 3.24 mg/g; mean of 8 cases, 2.85 mg/g; in abdominal hairs 2.95 to 4.61 mg/g; mean of 4 cases, 3.53 mg/g; in hairs on the thigh 2.46 and 3.43 mg/g; mean of 5 cases, 2.95 mg/g; in beard 3.25 and 5.59 mg/g; mean of 3 cases, 4.56 mg/g; in hairs on the buttocks 2.34 and 2.94 mg/g; mean of 2 cases, 2.64 mg/g.

Hairs from 5 hirsute women were studied for cholesterol contents before and after ACTH treatment. Table VI shows that though in the individual cases ACTH treatment moderately increased or decreased the cholesterol content, the mean values were practically identical before and after treatment.

Table VI

Cholesterol contents in hairs of hirsute women before and after ACTH treatment

Patient's initials	Age, years	Hairs (mg/g)					
		head		pubic		axillary	
		before	after	before	after	before	after
		ACTH treatment					
B. É.	27	2.51	2.49	—	1.42	2.64	2.51
N. J.	20	1.64	1.15	1.76	2.01	2.93	3.24
N. E.	23	1.91	2.36	1.40	2.26	2.34	2.80
K. Zs.	18	1.72	1.28	1.47	1.93	2.60	2.46
M. E.	20	1.42	1.70	1.28	1.57	3.10	2.61
Mean		1.84	1.80	1.48	1.84	2.72	2.72
Extremes		1.42—2.51	1.15—2.49	1.28—1.76	1.42—2.26	2.60—3.10	2.46—3.24

Discussion

Little is known of the significance of cholesterol in human skin and hairs. CLAYTON *et al.* (1963) have demonstrated the ability of rat skin to synthesize cholesterol from acetate. This shows that the skin of the rat is rich in enzymes. By treatment with triparanol, these authors successfully inhibited the enzyme responsible for reduction of the 24,25-double bond in the sterols. Similar results have been obtained by HORLICK and AVIGAN (1963). These findings and the cyclic growth of hairs constitute additional proof of vigorous metabolic processes taking place in the skin. Hormonal influences are thought to be responsible for the differences in hair growth between the human male and female, but the causative biochemical processes are unknown.

In the present experiments more cholesterol was found in the hairy pubic than the hairless abdominal skin of endocrinologically normal persons. In hirsute women, pubic skin contained more cholesterol than did the hairy abdominal skin. However, in neither group was the difference statistically significant (Tables I and II).

According to MEYER (1931), the amount of cholesterol in dry normal skin is 367 mg/100 g. We found an average of 1.25 mg in 1 g of wet skin. Since skin contains about 60 per cent water, 1.25 mg/g of wet skin corresponds to 313 mg/100 g of dry skin, which value agrees well with that established by MEYER.

Hairs showed higher cholesterol contents than did the skin; the corresponding values were similar for males and females. In both sexes, the contents were the highest in axillary hairs, significantly less in hair and pubic hairs, and, in men, in hairs of the chest (Tables III and IV).

Hair and pubic and axillary hairs contained about as much cholesterol in hirsute as in endocrinologically normal women. Much higher were the cholesterol contents in hirsute women with characteristic masculine hair growth (chest, abdomen, thighs, beard). Thus, considerably more cholesterol was found in hairs of the chest of hirsute females than of normal males (Tables IV and V).

ACTH treatment caused no appreciable change in the cholesterol contents of hairs from hirsute women.

The present investigations were restricted to total cholesterol determination, and were not extended to cholesterol esters, and the cholesterol derivatives isolated from the skin by earlier workers. MOORE and BAUMANN (1952) isolated 7-dehydrocholesterol from rat skin. IDLER and BAUMANN (1952) demonstrated in the rat skin the presence of $\triangle^7$-cholestenol, NEIDERHISER and WELLS (1959) that of 4 α-methyl-$\triangle^7$-cholestenol (methostenol), and STOKES *et al.* (1958) of 24-dehydrocholesterol (desmosterol). GAYLOR (1960) determined the proportions of the chief cholesterol derivatives in the rat skin and found 52 per cent cholesterol, 31.2 per cent $\triangle^7$-cholestenol, 7.5 per cent cholestanol, 2.4 per cent desmosterol, and 1.9 per cent 7-dehydrocholesterol. Studies of these

sterols would yield interesting results and may explain the origin and significance of the substantial amounts of cholesterol found in the hairs of hirsute women.

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STEROIDS IN HUMAN SKIN AND HAIRS

IV. NEUTRAL 17-KETOSTEROIDS IN HUMAN HAIRS

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Extracts of the hairs of normal men and women, and of hirsute women, have been studied. Hair, axillary and pubic hairs were found to contain 17-ketosteroids; axillary hairs were the richest in them. Dehydroepiandrosterone and its artefact, 3-chloro-dehydroepiandrosterone, were identified by their R_f in different thin-layer chromatographic systems and by different colour reactions.

In hairs from hirsute women two 17-ketosteroids of low R_f value were detected; these were absent from the hairs of normal women. No qualitative differences were observed between body hairs before and after ACTH treatment.

In earlier experiments it has been shown that human skin extract purified with Girard's reagent T contains 17-ketosteroids (JULESZ *et al.*, 1966). In a study concerned with the cholesterol content in human body hairs, thin-layer chromatography revealed at the level of the standard 17-ketosteroids the appearance, in addition to cholesterol, of intensely fluorescent substances, probably 17-ketosteroids (TÓTH *et al.*, 1966). This raised the question whether hairs might contain androgen steroids; a point on which we have been unable to find information in the literature. Another of our observations was that human hairs contained more cholesterol, the principal steroid precursor, than did human skin (FAREDIN *et al.*, 1966). All this seemed to justify a detailed study of the 17-ketosteroid content of the hairs from normal men and women as well as women with hirsutism.

As Kieselgel G is not suitable for the fine separation of androgen-type steroids on thin layers, thin-layer chromatography with alumina as adsorbent (FAREDIN and TÓTH, 1966) was used in the present experiments, which allowed the separation of minute quantities of 17-ketosteroid present in biological substances.

Methods

Exposure and extraction of hairs

Hairs cut from different areas (head, armpits, pubic region) of normal men and women were collected in batches of 400 to 1600 mg and boiled under reflux in 40 ml of a 2.5 per cent solution of NaOH for 20 minutes. The solution was then neutralized with 3 ml of 11 n HCl and acidified with 6 ml of concentrated HCl, and boiled under reflux for another 15 minutes.

Cooling was followed by extraction with 4×20 ml of ether. The ethereal extract was washed with 3×20 ml of 2 n NaOH and 2×20 ml of distilled water, thereafter dehydrated with 3 to 5 g of Na_2SO_4, filtered, and evaporated to dryness.

Purification of hair extracts

The crude extract was dissolved in 5 ml of benzene and evaporated to dryness in vacuo. The dry residue was applied in 3×5 ml of benzene onto a 2 g Nymco florisil column suspended in benzene. The column was washed first with 15 ml of benzene and then with 10 ml of 0.5 per cent ethanol in benzene. Thereafter, the 17-ketosteroids were eluted from the column with 45 ml of 2 per cent ethanol in benzene. The eluate was evaporated to dryness, purified with Girard's reagent T according to PINCUS and PEARLMAN (1941), and the ketonic fraction was separated.

Thin-layer chromatography

The ketonic fraction was applied in benzene on a 0.25 mm layer of alumina spread on a chromatographic plate measuring 10 cm by 35 cm, and run in a mixture of ethyl acetate, n-hexane, glacial acetic acid and absolute ethanol (120 : 120 : 2 : 1) at 30° C for 3 to 4 hours. After chromatography, the glass plate was dried and the layer sprayed with a 1 : 1 mixture of 2 per cent m-dinitrobenzene in ethanol and 2.5 n KOH in methanol. After drying, there appeared the lilac-blue 17-ketosteroid spots (FAREDIN and TÓTH, 1966).

Results

In the first part of the present investigations, 17-ketosteroids, in the hairs of normal men and women were studied by thin-layer chromatography, with alumina as adsorbent. From males, 1432 mg of hair, 490 mg of axillary hairs, and 1263 mg of pubic hairs were studied. Fig. 1 presents the results. Proceeding from left to right, strip 1 contained the 17-ketosteroids from the pubic hairs; it shows 5 spots. On strip 2 were run the standards: 20 μg of 3-chloro-dehydroepiandrosterone (Cl.-D.E.A.); 20 μg of dehydroepiandrosterone (D.E.A.); and 20 μg of 11-hydroxyandrosterone (11-HO-A). To strip 3 was applied the extract of the axillary hairs; 10 spots can be seen on it. Strip 4 shows again spots of standard 17-ketosteroids; namely, in downward order, 20 μg of Cl.-D.E.A.; 20 μg of androsterone (A); 17 μg of etiocholanolone (E); and 11 μg of ketoandrosterone (11-O-A). Strip 5 reveals 4 spots of ketosteroids from hair.

From normal women were collected 1534 mg of hair, 482 mg of axillary hairs, and 1206 mg of pubic hairs. The results are presented in Fig. 2. Strip 1 refers to pubic hairs, strip 2 to standard 17-ketosteroids, viz.: 10 μg of Cl.-D.E.A., 10 μg of D.E.A., and 10 μg of 11-HO-A. On strip 3, 5 spots of the extract of axillary hairs can be seen. Strip 4 shows, in downward order, the spots of 10 μg of Cl.-D.E.A., 10 μg of A, 8.5 μg of E., and 5.5 μg of 11-O-A. Strip 5 relates to hair.

Fig. 3 presents the chromatograms of the pooled hairs from three different areas of two hirsute women, before ACTH treatment. Strip 1 relates to the extract of 481 mg of hair, strip 2 to the standards, 5 μg of Cl.-D.E.A., 5 μg of D.E.A., 6 μg of 11-HO-A, and 12 μg of ketoetiocholanolone (11-O-E). On strip 3 the extract of 172 mg of axillary hairs was run. Strip 4 shows the spots

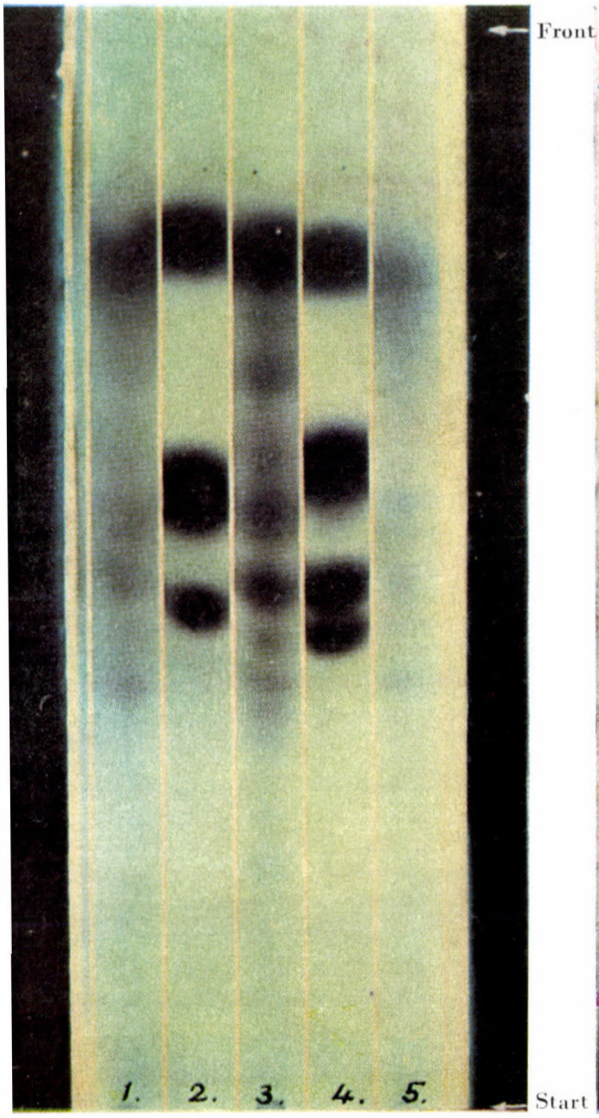

Fig. 1. Chromatogram of male hairs on alumina layer

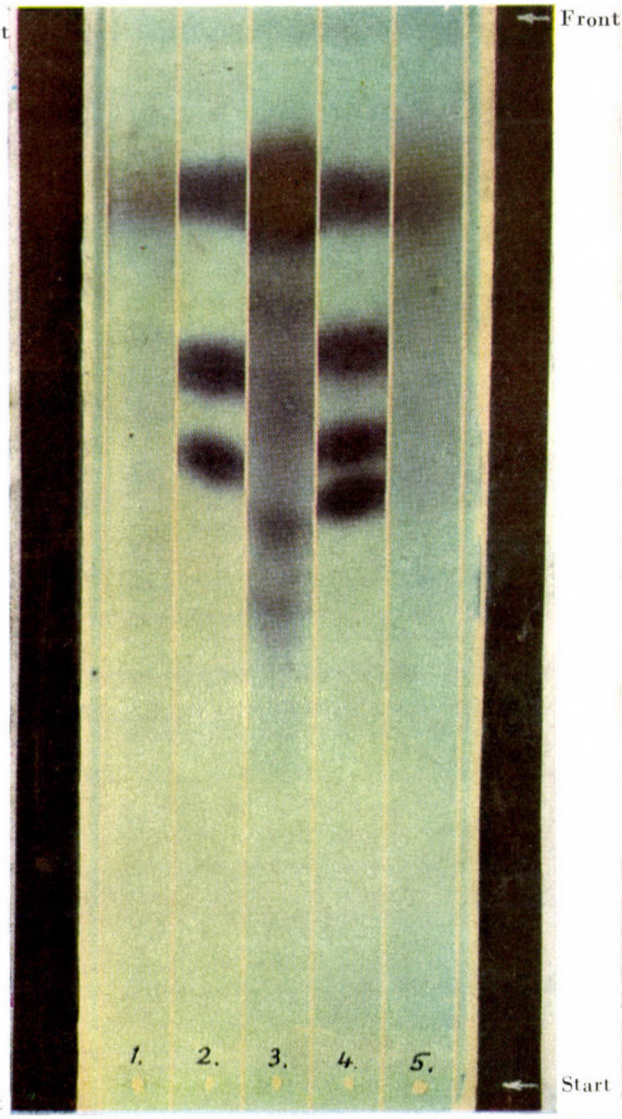

Fig. 2. Chromatogram of female hairs on alumina layer

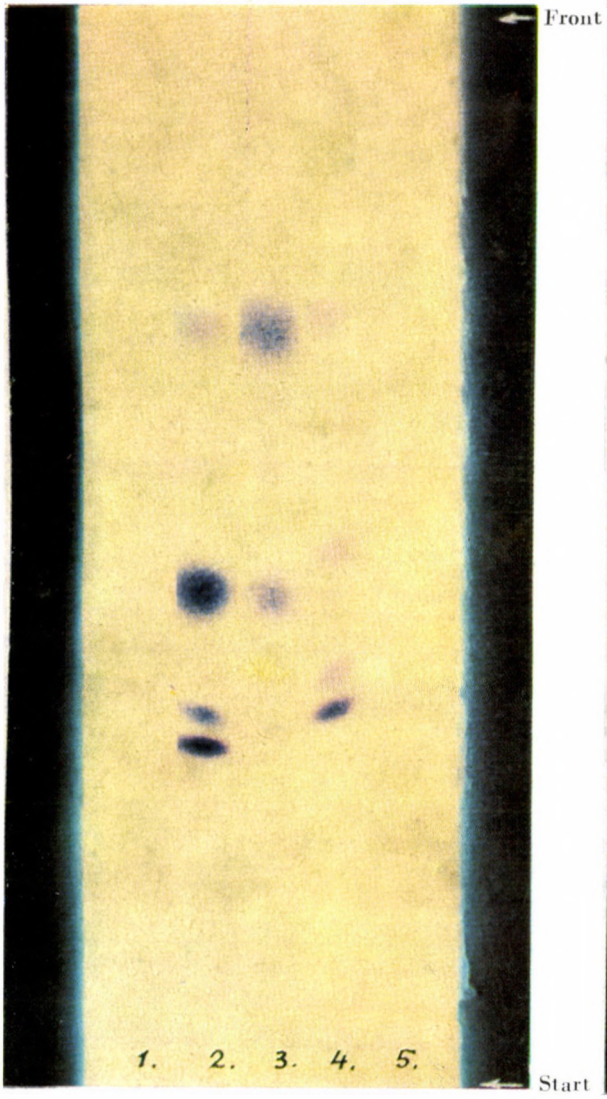

Fig. 3. Chromatogram of hairs from hirsute women, on alumina layer

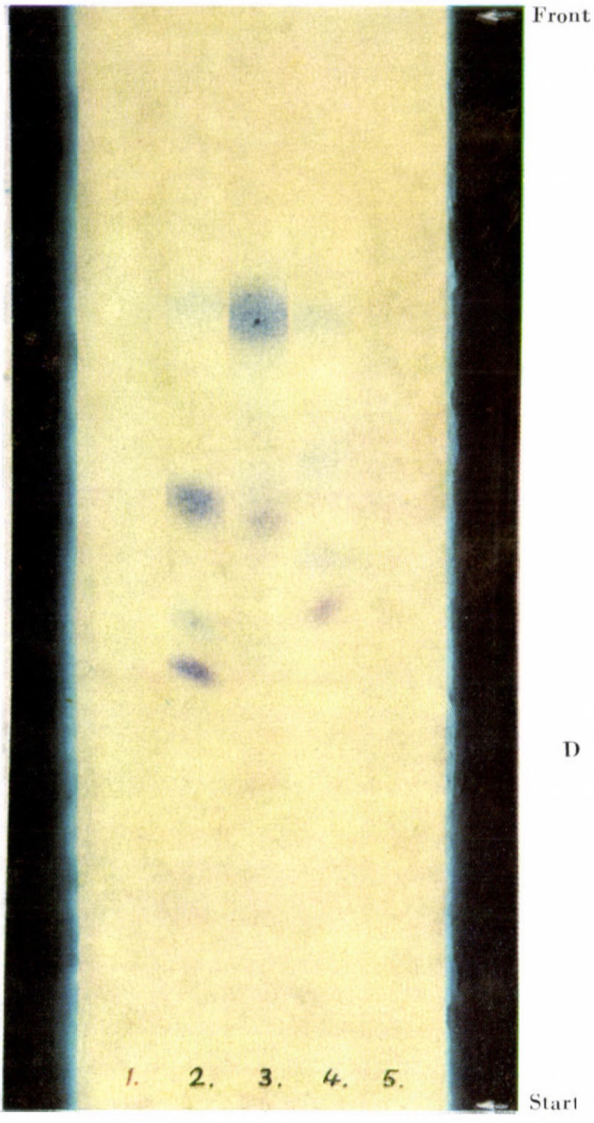

Fig. 4. Chromatogram of hairs from hirsute women, on alumina layer, after ACTH treatment

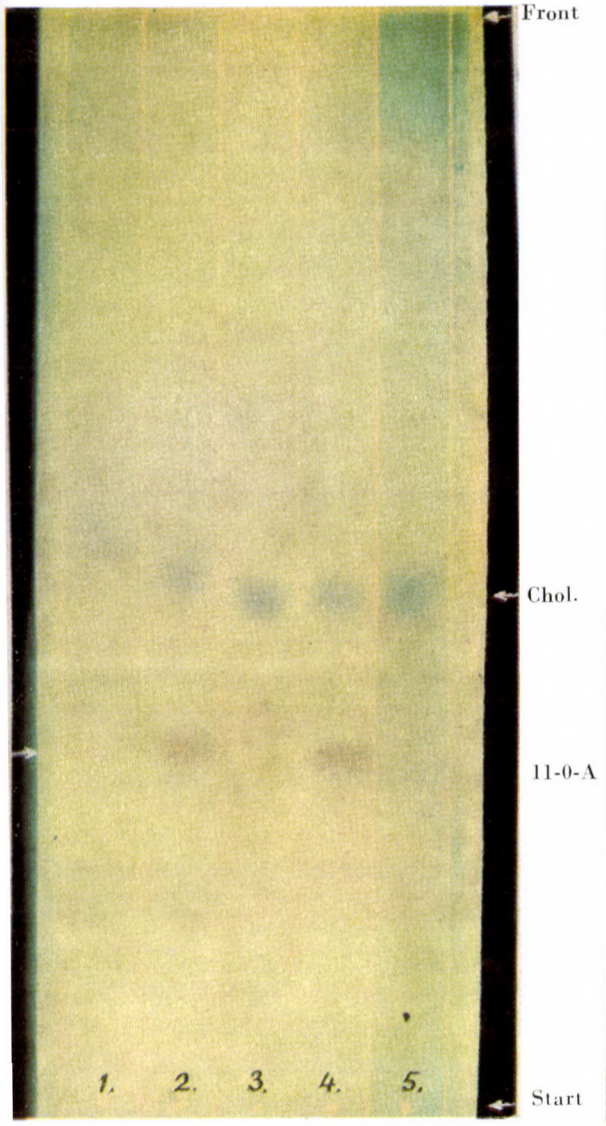

Fig. 5. Chromatogram of steroids on Kieselgel G layer following phosphoric acid colour reaction

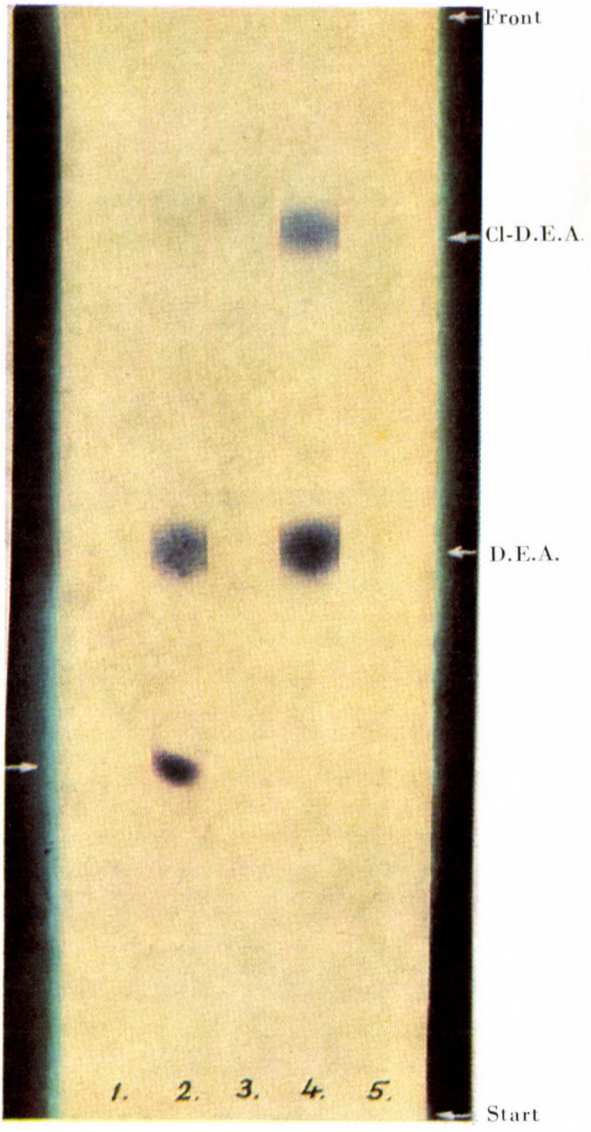

Fig. 6. Chromatogram of steroids on alumina layer following the Zimmermann reaction

of 5 μg of Cl.-D.E.A., 5 μg of A, 8 μg of E, and 11 μg of 11-O-A, strip 5 the extract of 467 mg of pubic hairs.

Fig 4 shows the results for the pooled hairs of the same two hirsute women after ACTH treatment in the order as in Fig. 3. The quantities worked up were: 455 mg of hair, 143 mg of axillary hairs, and 186 mg of pubic hairs.

Discussion

Figs 1 and 2 show that the hairs of normal men and women contain considerable quantities of 17-ketosteroids and that the richest in them are the axillary hairs in both sexes. In hairs from all the skin areas studied, 3-chlorodehydroepiandrosterone is present in the largest amount. This is an artefact arising from dehydroepiandrosterone upon the effect of hydrochloric acid hydrolysis. Both 3-chlorodehydroepiandrosterone and dehydroepiandrosterone were identified with the Zimmermann and the phosphoric acid colour reactions, and also with that of PATTERSON (1947) which is specific for these two steroids. The characteristic R_f of these two steroids chromatographed in two different systems (Kieselgel G and alumina), represents additional evidence.

In human hairs, particularly the axillary hairs, there are a number of other 17-ketosteroids. The two spots below that of Cl.-D.E.A. are presumably 5 α-androstane-3 : 17-dione and androst-4-ene-3 : 17-dione. Identification of these two 17-ketosteroids and the other spots, is in progress.

Apart from the spot of Cl.-D.E.A., two well-discernible but faint spots were present in the hair of hirsute women, at the level of the 11-oxy-17-ketosteroids (Fig. 3). No similar spots could be detected in three times that amount of hair of normal women. Along with the Cl.-D.E.A. spot, the axillary hairs gave several spots of lower R_f value. The pubic hairs also yielded a few ketosteroid spots of lower R_f value, which could not be detected in the pubic hairs from normal women. These spots faded away and are not visible in Figs 3 and 4.

The chromatogram obtained after ACTH treatment (Fig. 4) agrees with the former chromatogram (Fig. 3). These qualitative results did not yield quantitative information. Thus all we can conclude upon is that upon the effect of ACTH there appeared no 17-ketosteroids other than in the controls.

The experiments raise a number of problems. The first question is whether the 17-ketosteroids detected may not be regarded as mere artefacts arising from one of two 17-ketosteroids due to rough manipulations. This question is now being studied.

Nor can the possibility be ruled out that the detected 17-ketosteroids are artefacts formed under the effect of alkali or that of acid hydrolysis. It was shown earlier (TÓTH *et al.*, 1966) that after treatment with alkali, acid hydrolysis and ether extraction, 92.2 per cent of the added cholesterol was recovered. This makes it improbable that steroids originating from cholesterol degrada-

tion should appear in the chromatogram. To rule out this possibility, cholesterol subjected to different manipulations was run with standard steroids. Fig. 5 shows the chromatograms of steroids run on Kieselgel G layer following treatment with phosphoric acid. From left to right the following substances were run. Strip 1 : 150 μg of untreated cholesterol (extraction with ether from aqueous solution); strip 2 : 20 μg of standard D.E.A. (lower spot) and 80 μg of cholesterol; strip 3 : 150 μg of cholesterol after boiling in alkali for 20 minutes; strip 4 : 20 μg of standard D.E.A. and 80 μg of cholesterol; strip 5 : 150 μg of cholesterol after boiling in alkali for 20 minutes followed by boiling with hydrochloric acid for 15 minutes.

These tests revealed no artefacts whatever.

The effect of manipulations was controlled on alumina layer by the Zimmermann reaction. Fig. 6 presents the data; strip 1 : 150 μg of cholesterol without previous boiling; strip 2 : 20 μg of standard D.E.A. and 11 μg of 11-O-A; strip 3 : 150 μg of cholesterol after boiling in alkali; strip 4 : 10 μg of standard Cl.-D.E.A. and 20 μg of D.E.A.; strip 5 : 150 μg of cholesterol after boiling in alkali and subsequent acid hydrolysis.

In this system, no steroids could be detected to have formed from cholesterol.

These investigations supply evidence that the 17-ketosteroids found in human hairs are steroids not arising from the degradation of cholesterol.

Accordingly, our assumption that human hairs contain considerable quantities of not only cholesterol but also of 17-ketosteroids, has been verified. The axillary hairs are the richest in 17-ketosteroids; they are richer in cholesterol than is hair and the pubic hairs. Although these experiments are of a primarily qualitative character, they permit the statement that the human hairs contain 17-ketosteroids in much greater amounts than does the human skin (JULESZ *et al.* 1966).

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SERUM MUCOPOLYSACCHARIDES IN ALLOXAN AND STEROID DIABETES

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Neutral mucopolysaccharide fractions in the serum of rabbits — hexose, hexosamine, sialic acid, and seromucoid — have been studied for changes occurring in diabetes induced by treatment with alloxan, cortisone, and alloxan combined with cortisone. In alloxan diabetes hexosamine and sialic acid, and in steroid diabetes hexose and seromucoid, were found to rise in concentration. The changes observed in diabetes induced by alloxan and cortisone were similar to those observed in steroid diabetes. The morphological lesions were severest on alloxan + cortisone treatment and showed no parallelism with the changes in the serum levels of neutral mucopolysaccharide fractions.

In spite of continuous efforts, little progress has been made in the problems of vascular complications in diabetes. A number of authors have succeeded in demonstrating increases in the serum mucopolysaccharide (MPS) fractions mostly in patients with manifest complications (1 through 7). We have shown an increase in the neutral MPS fractions, principally the hexose and seromucoid fractions, in slight and moderate diabetic retinopathy [8]. However, MPS metabolism and the origin of serum MPS-protein complexes are still unclear in many respects, in spite of the fact that between serum and tissue MPSs there must be a dynamic relationship, for changes in tissue MPS metabolism are usually accompanied by changes in the composition of serum MPSs.

In view of this it seemed interesting to study the serum MPS fractions in experimental diabetes, particularly in cases accompanied by vascular changes.

Alloxan injected into animals is followed by a diabetes-like condition due to insulin deficiency, while cortisone administered for several weeks is known to induce diabetes and associates vascular changes morphologically similar to human micro-angiopathy.

Materials and methods

The serum neutral MPS fractions were estimated in a total of 51 female rabbits weighing 2200 to 2400 g each. The animals were divided into three groups.

In Group I, 21 animals were each given a single intravenous injection of 100 mg per kg body weight of alloxan (BDH, London). Fasting blood sugar level was then determined weekly for four weeks, whereafter the rabbits were exsanguinated. At the end of the experiment, the serum MPS fractions were once more determined and the tissues, especially the kidneys were subjected to histology. For protein-bound hexose assay in serum the orcin reaction was used following STARY *et al.* [9]; hexosamine was estimated by the method of RIMINGTON [10],

using Ehrlich's reagent; sialic acid with diphenylamine according to AYALA [11], and seromucoid according to WEIMER and REDLICH-MOSHIN [12] from the hexose contents.

In Group II, each of 10 rabbits received daily 7 mg of cortisone (Adreson, Organon, Oss.) for four weeks, fasting blood sugar and sugar excretion were examined weekly.

In Group III, 20 animals were each given a single intravenous injection of 100 mg per kg body weight of alloxan, followed immediately by 4-week treatment with cortisone, as in Group II.

Results

A single alloxan injection induced persistent diabetes in 10 of the 21 animals in the first group; 10 animals developed no hyperglycaemia or glycosuria; and 1 rabbit died of intercurrent disease.

In the cortisone-treated second group, the fasting blood sugar level was found to have risen in all the animals by the end of the third week.

All the animals in the third group developed diabetes in the first week with the fasting blood sugar ranging between 180 and 260 mg per 100 ml. Eight rabbits died of cachexia in 8 to 10 days. Since in these animals the serum MPS fractions were determined before the alloxan injection, their data have been neglected. The remaining 12 rabbits survived the cortisone treatment, though all of them were wasted and developed severe diabetes.

Histology

1. *Alloxan.* In the normoglycaemic and the diabetic rabbits, only mild glomerulonephrosis and thickening of the basement membrane were found. In three diabetic animals, slight fibrinoid necrosis of some glomerular sites but no hyaline droplets were seen. Some hyaline in the arteries and dystrophic tubular lesions were occasionally observed.

2. *Cortisone.* Gross inspection revealed pinpoint haemorrhages on the renal surface in two animals. In all the rabbits of this group glomerular lesions consisting in a thickening of the basement membrane; fibrinoid necrosis in some places and hyaline droplets characteristic of glomerulosclerosis in others were observed (Fig. 1). In addition, lesions indicative of vasculitis were present. Degenerative tubular lesions were revealed in every animal.

3. *Alloxan + cortisone.* Gross examination revealed pinpoint haemorrhages on the renal surface of three rabbits. Histology revealed similar but much more severe glomerular lesions than in the cortisone-treated group (Fig. 2).

Serum MPS

The results are presented and evaluated in four Tables. The changes were studied by comparing initial values and end values. The fact that the initial values for the individiual groups were different, made it difficult to analyse the results statistically. Thus, the hexose values for the cortisone-treated animals

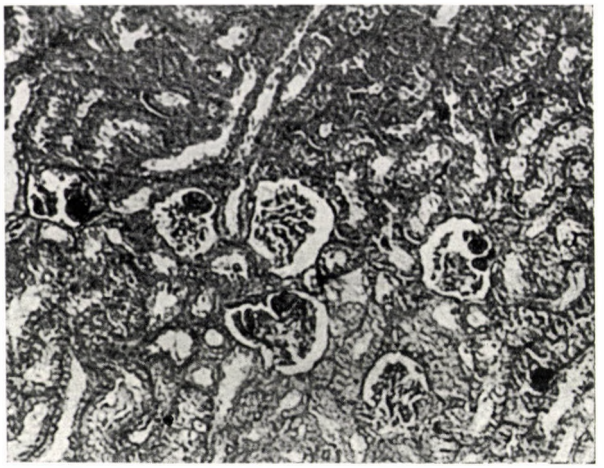

Fig. 1. Rabbit. Cortisone treatment. Focal fibrinoid swelling and globe-like processes in glomeruli. PAS staining

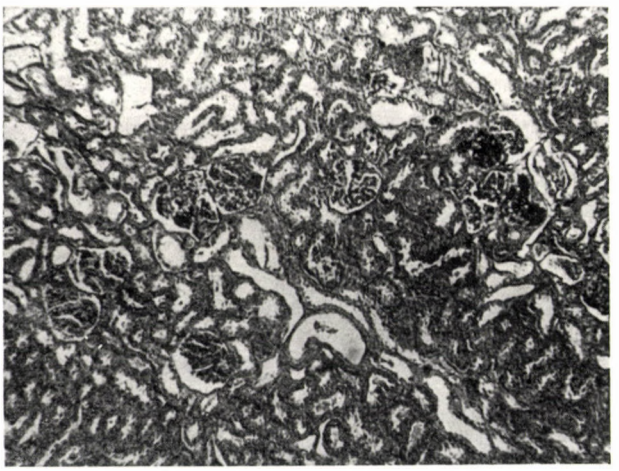

Fig. 2. Rabbit. Alloxan + cortisone treatment. Extensive fibrinoid necrosis in some glomeruli. Endes' trichrome staining

were lower, and the seromucoid values for the alloxan-treated diabetic rabbits higher, than the mean values for the other groups (Table I). For this reason, the results were analysed in two different ways: on the basis of the changes (Table III), and on the basis of the end values (Table II). The values in Table III differ from the difference between those in Tables I and II because some animals died during the experiment, and so less rabbits were examined at the end of the 4-week period.

The changes were evaluated with the one-sample "t" test; and in rela-

tion to each other, with the two-sample "t" test (top part of Table IV). The end values were analysed with the two-sample "t" test and, where the homogeneity of variances permitted it, with the analysis of variance (bottom part

Table I

Initial values

Mean and scatter (mg per 100 ml)

Group	Treatment		Hexose	Hexosamine	Sialic acid	Seromucoid
I	Alloxan;	n	12	12	12	12
	no diabetes	$\bar{x}$	126	94	38.0	11.2
	developed	s	8	10	12.4	2.3
II	Alloxan;	n	8	8	8	8
	diabetes	$\bar{x}$	134	86	48.1	18.5
	developed	s	14	12	6.8	3.1
III	Cortisone;	n	9	9	9	9
	diabetes	$\bar{x}$	104	87	52.8	12.6
	developed	s	8	11	6.7	2.4
IV	Alloxan + cortisone;	n	9	9	9	9
	diabetes	$\bar{x}$	130	79	51.2	12.0
	developed	s	16	9	8.1	2.9

Table II

End values

Mean and scatter

Group	Treatment		Hexose	Hexosamine	Sialic acid	Seromucoid
I	Alloxan;	n	9	9	9	9
	no diabetes	$\bar{x}$	128	86	54.8	12.9
	developed	s	8	6	5	2.2
II	Alloxan;	n	8	8	8	8
	diabetes	$\bar{x}$	99	106	75.5	10.2
	developed	s	12	12	11	2.6
III	Cortisone;	n	7	7	7	7
	diabetes	$\bar{x}$	209	86	62	29.1
	developed	s	41	18	11.2	8.3
IV	Alloxan + cortisone;	n	9	9	9	9
	diabetes	$\bar{x}$	161	82	58.6	17.9
	developed	s	52	11	9.4	4

of Table IV). Data obtained in the group treated with alloxan + cortisone were not compared with those obtained in the other groups because, excepting the values for sialic acid, the changes observed in this group were all within the limits of those seen in groups II and III.

Table III

Changes

Mean and scatter

Group	Treatment		Hexose	Hexosamine	Sialic acid	Seromucoid
I	Alloxan; no diabetes developed	n	9	9	9	9
		$\bar{x}$	2	−7	13.4	1.7
		s	12	8	13.9	2.6
II	Alloxan; diabetes developed	n	8	8	8	8
		$\bar{x}$	−35	20	27.4	−8.3
		s	18	10	12.3	3.6
III	Cortisone; diabetes developed	n	7	7	7	7
		$\bar{x}$	104	−2	8.9	16.1
		s	41	14	11.4	7.5
IV	Alloxan + cortisone; diabetes developed	n	9	9	9	9
		$\bar{x}$	31	3	7.4	5.9
		s	58	17	12.4	3.6

Table IV

Comparison of individual treatments

Abbreviations used

I = alloxan treatment: no diabetes developed.
II = alloxan treatment; diabetes developed.
III = cortisone treatment; diabetes developed.

Notes

Above the diagonal, comparison of values for changes; below the diagonal, comparison of end values.

Upper numerals in each square indicate the differences between the corresponding means in Tables III and II. These were obtained by deducting from the mean in the row the mean in the column. Lower numerals represent results of statistical analyses (p values).

a) Hexose Changes

	I	II	III
I		37 <0.1	−102 ≪1
II	−29 ≪0.1		−139 ≪1
III	81 <1	110 <1	

End values

b) Hexosamine

Changes

End values

	I	II	III
I		27 <0.1	−5 >40
II	20 <1		22 <5
III	0 <90	−20 <1	

c) Sialic acid

Changes

End values

	I	II	III
I		−14 <5	4.5 >40
II	20.7 <0.1		18.5 <5
III	7.2 >10	−13.5 <5	

d) Seromucoid

Changes

End values

	I	II	III
I		9.9 <0.1	−14.4 <1
II	−2.7 <5		−24.3 ≪1
III	16.2 <1	18.19 <1	

It has been mentioned that in a few cases the initial values differed from those in the other groups. The change in the value for sialic acid in Group I (13.4 mg per 100 ml), though significant, was hardly acceptable because the initial value was 10 to 15 mg less than the other values. In Group II, the initial value for seromucoid was about 6 mg higher than in the other groups, wherefore the decrease of 8 mg per 100 ml, though significant, was to be evaluated with caution. In Group III, the rise in hexose was definitely acceptable since the end value

for it exceeded by far the end values in the other groups; further, because if to the initial value 30 mg per 100 ml (the difference against the initial value in the other groups) were added, the rise was still significant, and still significantly greater than in the other groups.

Evaluations of results

In Group I no appreciable changes occurred. In Group II the values for hexose and seromucoid decreased and those for hexosamine and sialic acid increased. In Group III, in contrast to Group II, hexose and seromucoid were elevated, hexosamine was unchanged, and sialic acid showed a slight rise. In Group IV, the changes in hexose, hexosamine and seromucoid were within the limits observed in the preceding two groups; the change in sialic acid was almost identical with that seen in Group III.

Comparison of the changes revealed a significant difference between Group I and Group II for all the MPS components. If corrected in the above discussed manner, the significance increased for sialic acid but decreased for seromucoid.

Comparison of the data in Group III and Group I showed significant differences for hexose and for seromucoid; both these fractions were increased in Group III. Correction did not make the difference significant for sialic acid, and left that for hexose unaffected.

Comparison of Groups III and II revealed significant differences in the changes for each fraction. Correction did not interfere with significance in the case of either hexose or seromucoid. A rise in these fractions was considerable in Group III whereas in Group II a decrease was apparent. On the other hand, hexosamine and sialic acid were markedly elevated in Group II. In Group III the changes were less conspicuous.

Comparison of end values in Groups I and II revealed significant differences for all four MPS components. In the alloxan-diabetic animals, serum hexose and seromucoid levels were lower, and hexosamine and sialic acid levels higher. Between Groups III and I, a significant difference was found for hexose and seromucoid. Between Groups III and II, the difference was significant for all the four components. In Group III, the hexose and seromucoid levels and in Group II the hexosamine and sialic acid levels were higher.

In bringing about the results in Group IV, three factors had a part to play, each of which is in itself capable of influencing MPS metabolism. Alloxan alone caused practically no change in the serum MPS level by the end of the fourth week. This observation is in accordance with the data obtained in Group IV, the tendency of which was similar to those obtained in Group III. For the characteristic serum MPS level to arise, treatment with cortisone, respectively the diabetes accompanying it, seems to be decisive.

Conclusions

1. In animals that do not develop diabetes four weeks after a single injection of alloxan, no change occurs in the concentration of either of the serum MPS fractions studied.

2. In animals that develop diabetes after a dose of alloxan, the serum hexose and seromucoid levels decrease, and hexosamine and sialic acid levels increase.

3. In animals developing diabetes during 4-week cortisone treatment, hexose and seromucoid levels markedly rise, and the sialic acid level moderately increases.

4. Alloxan diabetes is associated with increased hexosamine and sialic acid levels, and steroid diabetes with increased hexose and seromucoid levels.

5. Characteristic of the changes in serum MPS observable in diabetes induced by combined alloxan and cortisone treatment is the fact that they are within the limits of the changes observed in cortisone diabetes and alloxan diabetes, though nearer to those in the former. This is all the more remarkable as

6. vascular lesions were the severest in diabetes induced by combined alloxan and cortisone treatment.

7. The severity of morphological lesions shows no parallelism with the changes in the levels of serum MPS fractions.

*

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MECHANISCHE ZEICHEN DER KARDIALEN INSUFFIZIENZ

Von

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HERZFÜRSORGESTELLE FÜR JUGENDLICHE (VORSTAND: DR. GY. BODROGI), BUDAPEST

(Eingegangen am 21. Mai 1965)

Bei kardialer Insuffizienz erscheint auf der Venenkurve die a-Druckwelle schneller nach der P-Welle als in Normalfällen. Die Ursache der Verkürzung der P-a-Strecke liegt darin, daß auf der Venenkurve außer der normalerweise vorkommenden a-Volumwelle auch eine a-Druckwelle auftritt, die infolge ihrer wesentlich größeren Fortpflanzungsgeschwindigkeit früher erscheint. Es ist angenommen, daß bei Dekompensation für das Erscheinen der sich in der Verkürzung der P-a-Strecke manifestierenden a-Druckwelle die Erhöhung der enddiastolischen Kammerfüllung bzw. der Anstieg des diastolischen Enddruckes verantwortlich ist.

Anhand der Analyse von einer großen Anzahl von Kurven und von in dem Vorhof registrierten Druckkurven, konnte im Einklang mit den Schrifttumsangaben nachgewiesen werden, daß in Fällen bzw. Zuständen, in denen während der Vorhofaktion der Kammerdruck erhöht ist, die a-Druckwelle auf der Venenkurve früher erscheint

Man ist seit langem bestrebt, durch Registrierung der Bewegungen des Herzens und der um das Herz liegenden Großgefäße auf die mechanische Herztätigkeit Schlüsse zu ziehen.

In Kenntnis des engen Zusammenhanges und der weitergehenden zeitlichen Koordination, die zwischen den Bewegungen der einzelnen Herzteile besteht und der strengen Aufeinanderfolge von Kontraktion und Relaxation der einzelnen Muskelgruppen ist es selbstverständlich, daß im Gleichgewichtszustand die verschiedenen Herzteile bzw. die einzelnen Abschnitte der Großgefäße stets übereinstimmende Bewegungen verrichten und daß die in identischer Ableitung aufgenommenen Kurven bei jeder Person stets identisch ausfallen. Die vom Herzen und von den Großgefäßen verfertigten Mechanogramme sind also gleichförmig und reproduzierbar. Aller Wahrscheinlichkeit nach hat außerdem die insuffiziente Herztätigkeit gewisse gesetzmäßige Veränderungen der Kurven zu Folge, die empirisch und auf Grund theoretischer Erwägungen erkannt werden können.

WEBER [12], der bereits 1935 auf Dekompensation charakteristische Veränderungen auf den Venenkurven beobachtete, wies darauf hin, daß bei Insuffizienz die die beiden Ebbewellen repräsentierenden x und y-Punkte auf einer höher gelegenen Stelle der Kurve erscheinen oder sogar verschwinden. Diese Beobachtung ist mit gewisser Beschränkung auch heute noch gültig. Bekanntlich kann nämlich bei gewissen Klappenfehlern (Pulmonalstenose, Trikuspidalstenose), Vorhofseptumdefekt, pulmonaler Hypertonie [2], sowie

nach Abklingen einer Karditis [4] ohne das geringste Zeichen einer Dekompensation y-Mangel vorkommen. Die Vorwölbung des absteigenden Schenkels der c-Welle und das frühzeitige Erscheinen der x-Welle deuten ebenfalls nicht immer auf linke Kammerinsuffizienz. Werden jedoch diese bekannten Zustände vor Auge gehalten, so kann die Herzinsuffizienz anhand der erwähnten Ebbewellenveränderungen unter Umständen diagnostiziert werden.

Die Bestimmung der zeitlichen Verhältnisse der Ejektionsphase hat sich zur Feststellung der kardialen Dekompensation ebenfalls gut bewährt [6].

Untersuchungsmaterial und Methodik

Wir haben von unserem Material 12 solche Fälle ausgewählt, wo es uns gelang, mehrere Mechanogramme während Dekompensation und im darauf folgenden kompensierten Zustand aufzunehmen. Einige Patienten von denen lagen öfters in unserem Institut, wegen Dekompensation; so konnten wir bei den selben Personen öfters die Mechanogramme aufnehmen. Zur Registrierung der Herztätigkeit dienten folgende Verfahren: Elektrokardiogramm, Phlebogramm, Kardiogramm, Karotiskurve, Phonokardiogramm und mitunter Ballistokardiogramm.

Ergebnisse

Anhand der genauen Analyse der Serienaufnahmen ließen sich auf der Venenkurve und auf dem Apexkardiogramm Veränderungen erkennen, die wir für die Dekompensation charakteristisch hielten.

Auf der Venenkurve ändert sich im Falle von Dekompensation der Zeitpunkt der Erscheinung der a-Welle in auffallender Weise. Diese Beobachtung steht mit der altbekannten Literaturangabe im Einklang, nach der bei kardialer Insuffizienz große a-Wellen auftreten können. Unsere Befunde ergaben jedoch, daß sich die a-Welle nicht nur bezüglich ihrer Größe verändert, sondern auch vorzeitig auftritt. Bei der Feststellung dieser Erscheinung kann dem P-Wellenbeginn (Beginn der Depolarisation des rechten Vorhofs) in der II. Standardableitung eine Bedeutung beigemessen werden. Anhand des bekannten kausalen Zusammenhanges zwischen Vorhofsystole und elektrischer Depolarisation haben wir den Beginn der a-Welle von diesem Punkt angemessen. Die Strecke vom Beginn der P-Welle der II. Ableitung bis zum Beginn der a-Welle der Venenkurve bezeichneten wir mit P-a. Anhand des Vergleiches dieser Abschnitte konnte festgestellt werden, daß bei Dekompensation die a-Welle regelmäßig früh auftrat, die P-a-Strecke in sämtlichen Fällen unter 0,10″ blieb und im allgemeinen zwischen 0,05—0,07″ schwankte. Nach Aufhören der Dekompensation erhöhte sich die P-a-Strecke über 0,10″. Bei gesunden Kindern dauerte die P-a-Strecke demgegenüber in sämtlichen Fällen länger als 0,10″ (0,12—0,14″).

Auf den Kurven der Dekompensierten fällt es gleich auf, daß die a-Welle höher und breiter ist, die Zeitmessung vom Beginn der P-Welle ermöglicht jedoch eine objektive Bewertung der a-Welle (Abb. 1).

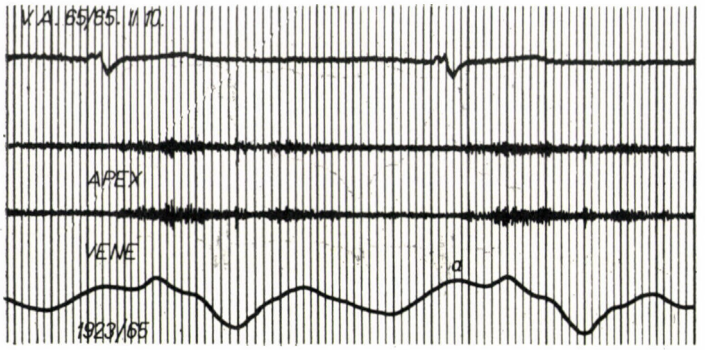

Abb. 1. Hohe und breite a-Welle. Die vom Beginn der P-Welle der II. Ableitung bis zum Beginn der a-Welle verstrichene Zeit beträgt 0,08″

Es sei betont, daß auf der Venenkurve parallel mit der früheren Erscheinung der a-Welle auch andere auf Dekompensation hinweisende Zeichen zu beobachten waren. In der Mehrzahl der Fälle war die x-Welle hoch und trat früh auf, mitunter wurde der diastolische Kollaps flach (Abb. 2).

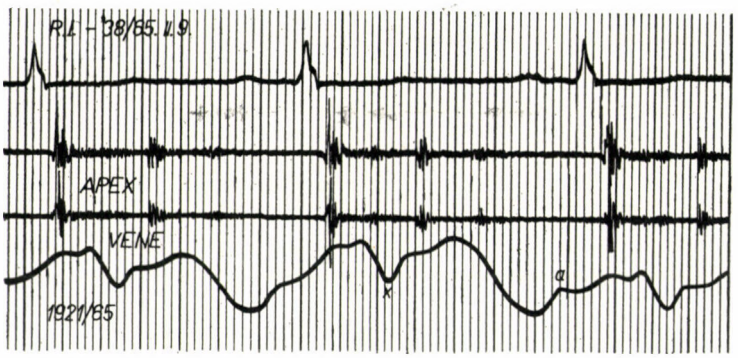

Abb. 2. Die a-Welle erscheint 0,06″ nach dem Beginn der P-Welle. Der x-Punkt tritt früher und höher auf; nach dem v-Punkt langsam abwärts verlaufender absteigender Schenkel

Auf dem Kardiogramm traten im Falle von Dekompensation ebenfalls Formveränderungen in Erscheinung. In Normalfällen zeigte die Apexkurve bis zum Beginn der Ejektion eine Erhöhung, sodann stellte sich nach dem Öffnen der Semilunarklappe der bekannte systolische Kollaps ein, worauf die Kurve steil abwärts verlief und — eventuell nach einer spätsystolischen Erhöhung — den Tiefpunkt, den sog. 0-Punkt im Moment der Öffnung der Atrioventrikularklappe erreichte, um danach in eine, auf rasche Füllung charakteristische Welle zu überschlagen (Abb. 3).

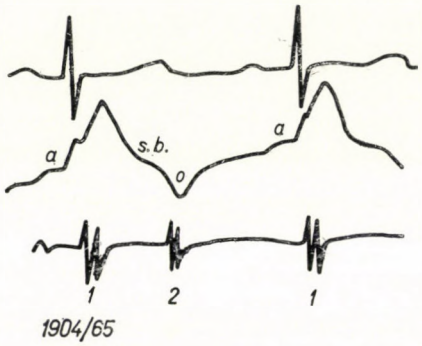

Abb. 3. Schematische Darstellung: a-Welle, — die der Semilunaröffnung vorangehende 2 Phasen und der darauffolgende systolische Kollaps mit spätsystolischem Aufstieg (s. b.), der den Tiefpunkt beim Öffnen (0) der Atrioventrikularklappe erreicht — deutlich differenzierte Füllungsphasen der beiden Kammern

Bei Insuffizienz waren die Veränderungen auf dem Kardiogramm nicht so einheitlich, wie auf der Venenkurve; angesichts ihrer ausgeprägten Tendenz sind die beobachteten Formveränderungen jedoch erwähnenswert.

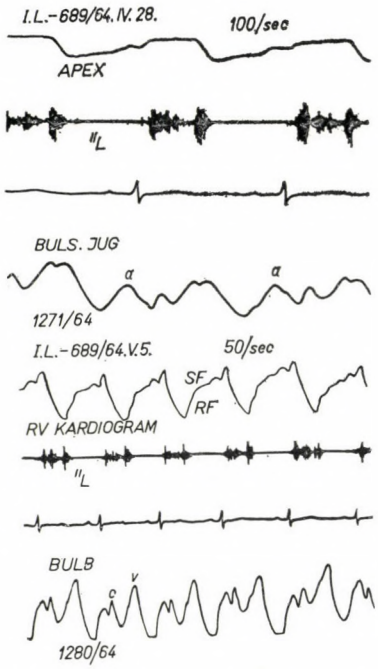

Abb. 4. In der obersten Reihe der obenstehenden Abbildung: Apexkardiogramm. Die beiden diastolischen Füllungsphasen sind verschmolzen. Das untere Kardiogramm wurde in kompensiertem Zustand verfertigt. Deutlich wahrnehmbare Differenzierung der raschen RF und langsamen SF Füllungsphase

In manchen Fällen war anstatt dem systolischen Absturz eine Plateaubildung zu beobachten, mitunter begann der systolische Absturz bereits nach dem Q-Punkt. In anderen Fällen begann der diastolische Anstieg vor dem 0-Punkt, am Ende der Systole; diese Strecke enthielt auch die WIGGERSsche protodiastolische und isometrische Relaxationsstrecke. Nach Wiederherstellung der Kompensation wies die Kurve wesentliche Formveränderungen auf: Der systolische Absturz beginnt lediglich später, der 0-Punkt liegt am entsprechenden Platz, und die beiden Füllungswellen zweigen sich in der Diastole ab.

Bei Dekompensation sind die Grenzen der raschen und langsamen Füllungsphase häufig verwischt bzw. es ist keine Abtrennung festzustellen, anläßlich der Wiederherstellung der Kompensation können sie jedoch voneinander deutlich abgesondert werden (Abb. 4).

Besprechung

Um die bei dekompensierten Kranken beobachtete Venenkurvenveränderung (frühzeitige a-Welle) klären zu können, wollen wir vorerst die Auffassungen bezüglich des Zustandekommens der Venenkurvenwellen überblicken. In dieser Frage hat sich auch heute noch keine einheitliche Meinung durchgesetzt; die Venenwellen werden mit Druck- [11] oder mit Volumveränderungen [13] erklärt. GROEDEL [7] nahm an, daß die Venenwelle sowohl Druck- wie auch Volumkomponente repräsentiert. LUISADA [10] betonte, daß innerhalb derselben Welle der aufsteigende Schenkel auf Druckveränderung, der absteigende dagegen auf Volumveränderung deuten kann.

Wir schließen uns wesentlich der Ansicht von GROEDEL [7] an. Unseres Erachtens entsteht die Venenwelle wahrscheinlich in der Weise, daß gewisse Wellen, die bei intaktem Kreislauf lediglich reine Volumveränderungen bedeuten, im dekompensierten Zustand Druckveränderungen repräsentieren. Bezüglich der a-Welle vertreten wir die Meinung, daß diese bei kompensiertem Kreislauf als reine Flutwelle erscheint. Ergänzungshalber sei darauf hingewiesen, daß der die venöse Strömung aufrechterhaltende Faktor letzten Endes ein stets abnehmender Druckgradient ist, der seinen tiefsten Punkt im Vorhof bzw. während der Diastole in der Kammer erreicht. Den Strömungsgesetzen entsprechend vollzieht sich die während der Vorhofsystole im Vorhof auftretende Druckveränderung in Richtung des geringeren Druckes, d. h. kammerwärts, woraus folgt, daß in diesem Zeitpunkt in Richtung der Venen weder Rückströmung, noch Drucksteigerung zustandekommt. Die Vorhofsystole bedeutet demnach dasselbe, wie die Errichtung eines Dammes in einem Flußbett: hinter dem Damm entsteht wegen der ständig dahingelangenden Flüssigkeit eine Flutwelle (Volumveränderung), die sich in retrograder Richtung langsam fortpflanzt. Auf diese Weise kommt — infolge des augenblicklichen Abflußhindernisses — im Zeitpunkt der Vorhofsystole die Druckveränderung venen-

wärts nicht zur Geltung, in dem distal vom Vorhof liegenden Venen wächst lediglich das Volumen an, d. h. es tritt eine Flutwelle auf, die für ihre Fortpflanzung eine gewisse, gut bestimmbare Zeit benötigt. Die zeitliche Verzögerung der Volumwellen in Richtung der Peripherie ist eine unzweifelhafte Tatsache: dies beweisen auch unsere vorangehenden Untersuchungen über die Pulsation der Schultervenen [3].

Anders stehen die Dinge bei Rechtkammerinsuffizienz. In diesen Fällen hat der Vorhof, um seinen Inhalt in die Kammer befördern zu können, wegen des erhöhten diastolischen Enddruckes der rechten Kammer einen stärkeren Druck zu überwinden. Der in den Venen herrschende Druck ist selbstverständlich noch höher, da der Druckgradient nur auf diese Weise gesichert werden kann. Diese erhöhten Druckverhältnisse haben es zur Folge, daß das Dehnungsvermögen der Venenwand sich allmählich erschöpft. Im Moment der Vorhofsystole ist die Distensibilität der Venenwand zur Aufnahme der durch die Vorhofaktion retrograd transportierten Blutmenge mittels einfacher Volumveränderung bereits ungenügend, und es entsteht eine Druckerhöhung, die auch retrograd, innerhalb der Venen zur Geltung kommt. Dies bedeutet, daß im Falle von über der Norm liegenden Druckwerten in der Vene infolge der Vorhofsystole eine Druckveränderung entsteht, was die Erscheinung der a-Druckwelle herbeiführt. Da jedoch die Fortpflanzungsgeschwindigkeit der Druckwelle jene der Volumwellen wesentlich übertrifft, ist es leicht verständlich, daß die a-Druckwelle früher auftritt, als die a-Volumwelle.

Wir können die Richtigkeit der oben beschriebenen These mit mehreren, indirekten Beobachtungen unterstützen.

Bekanntlich erscheint auf den von den um das Herz entstandenen Bewegungen verfertigten, Druckveränderungen repräsentierenden Mechanogrammen mitunter auch eine vorhofaktionsbedingte Druckwelle. Auf diese Weise treten vor dem steil aufsteigenden Schenkel der hohen Pulswelle häufig 2 kleine Wellen in Erscheinung; eine dieser Wellen beginnt nach dem R-Gipfel und entspricht unzweifelhaft der isometrischen Spannungsphase. Wird die Aufnahme mit einem empfindlichen und auch etwas höhere Bewegungsfrequenzen übertragenden Rezeptor verfertigt, ist an dieser Stelle manchmal eine winzige Inzisur ersichtlich, die gleichzeitig mit dem Schließen der Atrioventrikularklappe auftritt. Vor dieser Welle bzw. vor dieser niedrigen Inzisur tritt gemeinsam mit der Vorhofaktion 0,06″ nach Beginn der P-Welle mitunter noch eine niedrige positive Welle auf.

In unserem Material der letzten Jahre war auf der Karotiskurve in 25 Fällen eine ausgeprägte a-Welle zu beobachten. Da die Vorhofaktion die Kammerfüllung steigert, bedeutet das Erscheinen dieser Welle selbstverständlich eine durch die erwähnte Aktion ausgelöste Druckveränderung, die durch die geschlossene Aortenklappe auch auf der Karotiswelle zur Geltung kommen kann.

Von Interesse ist außerdem unsere Beobachtung bezüglich der Verzögerung der a-Welle, was unzweifelhaft eine Druckveränderung bedeutet (Abb. 3). Anhand der eingehenden Analyse von 25 Kardiogrammen, auf denen diese Erscheinung zu beobachten war, konnte festgestellt werden, daß vom Beginn der P-Welle der II. Ableitung bis zum Beginn der a-Welle 0,06″ erforderlich sind. Diese Welle ist offensichtlich eine Folge jener Druckveränderung, die die Vorhofaktion auf die Kammerwand ausübt. Die P-a-Strecke beträgt auch in diesen Fällen 0,06″ (Abb. 5).

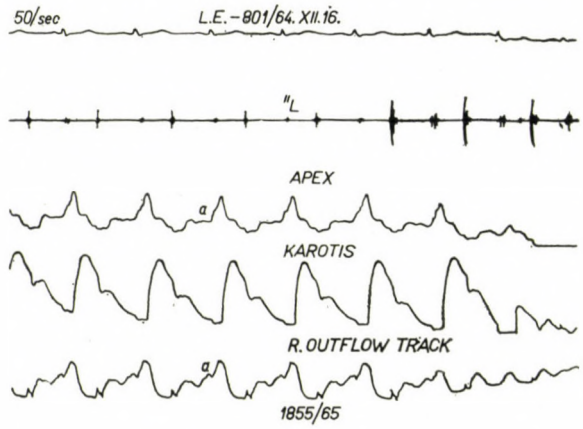

Abb. 5. Elektrokardiogramm, Phonokardiogramm, Apex-, Karotis- und Rechtskammerkurve. Auf beiden Kardiogrammen deutlich erkennbare a-Wellen, die 0,06″ nach dem Beginn der P-Welle beginnen

Auf Phonokardiogrammen von niedriger Frequenz — besonders wenn diese bei Kindern mit dünnem Brustkorb verfertigt werden — ist nicht selten eine auf die Vorhofaktion folgende Schwingung von niedriger Frequenz und kleiner Amplitude zu beobachten. Diese ist die subaudibile Komponente des Vorhoftons, die der infolge der Vorhofaktion entstehenden Kammerfüllung entspricht und demzufolge in jenem Moment auftritt, in dem die durch die Vorhofaktion ausgelöste Druckerhöhung eine maximale Erweiterung der Kammerwand bewirkt, worauf diese anschwingt. Selbst diese geringe Vibration bedeutet eine Druckveränderung und die vom Beginn der P-Welle berechnete Zeit beträgt im Durchschnitt 0,10″; diese Zeitperiode ist ebenso lang wie der Gipfel der a-Welle (Maximum des Vorhofdrucks).

Bekanntlich kann die Vorhofaktion auch unter Normalverhältnissen einen hörbaren Ton produzieren. Die Ursache dieser Erscheinung liegt wahrscheinlich darin, daß infolge der durch die Vorhofaktion verursachten Druckerhöhung die Klappensegel sich anläßlich der Kammer-Enddiastole plötzlich erheben und dadurch eine Vibration verursachen. Diese Schwingungen treten

in der Regel 0,16—0,20″ nach der P-Welle auf und fließen somit mit dem 1. Ton zusammen. Falls nun irgendwelche Schädigung des Herzmuskels vorliegt (z. B. Anoxie), so kann sich die hörbare Komponente des Vorhoftons vom 1. Ton abtrennen und vor der Q-Welle als präsystolischer Ton bzw. Galopprhythmus erscheinen. KINCAID-SMITH und BARLOW [8, 9], die die Richtigkeit dieser These bewiesen, haben außerdem festgestellt, daß sich nach Aufhören der Ischämie der aurikuläre Ton von der P-Welle entfernt und wieder dem 1. Ton beimischt. Im Falle einer ausgedehnten Myokardschädigung ist das frühe Erscheinen der hörbaren Komponente ausgeprägter: In diesen Fällen kann der Ton 0,06″ nach dem Beginn der P-Welle erscheinen. Dem Anschein nach finden sich bei Dekompensation ähnliche Verhältnisse. Der in diesem Zeitpunkt häufig auftretende präsystolische Galopprhythmus ist mitunter mit der subaudibilen Komponente gleichzeitig zu beobachten. Dies dürfte damit erklärt werden, daß bei Dekompensation sich die Klappensegel infolge der enddiastolischen Kammerdruckerhöhung früher erheben und dadurch die in Normalfällen in den 1. Ton eingeschmolzene hörbare Komponente wesentlich früher auftritt. Aller Wahrscheinlichkeit nach entsteht bei erhöhtem enddiastolischen Druck diese Klappenerhebung bereits in dem Zeitpunkt, in dem infolge der Vorhofaktion die subaudibile Komponente erscheint. Dies erklärt gleichzeitig die bekannte Tatsache, daß die Herzveränderung desto schwerer ist, je früher der Vorhofgalopp auftritt [5].

Alle diese Beobachtungen sprechen dafür, daß die durch die Vorhofaktion ausgelösten, mit der Druckveränderung zusammenhängenden Erscheinungen im allgemeinen 0,06″ nach dem Beginn der rechten Vorhofdepolarisation auftreten, ebenso wie in unseren dekompensierten Fällen die a-Druckwelle auf der Venenkurve erscheint. Dies unterstützt indirekterweise unsere Annahme, nach der im dekompensierten Zustand die a-Welle als Druckwelle 0,06—0,08″ nach dem Beginn der P-Welle auftritt.

Die Richtigkeit unserer Hypothese unterstützen auch andere Beobachtungen. Wie darauf die endokavitären Druckbestimmungen hinweisen, beginnt die a-Vorhofwelle 0,04—0,08″ nach dem Beginn der P-Welle. BAYER und WOLTER [1] stellten anhand der Analyse der intrakavitären Druckwellen fest, daß die a-Vorhofwelle 0,02—0,04″ nach der P-Welle auftritt. Unsere Untersuchungen ergaben, daß die a-Vorhofdruckwelle 0,06″ nach dem Beginn der P-Welle der II. Ableitung beginnt. Diese zeitliche Übereinstimmung spricht unzweifelhaft für den kausalen Zusammenhang, der einerseits zwischen der a-Vorhofdruckwelle und den Kammer- bzw. Karotiswellen, anderseits zwischen den auf dem Phonokardiogramm niedriger Frequenz manchmal beobachteten und den oben angeführten Wellen besteht. Diese Erscheinung unterstützt gewissermaßen unsere Hypothese, nach der die in dekompensiertem Zustand früher auftretende a-Welle eine, durch die Vorhofaktion ausgelöste Druckwelle repräsentiert.

Im Einklang mit dem bisher Gesagten steht auch unsere Beobachtung, die für unsere bezüglich des Frühauftretens der a-Welle vertretene Meinung spricht: Im Falle eines totalen Vorhofblocks hängt die Länge der P-a-Strecke auf der Venenkurve in erster Reihe von dem Umstand ab, auf welchen Abschnitt der Diastole die Vorhofaktion fällt. Falls die Vorhofsystole zu Beginn der Diastole eintritt, beträgt die P-a-Strecke z. B. 0,14″ und ist somit mit der Strecke identisch, die wir bei kompensiertem Kreislauf registrieren. Wenn jedoch die Vorhofaktion am Ende der verlängerten Diastole eintritt, in Fällen also, in denen annehmbare bedeutendere Füllung und erhöhter Kammerdruck vorliegen, so erscheint auf der Venenkurve die a-Welle wesentlich früher und in breiterer und höherer Form (Abb. 6). Wie auf Abb. 6 ersichtlich, verkürzte sich die P-a-Strecke nach der einen Vorhofsystole auf 0,06″, d. h. die a-Welle erschien in der gleichen Zeit, wie in unseren dekompensierten Fällen.

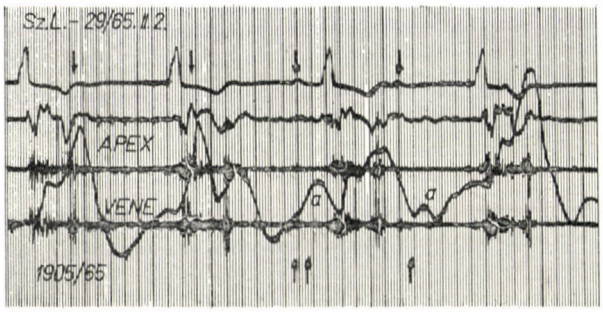

Abb. 6. Totaler Vorhof-Kammerblock. Die zu der zu Beginn der Diastole auftretenden Vorhofaktion gehörende a-Welle (nach dem dritten Kammerkomplex) ist niedrig und tritt 0,14″ nach dem Beginn der P-Welle auf. Die zu der am Ende der Diastole auftretenden Vorhofaktion gehörende a-Welle ist breit (vor dem dritten Kammerkomplex), hoch und tritt 0,06″ nach dem Beginn der betreffenden P-Welle auf

Unsere, anhand dieser Beobachtung vorgenommenen Serienmessungen wiesen darauf hin, daß zwischen der, den Beginn der Vorhofaktion bedeutenden Q-P-Strecke und der P-a-Strecke (dem sich von der Depolarisation des rechten Vorhofs bis zum Auftreten der a-Welle erstreckenden Abschnitt) ein enger Zusammenhang besteht. Im Falle einer längeren Q-P-Strecke wird die P-a-Strecke kürzer und die a-Welle breiter.

Die letzterwähnte Erscheinung steht mit der Beobachtung von KINCAID-SMITH und BARLOW [9] im Einklang, laut der das sog. P-G-Intervall (die Strecke zwischen dem Beginn der Vorhofwelle und dem Auftreten des 4. Tons) davon abhängt, in welcher Etappe der Diastole die Vorhofaktion auftritt. Nach einer früh in der Diastole auftretenden Vorhofwelle erscheint der aurikuläre Ton später, als wenn es spät in der Diastole zur Vorhofaktion kommt; auf der Venenkurve haben wir bezüglich des Beginns der a-Welle dasselbe beobachtet.

Die im Falle von Blockdissoziation beobachtete zeitliche Veränderung der a-Welle stimmt mit der Beobachtung von KINCAID-SMITH und BARLOW [9] und mit der Feststellung von DUCHOSAL [5] überein. Dies beweist auch die Richtigkeit unserer Auffassung, d. h. während der Zeit von erhöhtem diastolischem Druck auftretender Vorhofaktion führt auf der Venenkurven zur Erscheinung der (a) Druckwelle.

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ÜBER DIE WIRKUNG DER RADIOISOTOPENTHERAPIE AUF DIE INTENSITÄT DER TUBERKULINREAKTION

Von

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Die bei hyperthyreotischen und polyzythämischen Kranken durchgeführten Untersuchungen ergaben, daß die Radioisotopentherapie (4,5—10 mC ^{131}J bzw. 4—5 mC ^{32}P) die Intensität der Tuberkulinreaktion nicht beeinflußt.

Bekanntlich haben gewisse Strahlendosen die Verminderung bzw. den vollkommenen Ausfall der Immunreaktionen zur Folge. Da die per os bzw. parenterale Verabfolgung von inkorporierten Radioisotopen bei gewissen Krankheiten allmählich für ein Routinverfahren gilt, stellten wir uns die Frage, ob dies die allergischen Reaktionen der Patienten bedeutend beeinflußt. Nach der Strahlentherapie sind in den Leukozyten Chromosomschädigungen verschiedenen Typs zu beobachten. In Anbetracht, daß das Lymphoidsystem ziemlich strahlenempfindlich ist, und der Prototyp der allergischen Spätreaktionen, die Tuberkulinreaktion die Folgeerscheinung der lymphozellulären Immunreaktion (Sensibilisation) ist, haben wir zur Entscheidung der Frage bei hyperthyreotischen und polyzythämischen Kranken vor und nach der routinartigen ^{131}J- bzw. ^{32}P-Radioisotopentherapie die Gestaltung der Tuberkulinreaktion untersucht.

Methodik

Die Untersuchungen wurden teils an hyperthyreotischen und polyzythämischen Kranken, teils bei Meerschweinchen durchgeführt. Die Kranken waren frei von aktiver Tuberkulose. Die den Hyperthyreotikern verabreichte ^{131}J-Dosis schwankte zwischen 4,5—10 mC, während die polyzythämischen Kranken 4—5 mC ^{32}P erhielten. Die Versuchspersonen waren im Alter von 35—60 Jahren, die Geschlechtsverteilung war identisch. Die Tuberkulinpositivität 18 unserer Kranken war infektiösen Ursprungs. Die Tuberkulinprobe wurde mit der MANTOUXschen Methode mit 4fach verdünnter Alttuberkulinlösung 5—6 Tage vor der ^{131}J- bzw. ^{32}P-Therapie durchgeführt, sodann 24 bzw. 48 Stunden nach Beeindgung der Medikation in einem Abstand von einigen cm von der ersten Probe, auf die gleiche Weise wiederholt. Bei der Bewertung der Ergebnisse hielten wir uns an die internationale Vorschrift: negative Probe = weder Induration, noch Hautröte; zweifelhaft = 5 mm große, bzw. kleinere Induration; mäßig positiv = Induration mit einem Durchmesser von 6—10 mm und 1 mm Höhe; stark positiv = = Indurationsdurchmesser 11—20 mm, Höhe 2 mm. Insgesamt wurden 21 behandelte Kranken (11 hyperthyreotische und 9 polyzythämische) untersucht, 12 dieser Kranken erhielten das Tuberkulin 24 Stunden, und 9 Kranken, 48 Stunden nach Beendigung der Isotopentherapie. Die Kontrollgruppe bestand aus 52 an Hyperthyreose bzw. Polyzythämie leidenden Patienten, die ursprünglich zwecks Isotopentherapie aufgenommen wurden, infolge verschiedener Ursachen jedoch (falsche Einsendungsdiagnose, Verordnung einer anderen Therapie usw.) keine Radioisotopen erhielten.

Ergänzungshalber wurde bei Meerschweinchen die Frage untersucht, auf welche Weise die in der induktiven bzw. produktiven Phase verabfolgten ^{131}J-, ^{32}P- und ^{35}S-Dosen nach BCG-Vakzination die Stärke der zustandegekommenen Tuberkulinpositivität beeinflussen. Da sich in unseren vorangehenden Untersuchungen zur Behandlung einiger autoimmuner Erkrankungen das ^{35}S gut bewährte, haben wir den Tieren in vorliegenden Experimenten außer ^{131}J und ^{32}P auch dieses Radioisotop verabfolgt. Die Versuchstiere waren tuberkulinnegativ. Bei einer Gruppe der Meerschweinchen wurde die radioaktive Inkorporation in der induktiven Phase durchgeführt: 15 Tiere erhielten 1 Stunde vor der BCG-Vakzination (2 ml) 80, 60, 40, 20, 10 μC Aktivität enthaltende Dosen von ^{131}J, ^{32}P und ^{35}S. Der anderen Gruppe der Tiere wurden die Radioisotopen in der produktiven Phase zugeführt: 15 Tieren wurden die je 80 μC Aktivität enthaltenden ^{131}J-, ^{32}P- und ^{35}S-Dosen 10 Tage nach der Vakzination verabfolgt. Die Ergebnisse der Tuberkulinprobe wurden nach 48 Stunden abgelesen und folgendermaßen bewertet: weder Induration, noch Hautröte: negative Probe; Induration mit einem Durchmesser von höchstens 2 mm: zweifelhaft; Induration mit einem Durchmesser von 3 mm: mäßig positiv; Induration mit einem Durchmesser von 5 mm: stark positiv.

Zur Kontrolle dienten 15, mit Radioisotopen nicht behandelte, von den übrigen Tieren isolierte Meerschweinchen.

Ergebnisse

Die Intensität der vor und nach der Radioisotopentherapie durchgeführten Tuberkulinreaktion zeigte im Verhältnis zur Kontrollgruppe keine wesentliche Abweichung. Die Werte blieben zwischen den Grenzen der normalen Streuung. In den Fällen, in denen die Tuberkulinprobe nicht 24, sondern 48 Stunden nach der Isotopentherapie vorgenommen wurde, waren ähnliche Resultate zu verzeichnen (Tab. I).

Tabelle I a

Tuberkulinprobe vor und 24 Stunden nach der Isotopenbehandlung
(Untersuchungen bei Kranken)

Ergebnisse	Zahl der Fälle			
	Vor ^{131}J-Behandlung	Nach ^{131}J-Behandlung	Vor ^{32}P-Behandlung	Nach ^{32}P-Behandlung
Zweifelhaft	1	1	1	1
Mäßig	4	4	4	4
Stark	1	1	1	1
Insgesamt	6	6	6	6

Tabelle I b

Tuberkulinprobe vor und 48 Stunden nach der Isotopenbehandlung
(Untersuchungen bei Kranken)

Ergebnisse	Zahl der Fälle			
	Vor ^{131}J-Behandlung	Nach ^{131}J-Behandlung	Vor ^{32}P-Behandlung	Nach ^{32}P-Behandlung
Zweifelhaft	1	2	1	1
Mäßig	3	2	2	2
Stark	1	1	1	1
Insgesamt	5	5	4	4

Tabelle I c

Unbehandelte Kontrollpersonen

Ergebnisse	Zahl der Fälle	
	Erste Probe	Wiederholung der Probe
Zweifelhaft	5	7
Mäßig	29	32
Stark	18	13
Insgesamt	52	52

In den Tierexperimenten gestalteten sich die Ergebnisse in ähnlicher Weise: Während unter 15 tuberkulinpositiven Tieren 1 bzw. 2 tuberkulinnegativ wurden, befand sich in der entsprechenden Kontrollgruppe ebenfalls 1 Tier, das spontan tuberkulinnegativ wurde (Tab. II).

Tabelle II a

Isotopenbehandlung in der induktiven Phase
(mit BCG sensibilisierte Meerschweinchen)

Ergebnisse	Zahl der Fälle		
	^{131}J-Behandlung	^{32}P-Behandlung	^{35}S-Behandlung
Negativ	—	—	—
Mäßig	1	—	1
Mild	4	3	2
Stark	—	2	2
Insgesamt	5	5	5

Tabelle II b

Isotopenbehandlung in der produktiven Phase
(mit BCG behandelte Meerschweinchen)

Ergebnisse	Zahl der Fälle		
	^{131}J-Behandlung	^{32}P-Behandlung	^{35}S-Behandlung
Negativ	—	1	2
Mäßig	1	1	—
Mild	3	2	2
Stark	1	1	1
Insgesamt	5	5	5

Tabelle II c

Unbehandelte Kontrolltiere

Ergebnisse	Zahl der Fälle
Negativ	1
Mäßig	1
Mild	9
Stark	4
Insgesamt	15

Besprechung

Die Intensität der wiederholten Tuberkulinreaktion kann geringe, spontane Schwankungen aufweisen; diese Erscheinung konnten wir sowohl bei unseren Kranken, als auch bei den Kontrollpersonen beobachten. Die Tuberkulinprobe selbst verursacht im allgemeinen keine Sensibilisierung und steigert die vorhandene Sensibilität — falls die zweite Probe nicht an derselben Hautstelle vorgenommen wird — nicht. In Fällen, in denen die nacheinanderfolgenden Proben an identischen Hautflächen verfertigt werden, kann eine geringe aspezifische Beschleunigung zustandekommen, die jedoch in einigen Stunden abklingt [4]. Wird jedoch das zweitemal eine andere Impfungsstelle gewählt, so bleibt auch diese beschleunigende Abweichung weg. Anhand unserer Untersuchungen konnte somit festgestellt werden, daß die, durch die in der Therapie der Hyperthyreose bzw. der Polyzythämie angewandten Radioisotopen die Intensität der Tuberkulinreaktion nicht beeinflussen. In Anbetracht dessen daß die Chromosomschädigungen ebenfalls in den ersten 24—48 Stunden nach der Strahlentherapie am ausgeprägtesten sind [5], kann die Möglichkeit einer Spätwirkung ausgeschlossen werden, zumal da das Lymphoidsystem über eine bedeutende Regenerationsfähigkeit verfügt.

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EFFECT OF ENDOTOXIN PRETREATMENT ON SURGICAL LETHALITY IN ANIMAL EXPERIMENTS

By

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Injection of *Serratia marcescens* endotoxin was found to affect resistance to surgical stress in animal experiments. Normal resistance was restored in 48 hours. In animals pretreated with endotoxin for eight days resistance remained unaffected. In the development of resistance, the pituitary-adrenocortical system is essential, but in its maintenance other mechanisms are thought to be involved.

Evidence has been increasing that lipopolysaccharides (endotoxins) ol bacterial origin enhance the organism's non-specific resistance. After parenteraf administration of lipopolysaccharides, experimental animals have been found to survive infections (SHILO, 1959; ROWLEY, 1964), total body irradiation (SMITH, 1964), and traumatic shock (ZWEIFACH, 1957) lethal to control animals. Under certain well-defined experimental conditions it has been possible to discern two phases of this change in resistance, a phase of reduced resistance and a phase of enhanced non-specific resistance. One of the special patterns of such changes is endotoxin tolerance, in other words the failure of a repeated injection of endotoxin to elicit a toxic effect, or at least a major toxic effect, in animals pretreated with endotoxin (PETERSDORF, 1964). In the present investigations the effect of endotoxin injected at various intervals has been studied on surgical death in two groups of rats, one without pretreatment and one made tolerant to endotoxin.

Method

The experiments were carried out on 540 home-bred albino rats of both sexes, weighing between 110 and 160 g. The endotoxin was obtained by purification of a broth culture of *Serratia marcescens*. The purified endotoxin was tested for toxicity and in the whole course of the experiments LD_{10} and LD_{50} were applied. Calculated on the basis of dry material, the former corresponded to 1 mg per 100 g, the latter to 5 mg per 100 g. Experimental surgery included ligation of a jejunal loop, partial hepatectomy (these involving laparotomy), uni- and bilateral adrenalectomy and bilateral nephrectomy (lumbar operations). The results were expressed and tabulated in percentages of deaths. Tolerance to endotoxin was induced by daily injections of LD_{10} during a week. In these animals surgery was performed 48 hours after the last injection.

Results

In the first series we studied the influence of a single dose of endotoxin upon the effect of a second injection. When LD_{10} of *Serratia marcescens*

endotoxin had been administered simultaneously with LD_{50}, lethality was 60 per cent. When LD_{50} was given two hours after LD_{10}, lethality was 100 per cent. When, however, there was a 48-hour interval between LD_{10} and LD_{50}, survival was 100 per cent.

Injection of LD_{10} lipopolysaccharide thus resulted in hyperreactivity of the animals at 24 hours and in a tolerance to semi-lethal doses at 48 hours.

The results were similar when surgery was performed at various intervals after injection of LD_{10}. Table II shows that LD_{10} given two hours prior to operation had an adverse effect upon outcome.

Table I

Effect on endotoxin sensitivity of S. marcescens endotoxin pretreatment
Death rate of animals, per cent

Dosage	48-hour deaths per cent
1 mg/100 g, intraperitoneally	10
5 mg/100 g, intraperitoneally	52
1 mg/100 g, intraperitoneally, together with 5 mg/100 g intraperitoneally	60
1 mg/100 g, intraperitoneally + at 2-hour interval 5 mg/100 g intraperitoneally	100
1 mg/100 g, intraperitoneally + at 48-hour interval 5 mg/100 g intraperitoneally	0

Table II

Effect on surgical lethality of S. marcescens endotoxin pretreatment
Death rate of animals, per cent

Type of operation	Untreated	Treatment 2 hrs. before surgery	Treatment 48 hrs. before surgery
Unilateral adrenalectomy	0	25	1
Ligation of jejunal loop	6	52	7
Bilateral nephrectomy	0	78	9
Partial hepatectomy	28	100	18

Surgical death by partial hepatectomy seemed to be particularly suited for the study of changes in resistance. Table III clearly shows that the original resistance of the animals was restored between the 4th and 48th hours.

Table III

Effect on death from partial hepatectomy of S. marcescens endotoxin pretreatment
Death rate of animals, per cent

Type of operation	48-hour death rate, per cent
Partial hepatectomy	28
1 mg/100 g intraperitoneally, operation at 2-hour interval	100
1 mg/100 g intraperitoneally, operation at 4-hour interval	81
1 mg/100 g intraperitoneally, operation at 6-hour interval	70
1 mg/100 g intraperitoneally, operation at 48-hour interval	15

The same experiments yielded remarkable results when repeated in animals with induced tolerance to endotoxin. No increase in surgical deaths was noted in these animals, though they had received endotoxin two hours prior to operation. This shows that induced tolerance to endotoxin protects the animals from the adverse effects of endotoxin on surgical resistance as well.

By means of the present method it was hoped to clear the question whether endotoxins absorbed through the intestinal wall had some part in the disastrous consequences of intestinal obstruction and of renal failure. With this in view, the animals with jejunal loop ligation and those with bilateral nephrectomy were further studied, each animal being injected 20 ml of isotonic saline and 20 ml of isotonic glucose twice daily. If the endotoxin theory holds true, endotoxin tolerant animals must die later than the controls. This was, however, not the case. Death occurred at the same frequency in the first days after operation and all animals had succumbed by the sixth day.

Table IV

Surgical death 2 hour after a repeated injection of endotoxin in rats made tolerant by a 6-day course of S. marcescens endotoxin
Death rate of animals, per cent

Type of operation	Deaths in controls	Deaths in tolerant animals
1 mg/100 g intraperitoneally, ligature of jejunal loop at 2-hour interval	52	9
1 mg/100 g intraperitoneally, nephrectomy at 2-hour interval	78	14
1 mg/100 g intraperitoneally, partial hepatectomy at 2-hour interval	100	20

Table V

Death from intestinal obstruction and from renal failure in the first 6 days after operation in normal and endotoxin tolerant rats

Induced condition	Deaths in controls	Deaths in tolerant animals
Intestinal obstruction	100	100
Renal failure	100	100

Table VI

Lethality of endotoxin injection in normal and tolerant adrenalectomized animals
Death rate, per cent

Type of experiment	Untreated	Treatment 2 hrs. prior to operation	Treatment 6 hrs. prior to operation
Adrenalectomized, normal	0	100	100
Adrenalectomized, tolerant	0	16	25

The significance of the pituitary-adrenocortical system in the non-specific resistance of the organism justifies investigations into the possible correlations between endotoxin tolerance and the second phase of the general adaptation syndrome, *i.e.* the phase of resistance. Adrenalectomized animals have been found to exhibit an increased sensitivity to endotoxin (LEWIS, 1946). In our experiments even high dilutions of endotoxin proved fatal to adrenalectomized rats, whereas doses to the extent of LD_{50} were well tolerated by animals with induced tolerance to endotoxin.

Table VII

Ascorbic acid content of adrenal gland in normal and endotoxin tolerant rats 2 hours following injection of 1 mg/100 g of endotoxin

Type of experiment	Ascorbic acid content of adrenal gland, mg per 100 g
Physiological saline intraperitoneally	490 ± 52
Tolerant animals with physiological saline intraperitoneally	488 ± 30
Endotoxin intraperitoneally	365 ± 57
Tolerant animals with endotoxin intraperitoneally	478 ± 54

The adrenal cortex thus plays an essential role in inducing tolerance. Maintenance of it involves, however, other mechanisms.

It was remarkable that in endotoxin tolerant rats injection of endotoxin failed to cause a loss in adrenal ascorbic acid. We have thus no evidence to connect the favourable effect of induced endotoxin tolerance on surgical resistance with a possible ACTH-adrenocortical hyperfunction or hyperreactivity.

Discussion

The results of the present investigations may be summed up as follows.

1. A single injection of endotoxin results in an increased sensitivity to endotoxin at two hours, and in a reduced endotoxin sensitivity at 48 hours.

2. Surgical death rate is increased at two hours.

3. The sensitizing effect fails to appear in endotoxin tolerant animals.

4. There is no evidence to connect the tolerance with a hyperfunction or hyperreactivity of the ACTH-adrenocortical system.

Our preliminary results are in line with those of ABERNATHY (1957) and CHEDID (1964) who made mice tolerant to a second dose of endotoxin by a single injection of the substance. Increase in non-specific resistance of the animals to infections, radiological injury and traumatic shock as a further effect of a single injection, has been described by various observers, as quoted in the introductory part of the present report. It is hard to account for the reduction of non-specific resistance preceding its rise; we likewise ignore why in our animals the postoperative death rate was considerably higher than in the controls. The postoperative course may have been affected by a transitory circulatory disturbance due to the endotoxin effect. By demonstrating that endotoxin fails to reduce surgical resistance in endotoxin tolerant animals, we have been able to point out a further effect of endotoxin which is suppressed in endotoxin tolerance (PETERSDORF, 1964). This phenomenon, too, is little understood. Increased endotoxin clearance, enhanced RES activity, a higher titre of natural antibodies might have a part in it. Our view that the ACTH-corticoadrenal system plays no essential role in the maintenance of endotoxin tolerance is shared by EGDAHL (1958) and CHEDID (1964) and is consistent with the results of STARK (1965) who found a reduced ACTH production by the pituitary gland in endotoxin tolerant animals.

Finally, it must be stressed that the adverse effect of bacterial lipopolysaccharides on resistance, on the one hand, and the increased resistance, on the other, have important practical issues in clinical surgery. It is along these very lines, *i.e.* with a view to averting this undesirable loss of resistance while augmenting surgical resistance beyond its normal limits, that further studies are being pursued.

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INACTIVATION OF PENICILLIN BY SALIVA

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The penicillin inactivating effect of saliva is considerably diminished by boiling. On the addition of penicillinase, the added amount of penicillin is completely destroyed. The boiled saliva-buffer-penicillinase mixture exerts a more marked effect than the boiled saliva-buffer mixture does. Shaking saliva with dithizone dissolved in chloroform results in an increase of the inactivating capacity. A similar increase is induced by shaking the saliva with chloroform. In comparison to mixed saliva, parotid secretion has a weaker inactivating effect.

In previous experiments [1, 2] it has been shown that saliva of some animals as well as of man is capable of inactivating certain penicillins. Some connection was demonstrated between dental caries experience in the saliva donors, and the penicillin inactivating capacity of saliva.

Since the nature of this peculiar salivary factor has not been clarified, further experiments were carried out to examine changes in the penicillin inactivating capacity on subjecting the salivary sample to different treatments.

Materials and methods

Two and a half ml portions of saliva were collected from voluntary donors (patients and nurses of the Department of Stomatology) into flasks containing an equal volume of phosphate buffer pH 7.2. No attention was paid to the oral condition (caries and gingival status) of the donors, and to the time interval between the last meal and collecting the saliva. Aliquots of the saliva-buffer mixtures were subjected to different treatments. Thereafter crystalline potassium G-penicillin was added to each aliquot, to a final penicillin concentration of 5.0 U/ml.

The treatment procedures are summarized — together with their effects — in Table I.

Penicillin activity of the aliquots was estimated by the plate method. One tenth ml samples were put into each well of the plate previously inoculated with spores of *B. subtilis* ATCC 6633. After incubating the plate for 24 hours at 37 °C, the width of the inhibition rings around the wells was measured to the next 0.5 mm. The broader the inhibition ring, the higher the concentration of penicillin in the well, and thus the smaller the inactivating effect of the salivary sample. As our aim was a comparison of the different treatments, the widths of the inhibition rings were not converted into units of penicillin but were handled statistically directly.

Results

Results are summarized in Tables I and II.

Boiling the salivary sample prior to the addition of penicillin resulted in a diminished inactivation effect. This was the case with all the treatment proce-

dures applied. A total of 140 comparisons of unboiled and boiled salivary samples were made; in 138 instances the boiled sample was less inhibitory than the unboiled one. In two samples of dithizone treated saliva no difference was found between the unboiled and boiled salivary sample. This finding was in full agreement with our former experience [1, 2].

Addition of penicillinase to the mixture of saliva and buffer resulted in a complete inactivation of the added amount of penicillin. In the first experiments penicillinase-producing staphylococci were inoculated into, and incubated with, the saliva-buffer mixture. Later a standard penicillinase preparation

Table I

Effect of different treatments on inactivation of penicillin by human saliva

A) Means and standard errors

Code	Treatment of the saliva-buffer mixture	Number of saliva samples	Width of inhibition ring, and standard error in mm
A	No treatment = native	40	26.8 ± 0.62
B	Boiled	40	32.1 ± 0.50
C	Penicillinase, native (unboiled)	40	0
D	Penicillinase, boiled	40	28.6 ± 0.62
E	Shaken with dithizone, native (unboiled)	40	24.3 ± 0.57
F	Shaken with dithizone, boiled	40	29.8 ± 0.75
E_1	Like E (included in the 40 samples)	20	26.1 ± 0.67
F_1	Like F (included in the 40 samples)	20	33.2 ± 0.80
G	Shaken with chloroform, native (unboiled)	20	27.4 ± 0.37
H	Shaken with chloroform, boiled	20	33.5 ± 0.33

B) Differences of means

Codes of samples compared	Difference and standard error	Codes of samples compared	Difference and standard error
A — B	— 5.3 ± 0.8***	F_1 — H	−0.3 ± 0.86
C — D	−28.6 ± 0.62***	E_1 — G	−1.2 ± 0.77
E — F	— 5.5 ± 0.94***		
G — H	— 6.1 ± 0.5***	B — D	3.5 ± 0.8***
		A — D	−1.8 ± 0.88*
A — E	2.5 ± 0.85**	D — F	−1.2 ± 0.97
B — F	2.3 ± 0.9**		
A — F	— 3.0 ± 1.2**		

* = statistically probable
** = statistically very probable
*** = statistically significant

Table II

Width of inhibition rings of differently treated aliquots of identical salivary samples

Codes of samples compared	Difference of width in mm				
	more than 1.0	up to 1.0	0	up to −1.0	more than −1.0
A — B	0	0	0	2	38
C — D	0	0	0	0	40
E — F	0	0	2	1	37
G — H	0	0	0	0	20
A — E	28	0	2	3	7
B — F	28	0	0	7	5
D — B	4	2	1	5	28
A — D	14	2	3	3	18

If the first sample was more inhibitory, the difference was considered positive; if the second sample was more inhibitory, the difference was considered negative.

Table III

Inactivating effect of human mixed and parotid saliva

Source, and treatment of salivary sample	Width of inhibition zone in mm in examinee No.		
	1	2	3
Mixed, native	27	32	32
Parotid, native	39	39	39
Mixed, boiled	31	35	35
Parotid, boiled	40	41	41
Penicillinase-incubated mixed, native (unboiled)	0	0	0
Penicillinase-incubated parotid, native (unboiled)	0	0	0
Penicillinase-incubated mixed, boiled	29	23	28
Penicillinase-incubated parotid, boiled	38	35	35
Dithizone-shaken, mixed, native (unboiled)	28	25	29
Dithizone-shaken, parotid, native (unboiled)	28	25	31
Dithizone-shaken, mixed, boiled	32	34	33
Dithizone-shaken, parotid, boiled	35	33	35

Mixed saliva was collected by direct flow, parotid saliva by aid of a suction cup applied to Stenson's duct.

(Neutrapen Ricker) was used of which 0.8 U were added to each unit of penicillin. The mixture of saliva, buffer and penicillinase was kept at room temperature for 24 hours, then penicillin was added, and the mixture was filled into the wells, partly in the native, and partly in the boiled state. The native mixtures

caused no inhibition, while with the boiled ones marked inhibition zones were obvious after 24 hours incubation. These were narrower than with boiled mixtures of saliva and buffer not containing penicillinase. The difference was significant statistically. The inactivating effect of the boiled saliva-buffer-penicillinase mixture was, nevertheless, weaker than that of a native saliva-buffer mixture, without penicillinase; and in some instances the aforementioned boiled mixture exerted a stronger inactivating effect than the boiled (yet otherwise untreated) salivary sample.

These experiments have proved that the peculiar salivary factor was not penicillinase. No explanation can be offered for the significant increase of inactivation in the boiled mixture of saliva, buffer, and penicillinase as compared with the boiled mixture of saliva and buffer. Dilution of a factor in saliva by which penicillin might be protected, through the addition of penicillinase may be taken into consideration.

In order to remove metal traces which might be involved in destroying (or protecting) penicillin in saliva, aliquots of the saliva-buffer mixtures were shaken with 0.5 per cent dithizone, dissolved in chloroform of which 0.5 ml was added to each 5 ml of the mixture. Penicillin was then added to native and boiled samples of the supernatant. Samples shaken with dithizone displayed a more marked inactivating effect in the native and in the boiled state than corresponding samples not treated with dithizone. The dithizone-treated boiled sample's inactivating effect was, however, weaker than that of the native saliva-buffer mixture. In these comparisons individual samples behaved differently. In the case of 10 of the 40 salivary samples, inactivation was more marked with the native mixture than with the native dithizone-shaken sample, and in 12 with the boiled mixture than with the boiled dithizone-treated one.

Since by the above treatment not only the metals were removed from the specimens but by the chloroform used as a solvent also the lipids, 20 salivary samples were shaken out with chloroform alone (0.5 ml to 5.0 ml of the saliva-buffer mixture). The inactivating effect of these salivary specimens was similar whether they were shaken with dithizone dissolved in chloroform or with chloroform alone. This was true for both native (unboiled) and boiled samples.

These experiments have unequivocally demonstrated that penicillin is *protected* against destruction by a salivary factor which is of lipid nature. Metals apparently do not play any role in the process.

As regards the source of the inactivating factor, our former experiments pointed to its production by the oral flora. This conclusion was now further substantiated by comparing the inactivating effect of mixed saliva and of parotid saliva obtained directly from the duct by a suction cup. While the mixed saliva of three volunteers exerted a marked inactivating effect, significantly less inactivation was exerted by their parotid saliva, whether or not the latter had been boiled (Table III).

Discussion

According to the above findings, the partly thermolabile penicillin-inactivating factor of mixed saliva seems to be a product of the oral flora. This explains the differences in the inactivating effect, induced by different treatments. The factor is certainly different from penicillinase. Its action seems to be connected in some way with the lipid fractions contained in the mixed saliva.

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РЕЗЮМЕ

ХАРАКТЕРИСТИКИ ШУМА ПРИ ЛЕГОЧНОЙ НЕДОСТАТОЧНОСТИ, НЕ ОСЛОЖНЕННОЙ ГИПЕРТОНИЕЙ ЛЕГОЧНОЙ АРТЕРИИ И ПРАВОГО СЕРДЦА

Дь. ВИЛАГИ, Т. ЛОНЬАИ, К. ЛОЖАДИ и Дь. БОДРОГИ

Шум при недостаточности клапанов легочной артерии не сопровождающейся гипертонией правого сердца, можно на основании начала, формы, длительности и частоты дифференцировать от шума при недостаточности аортальных клапанов и при функциональной легочной недостаточности (шум Грэхем—Стилля) (рис. 7). По наблюдениям авторов отдельные виды приобретенной недостаточности клапанов легочной артерии с течением времени могут усиливаться. В связи с расширением легочной артерии может возникнуть так называемый феномен Аустин—Флинта. Амилнитрит в общем пригоден для распознавания шума недостаточности клапанов легочной артерии, однако, усиление его интенсивности наблюдается не во всех случаях. В противоположность этому после повторного глубокого вдоха шум всегда усиливается. Авторы устанавливают, что механограммы весьма полезны при идентификации шума недостаточности клапанов легочной артерии.

ИССЛЕДОВАНИЕ КОНЦЕНТРАЦИИ УГЛЕВОДОВ, СВЯЗАННЫХ К БЕЛКАМ СЫВОРОТКИ И ЛИМФЫ ПРИ ЭКСПЕРИМЕНТАЛЬНО ВЫЗВАННОМ ВОСПАЛЕНИИ

Л. ВАРГА, И. ПИУКОВИЧ, Т. Э. ЗОЛТАН, М. ГАБОР и М. ФЁЛЬДИ

В полном согласии с литературными данными, авторы наблюдали, в опытах на животных при воспалении, вызванном скипидаром, повышение уровня гликопротеидов в сыворотке. Параллельно повышению концентрации сывороточных гликопротеидов повышается также в грудном протоке содержание углеводов, связанных к белкам. Содержание гликопротеидов в шейном стволе, происходящем из области воспаления, было по истечении 24 и 48 часов достоверно ниже, чем в сыворотке или в грудном протоке. На основании этих результатов полагается, что в области воспаления организм удерживает гликопротеиды, в целях использования их для процессов регенерации.

СТЕРОИДНЫЙ ОБМЕН ЧЕЛОВЕЧЕСКОЙ КОЖИ И ВОЛОСЯНОГО ПОКРОВА В НОРМЕ И В ПАТОЛОГИЧЕСКИХ УСЛОВИЯХ

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М. ЮЛЕС, И. ФАРЕДИН и И. ТОТ

Исследовались хромогены Циммермана в коже, вырезанной из кожи живота (безволосной) и из волосатой кожи лобка эндокринологически здоровых женщин. По результатам исследований как волосатая, так и безволосная кожная ткань содержит хромогены Циммермана, причем в волосатой кожной ткани количество этих хромогенов значительно выше.

В волосатой коже живота и в лобковой кожной ткани женщин с гирсутизмом хромогены Циммермана можно выявить в значительно большем количестве, чем в соответствующих контрольных тканях.

Вытяжки кожи исследовались методом хроматографии на бумаге и на тонких слоях. При хроматографии на тонком слое кремнистого геля Г было выявлено значительное количество холестерина и ряд стероидов, присутствующих в меньшем количестве. На слое окиси алюминия удалось выявить несколько 17-кетостероидов. Идентификация и выяснение роли этих стероидов проводится в настоящее время.

СТЕРОИДНЫЙ ОБМЕН ЧЕЛОВЕЧЕСКОЙ КОЖИ И ВОЛОСЯНОГО ПОКРОВА В НОРМЕ И В ПАТОЛОГИЧЕСКИХ УСЛОВИЯХ

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И. ТОТ, И. ФАРЕДИН и М. ЮЛЕС

Исследовались вытяжки человеческой кожи и волос при помощи методов хроматографии на столбе и на тонких слоях. Установлено, что кожа и различные волосы содержат значительное количество холестерина. В хроматограммах на тонких слоях был выявлено, что эти органы, помимо холестерина, содержат еще и другие стероиды.

Авторы разработали метод для определения количества «общего холестерина» в небольших количествах человеческой кожи и волос. При помощи нового метода проводились параллельные исследования на образцах человеческой кожи и различных волос. Отклонения между параллельными исследованиями не превысили 12,5%. При этом методе добавленный холестерин в каждой фазе исследования (щелочная обработка, экстрагирование, хроматография на столбе флорисила) можно получить обратно с 92%-ным выходом.

СТЕРОИДНЫЙ ОБМЕН ЧЕЛОВЕЧЕСКОЙ КОЖИ И ВОЛОСЯНОГО ПОКРОВА В НОРМЕ И ПРИ ПАТОЛОГИЧЕСКИХ УСЛОВИЯХ

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И. ФАРЕДИН, И. ТОТ и М. ЮЛЕС

Исследовалось общее содержание холестерина в коже и волосяном покрове здоровых женщин и женщин с гирсутизмом, а также в волосяном покрове здоровых мужчин. Установлено, что в лобковой коже здоровых женщин и больных гирсутизмом содержание холестерина больше, чем в коже живота. Выявлено также, что волосы содержат большое количество холестерина, и что у здоровых женщин и мужчин бол ше всего холестерина содержится в подмышечных волосах. Волосы, появляющиеся при гирсутизме и отсутствующие у здоровых женщин содержат весьма большое количество холестерина. На основании результатов исследований кажется, что АКТГ не вызывает эффекта на содержание холестерина в волосах.

СТЕРОИДНЫЙ ОБМЕН ЧЕЛОВЕЧЕСКОЙ КОЖИ И ВОЛОСЯНОГО ПОКРОВА В НОРМЕ И ПРИ ПАТОЛОГИЧЕСКИХ УСЛОВИЯХ

IV. Нейтральные 17-кетостероиды в волосяном покрове человеческого тела

М. ЮЛЕС, И. ФАРЕДИН и И. ТОТ

Исследовались вытяжки волос здоровых мужчин и женщин, а также женщин, страдающих гирсутизмом. Выявлено, что волосы, подмышечные, лобковые волосы женщин и мужчин содержат 17-кетостероиды, и что среди этих видов волос больше всего кетостероидов содержат подмышечные волосы. Дегидроандростерон и его артефакт — 3-хлородегидроандростерон — идентифицировались различными методами хроматографии на тонких слоях и различными цветовыми реакциями. В волосах женщин, страдающих гирсутизмом, были выявлены два 17-кетостероида с такой низкой величиной Rf, которую

не удалось обнаружить в волосах здоровых женщин. При лечении АКТГ не было отмечено изменения по сравнению с качественной картиной волос, определенной до начала лечения.

ИССЛЕДОВАНИЯ МУКОПОЛИСАХАРИДОВ В СЫВОРОТКЕ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ АЛЛОКСАНОВОМ И СТЕРОИДНОМ ДИАБЕТЕ

Л. ЯКАБ, М. БРЕТАН, Я. ФЕХЕР и Л. КАММЕРЕР

При помощи введения аллоксана, кортизона и аллоксана + кортизона авторы вызывали у кроликов экспериментальный сахарный диабет и исследовали изменения нейтральных фракций мукополисахаридов (гексозы, гексозамина, сиалиновой кислоты и серомукоида). Установлено, что при аллоксановом диабете повышается концентрация гексозы и серомукоида. При диабете, вызванном совместной дачей аллоксана + кортизона, изменения мукополисахаридов в сыворотке более близки к изменениям, наблюдаемым при стероидном диабете. Морфологические изменения наиболее тяжелые после совместной дачи аллоксана + кортизона и не происходят параллельно изменениям концентрации отдельных фракций нейтральных мукополисахаридов в сыворотке.

МЕХАНИЧЕСКИЕ ПРИЗНАКИ НЕДОСТАТОЧНОСТИ СЕРДЦА

Дь. БОДРОГИ, Г ДИОШСИЛАДЬИ и К. КОЧИШ

При недостаточности сердца на венозной кривой (а) после зубца Р зубец давления появляется раньше, чем в норме. Причиной укорочения зубца Р является то, что помимо наблюдаемой в норме зубца объема (а) имеется также зубец давления, который, так как его скорость распространения значительно выше, появляется раньше. По мнению авторов при декомпенсации сердца появление зубца давления, вызывающего укорочение зубца Р-обуславливается повышением наполнения желудочков в конце диастолы, то есть повышением давления в конце диастолы.

Наблюдения авторов, сделанные при исследовании других кривых и кривой давления, измеренной в полости предсердия, а также литературные данные доказывают что более раннее появление зубца венозной кривой (а) наблюдается в условиях повышенного желудочкового давления во время предсердного действия.

ДЕЙСТВИЕ РАДИОТЕРАПИИ НА РЕЗУЛЬТАТ ТУБЕРКУЛИНОВОЙ ПРОБЫ

К. ПОНГОР и Дь. ПЕТРАНЬИ

Согласно результатам исследований, проводившихся у больных гипертиреозом и полицитемией, при даче 4,5—10 μC J^{131} или 4—5 μC P^{32} результат туберкулиновой пробы не изменяется.

ДЕЙСТВИЕ ПРЕДВАРИТЕЛЬНОЙ ДАЧИ ЭНДОТОКСИНА НА ОПЕРАЦИОННУЮ СМЕРТНОСТЬ ПОДОПЫТНЫХ ЖИВОТНЫХ

П. КЕРТАИ, К. УЙХЕИ и Й. ГЕРХАРДТ

После впрыскивания эндотоксина **Serratia marcescens** уменьшается резистентность подопытных животных в отношении операционной нагрузки. По истечении 48 часов после впрыскивания эндотоксина сопротивляемость животных нормализуется. Предварительная дача эндотоксина в течение недели предотвращает уменьшение резистентности. В развитии резистентности определенную роль играет система гипофиз-кора надпочечников, но в поддерживании резистентности следует считаться с другим механизмом.

НОВЫЕ ЭКСПЕРИМЕНТЫ ПО ИССЛЕДОВАНИЮ ИНАКТИВИРУЮЩЕЙ СПОСОБНОСТИ СЛЮНЫ В ОТНОШЕНИИ ПЕНИЦИЛЛИНА

Ц. АДЛЕР-ХРАДЕЦКИ, Б. КЕЛЕНТЕИ и П. АДЛЕР

Кипячение в значительной мере снижает способность слюны инактивировать пенициллин. При добавлении пенициллиназы смешенный со слюной пенициллин можно полностью инактивировать. Однако прокипяченная смесь слюны—буферного раствора — пенициллиназы инактивирует добавленный к слюне пенициллин более сильно, чем прокипяченная смесь слюны и буферного раствора. После встряхивания слюны с дитизоном, растворенном в хлороформе, повышается инактивирующая способность слюны в отношении пенициллина. Если слюну встряхивать лишь с хлороформом, получается такое же повышение ее инактивирующей способности. По сравнению со смешанной слюной инактивирующая способность слюны, полученной при помощи присоса из протока околоушной железы, оказалась меньшей.

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Tabletten

DAS BESTE ATARAKTIKUM

Gegen
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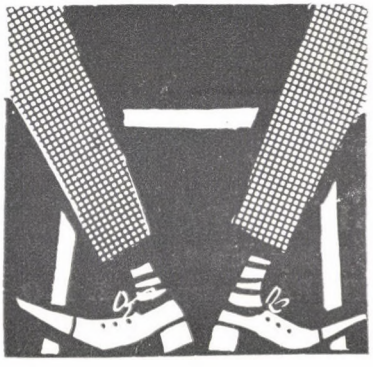

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BEHAVIOUR OF SERUM LIPIDS AND SERUM PROTEINS IN METHYLCELLULOSE- AND ENDOTOXIN-TREATED DOGS AND IN DYSPROTEINAEMIC HUMAN SUBJECTS

By

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(Received June 11, 1965)

The effects of methylcellulose and of *S. typhi* endotoxin have been studied in dogs. Both substances given separately or together have been found to cause monocytosis, endotoxin had moreover an anaemizing effect. Purified endotoxin failed to attenuate pulmonary arteritis and periarterial granulomas as did in earlier experiments, filtered *E. coli* broth cultures. No histological changes due to endotoxin were demonstrable in the various systems. Methylcellulose was intensively stored in the RES of the liver, still more of the spleen and formed spheroid deposits in the renal glomeruli. In the kidneys of animals treated with endotoxin and methylcellulose together, the renal deposits were distinctly reduced. The substances affected the physicochemical structure of the serum proteins and lipoproteins in a similar manner. Combined administration of the two substances was found to attenuate the quantitative changes in the protein and lipoprotein patterns. Fifteen out of 26 patients with dysproteinaemia displayed a characteristic abnormality marked by the appearance of three lipoprotein fractions on the lipoproteinogram, and a blurred precipitation band in the immune electrophoretic pattern.

It has been shown [3, 4] that solutions of methylcellulose (Mc) injected intraperitoneally are stored in, and induce hypertrophy of the RES. Protracted intravenous methylcellulose treatment of dogs leads to proliferative inflammation and perivascular granulomatous changes of, the pulmonary vessels associated with abnormal serum protein and serum lipid patterns, as revealed by paper-electrophoretic and immune-electrophoretic studies [2]. Methylcellulose being a substance foreign to the organism, we intended to study the effect of a macromolecular substance which may occur naturally in the organism, as for instance bacterial endotoxin. The filtrate of a broth culture of *E. coli* is a complex substance containing endotoxin. When dogs were treated with both such a filtrate and methylcellulose, the intravascular and perivascular changes induced by the latter substance were found to be slighter than without endotoxin [5, 6]. The problem arose whether the effect was due to the endotoxin or to other substances present in the broth.

Endotoxins are lipopolysaccharides of bacterial origin [7, 40] which produce a variety of phenomena, such as vasoconstriction and vasodilation, local and general Shwartzman phenomenon with bilateral cortical necrosis of the kidneys [38], stimulation of RES-phagocytosis [26], reduction of the plasma fibrinogen level [39], induction of fibrinolysis [24, 25], endogenous

release of heparin [28, 29, 33]. Cats and dogs are immune to the acute toxic effects of endotoxin [38].

The investigations to be reported in the present paper have been concerned with the serum protein and lipid pattern under the effect of methylcellulose and purified bacterial endotoxin. It has been furthermore investigated whether the influence of purified endotoxin on the proliferative methylcellulose induced changes of the pulmonary arteries is similar to that of filtered *E. coli* broth cultures. The effects of these substances on blood counts as well as on the histological structure of the kidneys, liver and spleen in dogs have also been studied.

Since both substances are capable of inducing hyperplasia of the RES parallel with changes in the serum protein and lipid patterns, we found it of interest to study the blood lipids and proteins also in human disease associated with hyperplasia of the RES or its granulation (reticuloses).

Material and methods

Thirty mongrel dogs of both sexes weighing between 9 and 12 kg and kept on a mixed diet were used. Eight animals received 2.5 ml per kg body weight of a 1 per cent solution of methylcellulose intravenously twice weekly. Six animals were treated intravenously with 10 μg per kg body weight of purified bacterial endotoxin in a sterile saline solution. The endotoxin was obtained from the 0 : 901 strain of *S. typhi*, by extraction according to BOIVIN and MESROBEANU and further purification by alcoholic precipitation. The amount releasing a Shwartzman-reaction was 3 μg. Further 6 dogs received injections of endotoxin in the above doses twice weekly, on the days subsequent to those on which methylcellulose had been given. The 10 controls received 2.5 ml per kg body weight of saline intravenously twice weekly. Samplings were done before and after treatment. Blood was collected in plastic tubes or siliconed glass tubes.

Blood chemistry included 1. serum total proteins by the biuret reaction, 2. protein fractions by paper electrophoresis, 3. protein-bound hexose by the method of STARY et al., 4. serum glutamic oxalacetic transaminase (SGOT) by the method of DUBACH, 5. thymol test according to MACLAGAN, 6. serum total lipids by the method of KUNKEL *et al.*, 7. lipoprotein fractions by paper electrophoresis, 8. serum total cholesterol by the method of ZLATKIS *et al.*, 9. phospholipids by the method of YOUNGBURG and YOUNGBURG, and 10. free fatty acids by the method of DOLE. The value for total protein was calculated by means of a Beckman—Spinco photocell apparatus devised for evaluation of paper electrophoresis.

In addition to these, the red blood cell, white blood cell, platelet and differential counts were estimated.

Results obtained before and after treatment were evaluated by Student's t-test.

At the conclusion of treatment the animals were killed by air embolism. The internal organs (heart, kidneys, lung, spleen, liver) were weighed, their specimens embedded in paraffin and stained with haematoxylin-eosin for histological study. The kidneys of the dogs which had been treated with methylcellulose and methylcellulose + endotoxin, were stained with congo red [22]. The animals did not display any significant circulatory change in the course of the studies. Temperatures reaching peaks of 40°C were noted 2 to 4 hours after the injection.

In a number of animals the lipoproteins were studied by ultracentrifugal analysis for the determination of Gofman's Sf-values, using a Phywe 131 type centrifuge. In one animal of each group treated with methylcellulose, physiological saline, methylcellulose and endotoxin and endotoxin alone, lipid extracts of the serum were subjected to thin-layer chromatography in silicagel medium in a chloroform-methanol, 2 : 1 v/v phase before and after treatment. The layer thickness of the silicagel (G. Merck) was around 0.25 mm. The layer was activated at 110°C during 25 minutes. The chromatogram was evolved in petrolether-ether-acetic acid 90 : 10 : 1 v/v. The front was run to a distance of 15 cm. The spots were developed by phosphomolybdenic acid and subsequent heating to 110°C [32, 35].

In 26 patients with dysproteinaemia, serum proteins and lipids were studied by paper electrophoresis and immune electrophoresis, according to GRABAR and WILLIAMS as modified by SHEIDEGGER. Paper electrophoresis was carried out on Schleicher and Schüll's type 2043 B paper, in a veronal buffer of 0.1 ionic strength at pH 8.6, for 16 hours at 250 V; current intensity per strip was 1.5 to 2.0 mA. For protein electrophoresis 0.02, for lipoprotein electrophoresis 0.05 ml blood serum was used. Proteins were stained with acid fuchsin, lipoproteins with Sudan black (after SWAHN). For immune precipitation, antihuman rabbit serum was used. The system was run on an agar-gel medium.

Results

A) Animal experiments

In the methylcellulose group, the RBC, WBC and platelet counts remained essentially unchanged. The average absolute lymphocyte count showed a decrease, the monocyte count, an increase from 66 to 401. In the endotoxin group RBC showed a reduction by 1,200,000, the WBC a rise from 6600 to 8600; the rise in the monocyte count from 33 to 582 was remarkable. In the group treated with methylcellulose and endotoxin a slight reduction of the RBC (by 500,000) and the lymphocyte count associated with a rise from 22 to 359 of the monocyte count occurred. No significant changes in the blood counts of the control animals were found at the conclusion of treatment. Results for blood counts are presented in Table I. No statistical evaluation has been carried out.

Table I

Blood counts. Mean values before and after treatment

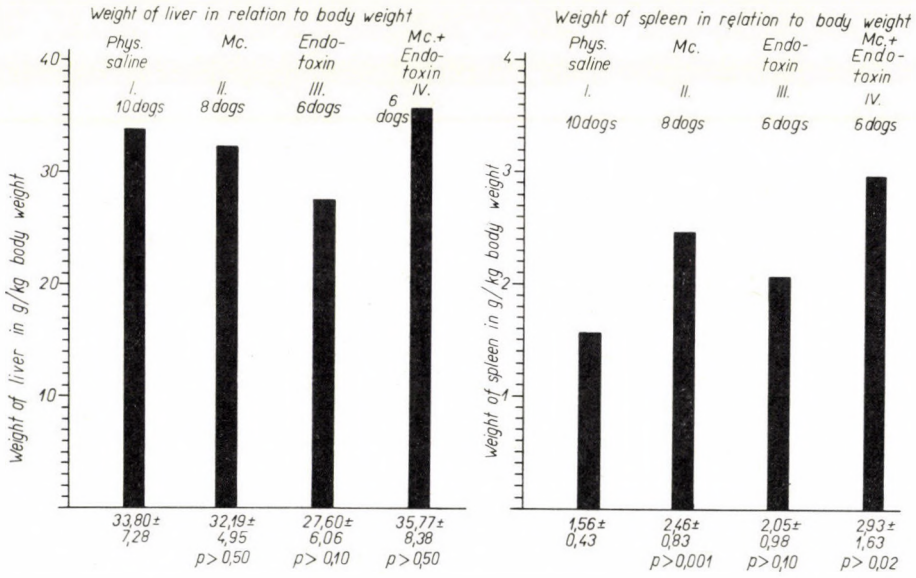
Treatment No. of animals	Before treatment								
	RBC	WBC	Platelet	Differential count (abs. numbers)					
	mill.	thousand		Meta-myel.	Band Form	Segm.	Eo	Ly	Mo
Methylcellulose 8	5.37	7120	195	0	100	4446	108	2400	66
Endotoxin 6	5.7	6600	206	0	99	3861	132	2475	33
Methylcellulose + endotoxin 6	5.4	7500	226	7	120	4455	8	2888	22
Physiological saline 10	5.6	7100	247	7	280	4210	85	2518	0
	After treatment								
Methylcellulose 8	5.42	7300	230	88	110	4742	117	1842	401
Endotoxin 6	4.5	8600	238	54	54	4953	137	2820	582
Methylcellulose + endotoxin 6	4.9	6200	228	93	93	3999	186	1470	359
Physiological saline 10	5.6	7200	260	73	187	4484	144	2168	144

The histological studies showed a distinct storage of methylcellulose in the RES elements, particularly in the liver and spleen, both in animals treated with methylcellulose and in those with methylcellulose and endotoxin. In the spleen there was an increased number of histiocytes with a characteristic foamy protoplasm. Storage was assessed by the relation of liver and spleen

Table II

Statistical evaluation by Student's t-test

Groups II, III, and IV have been correlated with group I.



weight to total body weight. Enlargement of the spleen in relation to the controls was notable in the animals treated with methylcellulose and still more so in those which had received methylcellulose and endotoxin. Liver weight showed no significant change (Table II). Proliferative arteriitis and perivascular granulomas in the lungs described earlier by us [3] were present in every instance. The methylcellulose deposits in the glomerular capillary loops were most conspicuous. Fig. 1 shows a degenerated shrunken glomerulus with methylcellulose deposits. Congo red staining disclosed the presence of methylcellulose globules of various sizes in the majority of the glomerules in all sections. The deposits had considerably damaged the neighbouring loops.

In the animals treated with endotoxin alone, there was no vascular or systemic change. In dogs treated with methylcellulose and endotoxin, pulmonary arteriitis and granulomatous periarteriitis of the same extent was found as in those which had received methylcellulose alone, in opposition to our previous observations with *E. coli* filtrate [5, 6].

In the glomerules of animals treated with methylcellulose and endotoxin haematoxylin-eosin staining failed to reveal methylcellulose deposits and the glomerules were practically unaffected, with the exception of a single dog which showed thickening of Bowman's capsule in a single glomerulus (Fig. 2). Congo red staining revealed methylcellulose storage in the glomerules of animals treated with methylcellulose and endotoxin, nevertheless the picture

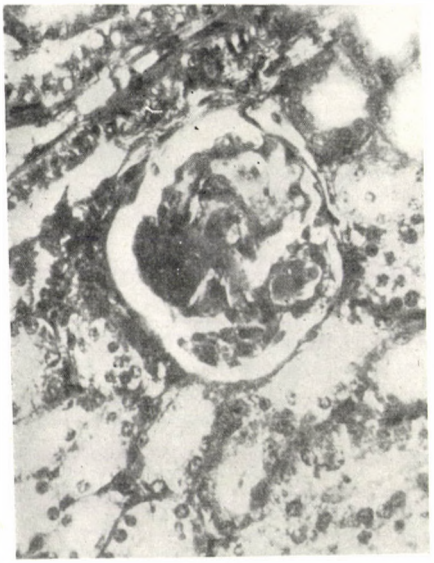


Fig. 1. Histologic appearance of a glomerulus in a dog after treatment with methylcellulose. Spheroid deposits of methylcellulose are seen in the glomerular loops (haematoxylin-eosin, ×300)

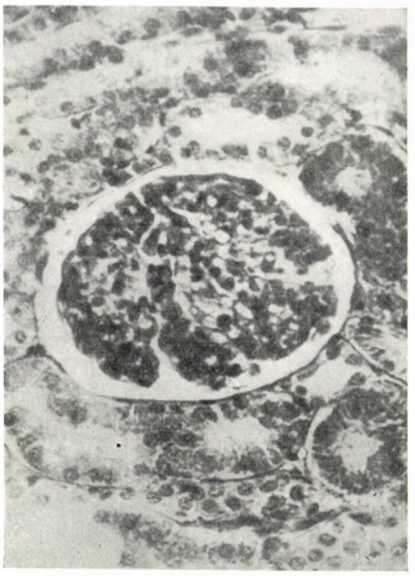


Fig. 2. Glomerulus of a dog after combined administration of methylcellulose and *S. typhi* endotoxin, with practically no structural change and slight methylcellulose deposit (haematoxylin-eosin, ×300)

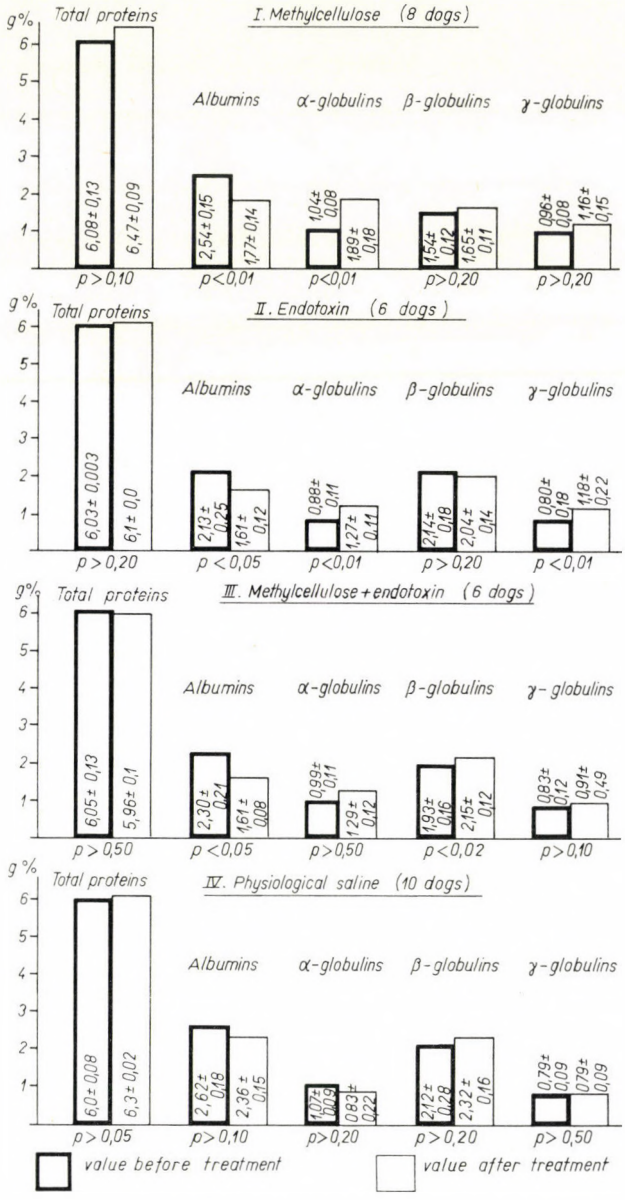
was entirely different from that seen after exclusive dosage of methylcellulose. Most glomerules contained methylcellulose deposits, but the substance occurred in the form of fine granules; large clumps were rare. The organs of control animal No. 10 showed no significant change.

Fig. 3 shows the serum protein and lipoprotein electrophoresis of a normal and of a methylcellulose treated dog [15]. The serum protein pattern after methylcellulose treatment showed intensive beta- and gamma-globulin spots, the serum lipids displayed three lipoprotein bands. In the serum of the other 7 dogs which had received methylcellulose, increase in beta- and gamma-globulins was less marked.

Table III presents a quantitative analysis of serum proteins. Administration of methylcellulose resulted in a significant reduction of albumin, a significant rise of alpha-globulin, and a less significant rise of beta- and gamma-

Table III

Effect of treatments on serum proteins



globulins. Endotoxin reduced albumin significantly, increased alpha-globulin to the same, and gamma-globulin to a somewhat greater extent, than did methylcellulose. On the combined administration of the two substances the reduction of albumin was less marked, while alpha- and gamma-globulins

showed practically no increase, — in brief, a tendency to normalization was recognizable in the protein pattern.

Table IV presents data for serum lipids. For some unknown reason, possibly upon the influence of some stressor substance, total lipid values were considerably raised in all four groups. Methylcellulose reduced the total chol-

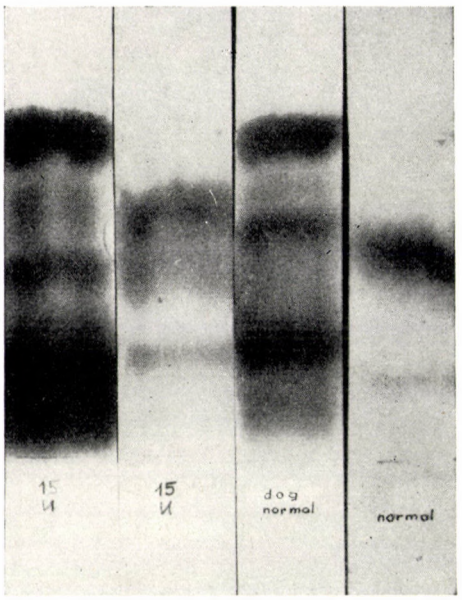


Fig. 3. Paper electrophoresis of serum proteins (15) and lipoproteins (15) of a dog after methylcellulose treatment, and of a normal dog

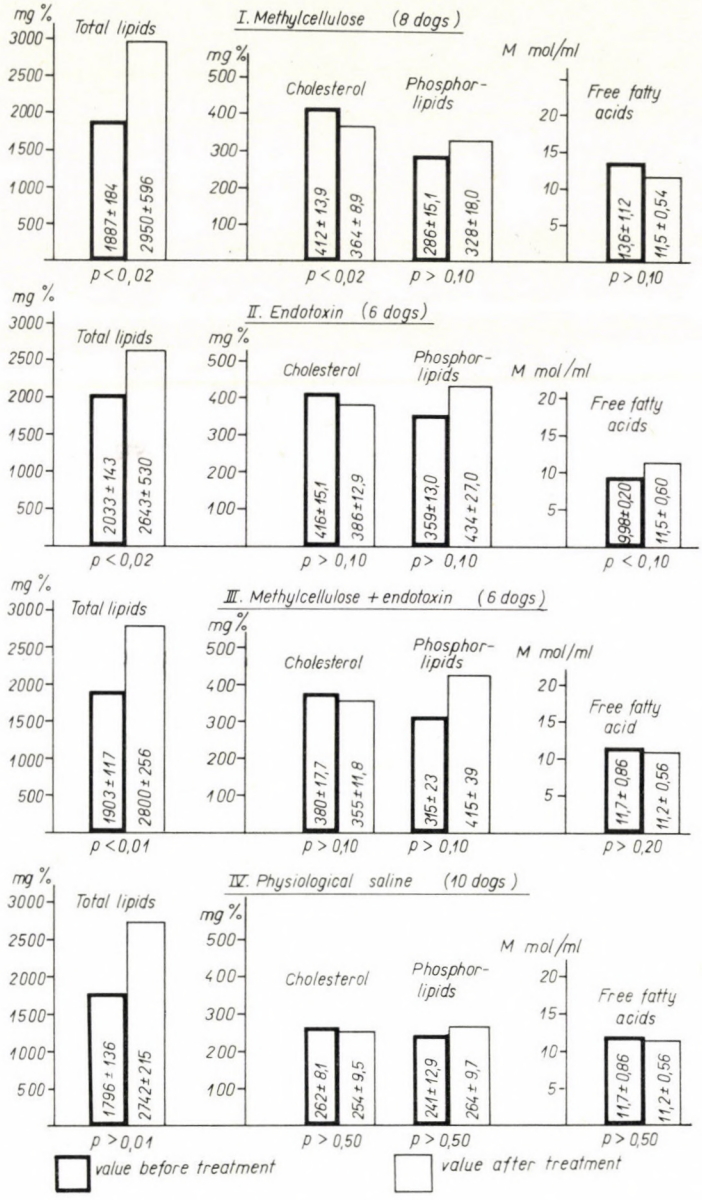
esterol values and left those of the phospholipids and free fatty acids unaffected. No significant change in the serum lipid fractions was induced either by endotoxin alone or by the combined administration of the two substances.

Table V registers the values for protein-bound hexose, SGOT, and the thymol test. Administration of endotoxin alone or in combination with methylcellulose caused a significant reduction of SGOT within the normal range.

As seen in Table VI, in 4 out of 8 animals treated with methylcellulose, in 3 out of 6 animals treated with endotoxin, and in 4 out of 6 animals treated with the two substances, a triad of lipoprotein fractions appeared. Upon the administration of methylcellulose, alpha-lipoproteins showed a significant rise, while beta-lipoproteins a fall. Endotoxin caused no quantitative change in the lipoprotein level and in combination with methylcellulose it reduced the alpha-lipoprotein level and raised that of beta-lipoproteins. Combined administration of the two substances thus had an effect opposite to that of methylcellulose on lipoproteins. Table VI also shows the amounts of $alpha_1$- and $alpha_2$-lipoproteins; these have not been evaluated statistically.

Table IV

Effect of treatment on serum lipids



Ultracentrifugation of the pooled serum of four healthy dogs revealed a component with an Sf of 16.5; in the serum of one dog treated with endotoxin, a component with an Sf of 12; and in the serum of a dog treated with methylcellulose, a component with an Sf of 7.7.

Table V

Effect of treatments on protein-bound hexose-, SGOT- and thymol values

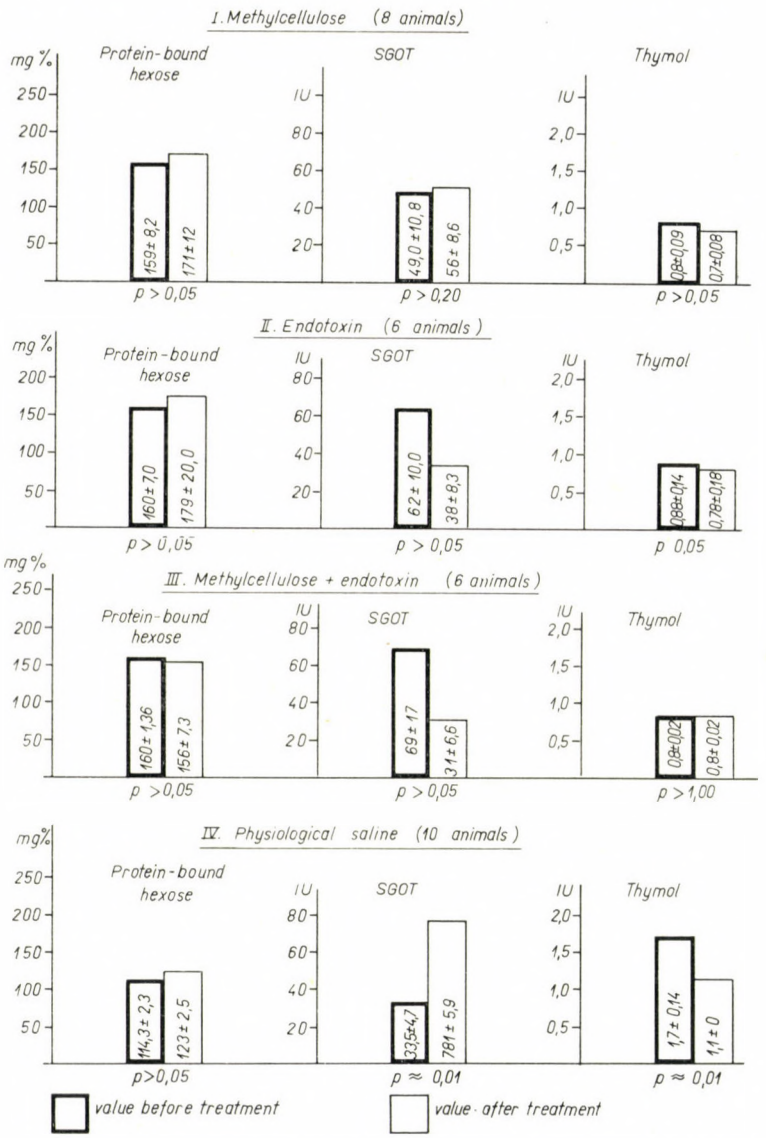
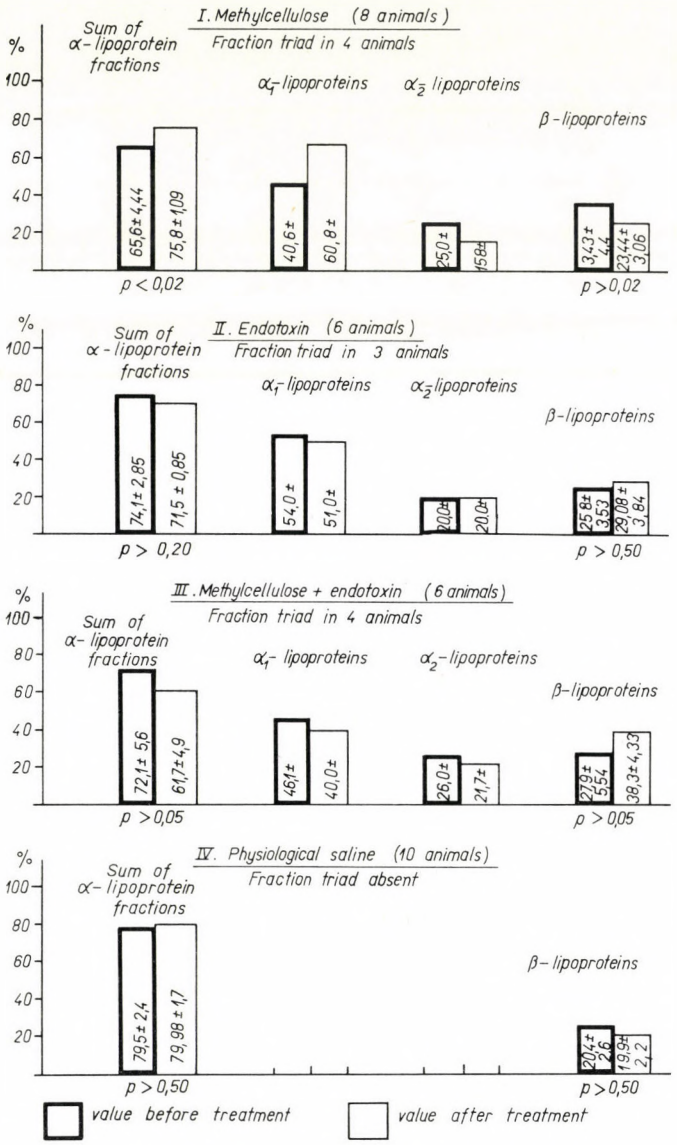


Fig. 4 shows the results of thin-layer chromatography of the lipid extract, of the serum of four animals before (I) and after (II) treatment. The firss animal was treated with methylcellulose, the second with physiological salinet the third with methylcellulose and endotoxin, the fourth with endotoxin alone. The order of the fractions was as follows. 1. Sterol ester (large homogeneous fraction), 2. fatty acid methyl ester (artificial), 3. neutral fat (tri-

Table VI

Effect of treatments on lipoprotein pattern



glyceride), 4. free fatty acids, 5. cholesterol, 6. phosphatides (start). The fatty acid methyl ester fraction was missing from the control rabbit serum as well as from that of the dog obtained at the conclusion of treatment with physiological saline. The spot of sterol ester fraction seemed to be larger after treatment with methylcellulose and endotoxin and with endotoxin alone, that of neutral

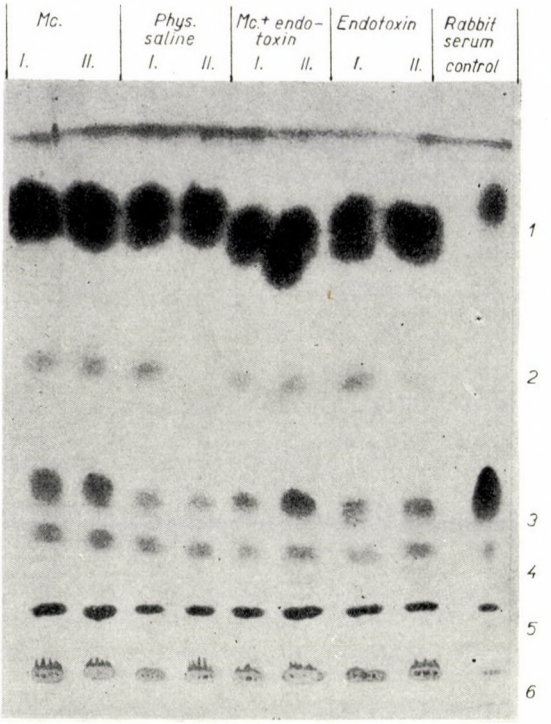


Fig. 4. Thin-layer chromatograms of lipid extracts obtained from sera of dogs before (I) and after (II) treatment with methylcellulose, physiological saline, methylcellulose and endotoxin, and endotoxin, and from normal rabbit serum

1. sterol ester
2. fatty acid methyl ester
3. neutral fats
4. free fatty acids
5. cholesterol
6. phosphatides start

fat and of cholesterol after treatment with methylcellulose and methylcellulose and endotoxin. In the zone between the free fatty acids and cholesterol the appearance of two faint lipid bands has been noted but their nature is unknown. No quantitative analysis has been made in the thin-layer chromatographic studies.

B) Patients with dysproteinaemia

The criterion of dysproteinaemia was in the case of myeloma an increase in abnormal (myelomatous) alpha-, beta- or gamma-globulins, in the case of haemoblastoses or reticuloses, an increase in gamma-globulin or the demonstration of abnormal globulins, including macro- or kryoglobulins, and, in the case of collagen disease, increase of gamma-globulin and the presence of paraproteins. The 26 cases under study included: 1 case of Waldenström's hyper-

globulinaemic purpura confirmed by autopsy, 7 cases of multiple myeloma, 10 cases of haemoblastosis or malignant reticulosis and 8 cases of collagenosis of various types (systemic lupus erythematosus, polyarteriitis nodosa, rheumatoid arthritis). All these conditions are characterized by a primary or secondary (reactive) proliferation of the RES.

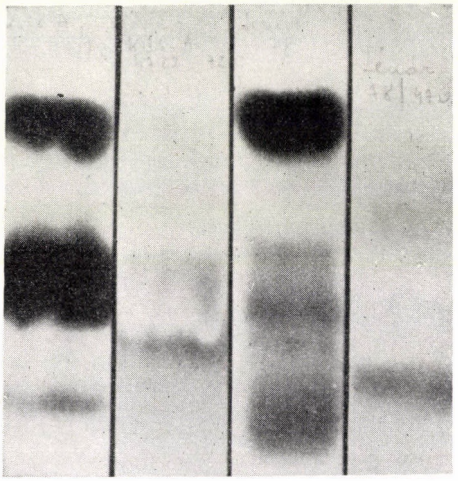


Fig. 5. Paper electrophoresis of serum proteins (Kiss, A. 4721/72) and lipoproteins (Kiss, A. 4722/72) in a patient with multiple myeloma, compared with the proteinogram (Lénár 78 and lipoproteinogram (Lénár 78/4705) of normal human serum

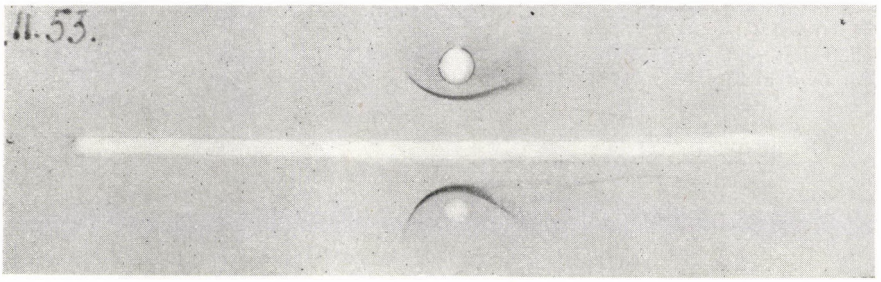


Fig. 6. Immune electrophoretic pattern of lipoproteins in normal human serum. The lipoproteins of slow mobility are characterized by a more intensive but sharp precipitation arc, those of fast mobility by a less intensive precipitation arc

Fig. 5 shows the protein and lipid patterns of a patient with beta-myeloma in comparison with those of a healthy subject. The broad spot of the beta-globulins is marked; the lipoprotein strip displayed in 7 out of 26 cases instead of the usual two bands two intensive lipid bands at the level of the beta-globulin spot and one faint band at that of albumin.

On agar-gel medium the lipoproteins usually dissociate into two groups, one of slower and the other of faster migration, giving with immune sera

a broad precipitation arc (Fig. 6). The abnormal immune electrophoretic lipoproteinogram is marked by a completely disrupted pattern of both groups and with immune serum it gives a broad band instead of a sharp precipitation arc. Such a protein pattern originates in a failure of the precipitate to dissolve in an antigen excess and it is a sign of a physicochemical abnormality of the protein [14]. Though no such phenomenon has been described in connection

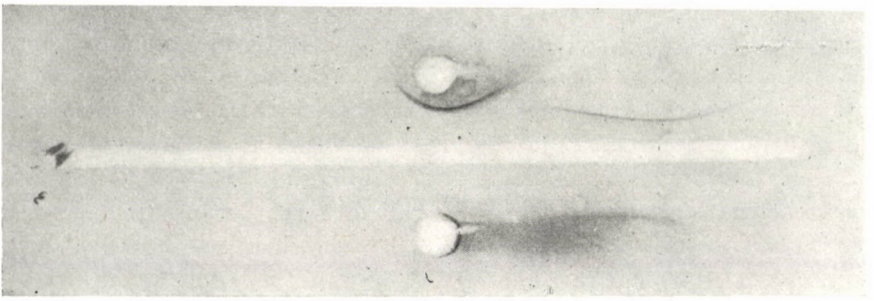


Fig. 7. Immune electrophoretic pattern of serum in a case of Waldenström's hyperglobulinaemic purpura. The lipoproteinogram shows a broad, blurred precipitation band in contrast to the sharp precipitation arcs in normal serum

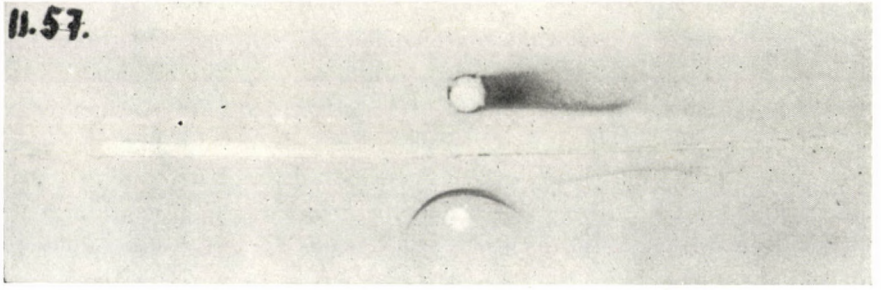


Fig. 8. Immune electrophoretic pattern of serum lipoproteins in a case of myeloma multiplex, with broad, blurred precipitation arcs, in contrast to the sharp precipitation arcs in the normal lipoproteinogram

with lipoproteins, we feel justified in interpreting a broad precipitate with blurred outlines as an abnormal lipoprotein pattern. Fig. 7 shows the immune electrophoretic lipoproteinogram of a patient with Waldenström's hyperglobulinaemic purpura and Fig. 8 that of a patient with myeloma multiplex. The broad blurred precipitation bands of the abnormal sera on one half of the figure are shown against the sharp precipitation arcs of normal sera on its other half. The lipoprotein abnormalities described above were demonstrable in 4 out of 8 cases of myeloma multiplex, in 5 out of 10 cases of haemoblastosis and malignant reticulosis and in 4 out of 8 cases of collagenosis.

Discussion

The observed increase in the monocyte count upon the administration of methylcellulose or of endotoxin, as well as of the two substances together is interpreted as a sign of reticular or rather lymphoreticular hyperactivity. Endotoxin has been found to induce a slight anaemia, presumably by damaging the bone marrow.

The fact that methylcellulose did not cause anaemia is not in contrast with our earlier findings [1], since the substance induced haemolytic anaemia associated with hypersplenism chiefly in rats whereas in dogs anaemia occurs largely as a consequence of protracted treatment with massive doses and even then it results from chronic renal damage rather than from hypersplenism [41].

From the histologic changes, those found in the lungs and kidneys deserve to be dealt with in more detail. Since the attenuating influence of filtered *E. coli* broth cultures on pulmonary arteriitis and granulomas could not be reproduced by purified bacterial endotoxin, it had to be presumed that the stressor effect of the *E. coli* filtrate was far stronger than that of the endotoxin doses applied. The inhibitory effect of *E. coli* broth filtrate upon pulmonary arteriitis and peri-arteriitis has been ascribed by us to cortisone mobilization. The fact that we have been able to inhibit the pulmonary changes by means of cortisone, confirms us in this belief [5, 6].

It is generally believed that macromolecules, thus also methylcellulose, are retained by the glomerules and this would account for the renal damage sustained in this manner [27]. HALL and HALL [16—20] induced renal changes similar to those in glomerulonephritis or glomerulosclerosis by means of methylcellulose and NaCl, and these were aggravated by splenectomy. In earlier experiments on albino rats we were able to increase the glomerular storage of methylcellulose by massive doses of cortisone until massive spheroid deposits reminiscent of those seen in Kimmelstiel—Wilson's syndrome had appeared in the glomerular loops [21, 23]. On the evidence of our present studies, endotoxin in contrast to cortisone, has a strong inhibitory effect on the storage of methylcellulose in the glomerular endothelium. This finding might be connected with the transglomerular passage of macromolecules. Endotoxin, being an activator of the RES, may enhance the phagocyting and storing functions of this system the affinity of which to methylcellulose would also increase in this manner, or, else, it stimulates the transporting activity of the glomerular endothelium. The capacity of endotoxin to reduce the plasma fibrinogen level may also be regarded as a factor inhibiting coalescence and deposition of methylcellulose molecules in the glomerular loops. Finally, a competitive inhibition on the part of endotoxin, barring the entry of methylcellulose molecules into the glomerular endothelium, may also be considered as a possibility.

The changes caused by methylcellulose and endotoxin in the serum protein pattern are very similar. Both macromolecules seem to affect the metabolism and physicochemical properties of the protein components. It is, however, a point of interest that the effect of the two substances when given together, so far from being additive, even seemed to be mitigated. It is well within the possibilities that the two substances act on different subcellular structures which may result in a tendency to normalization of the protein abnormalities.

The changed physicochemical behaviour under the influence of both methylcellulose and endotoxin is reflected by the lipoproteinogram of triple distribution. A similar pattern has been described in human liver disease [11]. On the evidence of ultracentrifugal analysis showing a reduction in the flotation constant (Sf) as well as of the slighter but nonetheless distinct lipid changes revealed by thin-layer chromatography, both polymers must be assumed to cause similar physicochemical modifications in the lipid components.

The serum level of alpha-lipoproteins rises under the effect of methylcellulose at the expense of beta-lipoproteins, while endotoxin has an opposite effect. Under the effect of a combination of the two substances, the level of alpha-lipoproteins remained unchanged while that of beta-lipoproteins rose.

Among the 26 dysproteinaemic patients, a lipoprotein abnormality was found by paper electrophoresis in 7, by immune electrophoresis in 15 cases. This supports our view that anomalies of protein synthesis are associated, at least in a number of cases, with abnormalities of the lipoprotein pattern.

*

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ÜBER DIE WIRKUNG DER VORHOFSYSTOLE AUF DAS SCHLAGVOLUMEN

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Bei 12 Kranken wurden vor und nach Wiederherstellung des Sinusrhythmus mittels Elektrokardioversion die Gestaltung des Zeit- (Herzminuten-)volumens und Schlagvolumens untersucht. Es wurde festgestellt, daß sich nach Normalisierung des Sinusrhythmus beide Werte signifikant erhöhten, der Anstieg des Schlagvolumens betrug durchschnittlich 28%. Bei weiteren 6 Patienten, bei denen die Kardioversion erfolglos blieb und der Sinusrhythmus nicht normalisiert werden konnte, ließ sich die Erhöhung der erwähnten Werte nicht beobachten.

». . .wenn nur mehr die Vorkammer allein schlägt und die Herzspitze mit einer Schere abgeschnitten wird, so fließt aus der Wunde bei jedem Vorkammerschlag Blut«, — schrieb WILLIAM HARVEY 1628. Seitdem wurde die Vorhoffunktion von zahlreichen Verfassern im Tierexperiment [5, 9, 21, 24] und beim Menschen [3, 4, 6, 8, 13] untersucht. HENDERSON [11] behauptete noch, daß infolge der Vorhofkontraktion nur einige Tropfen Blut in die Kammer gelangen. HIRSCHFELDER [12] fand bei experimenteller Mitralstenose, daß für 1/10—1/4 des diastolischen Kammervolumens die Vorhofsystole verantwortlich ist. STRAUB [24] konnte bei Katzen während der Vorhofsystole eine diastolische Volumenvergrößerung der Kammer registrieren. LEWIS [14] meinte, daß die bei Vorhofflimmern beobachtete Zeitvolumverminderung nicht durch das Fehlen der Vorhofsystole, sondern durch die Frequenzsteigerung verursacht wird. Diese Hypothese wurde von SKINNER und Mitarb. [22] widerlegt, da bei ihren Untersuchungen während des bei narkotisierten Hunden herbeigeführten Vorhofflimmerns nebst unveränderter Kammerfrequenz sowohl das Schlag- wie das Zeitvolumen abnahm. Die an Herz- und Lungenpräparaten durchgeführten Untersuchungen von GESELL [5] ergaben, daß im Falle normaler Vorhofsystolen das Zeitvolumen bei jedem beliebigem venösem Druck um 50% höher war. STEWART [23] wies mit Hilfe eines implantierten Herzkatheters, mit dem direkten FICKschen Verfahren während des Vorhofflimmerns eine 20—60%ige Verminderung des Zeitvolumens nach. HAWTHORNE [9] beobachtete bei unanästhesierten Hunden während der Vorhofsystole einen 30—40%-igen Anstieg des enddiastolischen Kammervolumens.

Seit der Verbreitung der Herzkatheterisierung stand die hämodynamische Untersuchung der Vorhoffunktion teils im Zusammenhang mit der

Chinidintherapie des Vorhofflimmerns, teils nach der Entdeckung der Elektrokardioversion im Mittelpunkt des Interesses. Anläßlich der Untersuchungen wurden vornehmlich die zwischen der im Laufe des Vorhofflimmerns bzw. die nach Behebung desselben bestimmten Zeitvolumenunterschiede registriert.

FERRER und Mitarb. [4] sowie WADE und Mitarb. [25] fanden bei rheumatischen Herzkranken, daß bei Vorhofflimmern das durchschnittliche Herzminutenvolumen niedriger ist als bei Sinusrhythmus. HECHT und Mitarb. [10] stellten fest, daß nach Vorhofflimmern der Sinusrhythmus das Herzminutenvolumen nur bei Belastung steigert, im Ruhezustand dagegen nicht. KORY und MENEELY [13] fanden bei 6 von 8 Kranken nach Behebung des Vorhofflimmerns mit Chinidinbehandlung sowohl im Ruhezustand als auch bei Belastung erhöhte Minutenvolumwerte. REEVE-HANSEN und Mitarb. [18] beobachteten bei 9 von 14 mit Chinidin behandelten Patienten nach Wiederherstellung des Sinusrhythmus auch im Ruhezustand eine mehr als 10%ige Erhöhung des Herzminutenvolumens. BROCK und MÜLLER [3] registrierten die Angaben von 20 Kranken im Ruhezustand und unter Belastung, vor und nach Behebung des Sinusrhythmus mit Chinidin. Während des Vorhofflimmerns erhöhte sich das im Ruhezustand gemessene durchschnittliche Zeitvolumen von 5,6 l/min bei Belastung auf 6,8 l/min. Während des Sinusrhythmus betrug der in Ruhezustand registrierte Wert 6,2 l/min, bei Belastung dagegen 8,1 l/min. Das Schlagvolumen machte während des Vorhofflimmerns im Ruhezustand 66 ml und bei Sinusrhythmus 78 ml aus; bei Belastung betrugen diese Werte durchschnittlich 51 bzw. 69 ml. In den 4 Fällen von GILBERT und Mitarb. [6] waren nach Wiederherstellung des Sinusrhythmus sowohl nach Belastung als auch im Ruhezustand bedeutend erhöhte Zeit- und Schlagvolumenwerte zu beobachten. Die, bei an Mitralstenose und anderen rheumatischen Erkrankungen leidenden Patienten durchgeführten Vergleichsuntersuchungen von SELZER [21] wiesen darauf hin, daß bei Vorhofflimmern das Herzminutenvolumen im allgemeinen geringer ist, als bei Sinusrhythmus.

MCINTOSH und Mitarb. [16] faßten sämtliche Literaturangaben über die Kardioversion neulich zusammen. Demnach erhöhte sich nach Wiederherstellung des Sinusrhythmus bei 77% der Kranken das Herzminutenvolumen im Ruhezustand durchschnittlich um 43% und bei 95% der Kranken bei Belastung um 26%. Es sei jedoch erwähnt, daß die einzelnen Verfasser nur über eine geringe Anzahl von Fällen berichteten und daß die Bestimmungen in einem Zeitabstand von 3 Stunden—16 Tagen vorgenommen wurden. Im Gegensatz mit der bisherigen Auffassung stellten GRAETTINGER und Mitarb. [8] fest, daß falls die Kammerfrequenz bei Vorhofflimmern und Sinusrhythmus identisch ist, bleibt auch das Zeitvolumen sowohl im Ruhezustand als auch bei Belastung unverändert. In diesem Zusammenhang vertritt BRAUNWALD [2] die Meinung ,daß die Ergebnisse jener hämodynamischen Untersuchungen, in denen die Wiederherstellung des Sinusrhythmus in den Zwecken der Herzkatheteri-

sierung dienenden Laboratorien vorgenommen wurde, nur mit Vorbehalt bewertet werden dürfen, da, falls es sich um einen submaximalen Streß handelt, mit Hilfe der das Herzminutenvolumen beeinflussenden zahlreichen Faktoren andere Reservmechanismen zur Geltung kommen können, die trotz des Fehlens der entsprechend tempierten Vorhofsystole das Herzminutenvolumen auf normalem Niveau zu halten vermögen. Anhand dieser Erwägungen gelangte BRAUNWALD [2] zur Ansicht, daß die Bedeutung der Vorhofkontraktion bei maximaler Muskelarbeit bewertet werden sollte. Wir wollten diese, den Patienten ungünstige Untersuchungsbedingung vermeiden und arbeiteten eine andere Methode aus.

Untersuchungstechnik

In unserer Klinik wurden im August und September 1964, 25 an Vorhofflimmern leidende, vor Elektrokardioversion stehende Kranken untersucht. In 18 dieser Fälle erhielten wir ein bewertbares Ergebnis (für nicht bewertbar wurden lediglich die Fälle betrachtet, in denen irgendein methodischer Fehler z. B. paravenöse Injektion usw. vorkam). Unter den 18 Kranken waren 14 Frauen und 4 Männer. Zur Kontrolle dienten die 6 Fälle (4 Frauen, 2 Männer), in denen sich der Herzrhythmus nicht normalisierte.

In die rechtsseitige A. femoralis wurde in Fluothan + Succinyl + Atropin-Narkose eine Punktionsnadel eingeführt, die durch einen dünnen Gummischlauch mit dem Hahn des Fraktionskollektors in Verbindung stand. Da die Patienten vor der Elektrokardioversion eine antikoagulante (Dikumarin) Behandlung erhielten, wurde ihnen im Laufe der Untersuchung kein Heparin zugeführt. Nach Verfertigung der EKG-Kurve wurde in die rechte Kubitalvene mit einer Spritze — vom Körpergewicht abhängig — 6—8—10 ml 5%ige Bromsulfalein- (Bromthalein Merck)-lösung rasch injiziert. Nach 1—3 sec. wurde der Fraktionskollektor in Gang gesetzt und der, mit der A. femoralis verbundene Hahn geöffnet. Die Probeentnahme erfolgte mit einer Geschwindigkeit von 90/min. Die Farbendilution ging in der üblichen Weise vor sich. Demnach erhielten die Patienten zwecks Behebung des Vorhofflimmerns mit dem LOWNschen Kardioverter einen Gleichstromschlag. In den Fällen, in denen sich auf der unmittelbar darauffolgend verfertigten EKG-Aufnahme die Normalisierung des Herzrhythmus erkennen ließ, wurde das Minutenvolumen durchschnittlich nach 15 ± 3 min. abermals bestimmt. Nach der zweiten Messung wurde die Nadel aus der A. femoralis entfernt und die Stichwunde mit einem Druckverband versorgt. Die Kranken wachten etwa nach 6—8 Minuten auf. Die Pulsfrequenz wurde mit Hilfe der EKG-Aufnahmen registriert.

Die Ergebnisse sind in Tabelle I dargestellt.

Die in Tabelle I angeführten ziemlich hohen Herzminutenvolumenwerte sind aller Wahrscheinlichkeit nach der Narkose (Atropinwirkung) und der teils konsekutiven Tachykardie zuzuschreiben. Da jedoch im übrigen die gleichen Untersuchungsbedingungen vorlagen, können die Ergebnisse der vor und nach dem Vorhofflimmern verfertigten Untersuchungen ohne weiteres verglichen werden. Nach der Behebung des Vorhofflimmerns war in 10 Fällen der Anstieg und in 2 (Fall 10 und 11) die Verminderung des Herzminutenvolumens zu verzeichnen; das Schlagvolumen stieg in 11 Fällen an und verminderte sich in 1 Fall (Fall 8). Bei dem letzteren Kranken war das Schlagvolumen bereits im Laufe des Vorhofflimmerns in bedeutendem Maße erhöht, woraus folgt, daß der Organismus die nach Normalisierung des Vorhofflimmerns auftretende — ebenfalls maximale — Frequenzsteigerung nicht weiter zu erhöhen vermochte.

Tabelle I

Werte des Minutenvolumens, der Pulsfrequenz und des Schlagvolumens

Nr.	Minutenvolumen, l/min.		Pulsfrequenz, min		Schlagvolumen, ml	
	VF	SR	VF	SR	VF	SR
1.	8,3	10,3	100	110	87	93
2.	7,3	9,5	120	130	60	73
3.	7,0	9,3	130	110	53	84
4.	9,9	10,9	120	120	82	90
5.	9,0	10,7	90	80	100	133
6.	9,1	10,1	80	70	113	144
7.	7,2	11,0	80	80	90	137
8.	11,0	11,9	80	100	137	119
9.	8,3	10,1	110	90	75	112
10.	9,6	8,1	110	80	87	101
11.	10,0	7,9	100	70	100	112
12.	8,7	10,0	115	80	75	125

VF = Vorhofflimmern
SR = Sinusrhythmus

Die statistische Analyse ergab, daß das Herzminutenvolumen nach Wiederherstellung des Sinusrhythmus signifikant höher ist, als während des Vorhofflimmerns; noch ausgeprägter, d. h. stark signifikant ist der Anstieg des Schlagvolumens. Die mit Hilfe des Studentschen t-Tests berechneten Angaben veranschaulicht Tabelle II.

Tabelle II

Statistische Analyse der Veränderungen des Zeit- und Schlagvolumens

	$\bar{x}$	S_x	$t_{[11]}$	p
Zeitvolumen	1,16	0,45	2,5	0,05
Schlagvolumen	22	5,5	4,0	0,01

Da sich die Möglichkeit erhob, daß die vorgefundenen Veränderungen durch den Stromschlag selbst verursacht worden waren, registrierten wir die Gestaltung der Pulsfrequenz und des Schlag- und Zeitvolumens auch in den 6 Fällen, in denen die Elektrokardioversion erfolglos blieb (d. h. die Normalisierung des Vorhofflimmerns nicht gelang). Die diesbezüglichen Daten sind in Tabelle III ersichtlich.

Tabelle III

Werte des Herzminutenvolumens, der Pulsfrequenz und des Schlagvolumens vor und nach Kardioversionsversuch

Nr.	Herzminutenvolumen, l/min		Pulsfrequenz/min		Schlagvolumen ml	
	v	n	v	n	v	n
1.	12,0	11,3	90	110	133	111
2.	10,4	12,0	60	70	173	171
3.	6,1	8,5	60	80	110	100
4.	9,3	9,0	120	120	77	75
5.	13,0	10,3	110	150	118	68
6.	10,1	11,8	70	80	144	147

v = vor Elektrokardioversionsversuch
n = nach Elektrokardioversionsversuch

Die Veränderungen des Zeitvolumens waren nicht charakteristisch, in der Hälfte der Fälle (Fall 2, 3, 6) waren erhöhte, in der anderen Hälfte (Fall 1, 4, 5) verminderte Werte zu verzeichnen. Das Schlagvolumen war entweder vermindert (Fall 1, 3, 5), oder im wesentlichen unverändert (Fall 2, 4, 6); die Verkürzung der diastolischen Füllung spielte nur in 1 dieser Fälle (Fall 5) eine Rolle, in dem sich eine paroxysmale supraventrikuläre Tachykardie entwickelte.

Diese Angaben weisen darauf hin, daß für die Erhöhung der Schlagvolumwerte, im Falle erfolgreicher Kardioversion keineswegs die Prozedur selbst verantwortlich ist.

Anhand der Gestaltung der Gesamtwerte des Zeitvolumens kann festgestellt werden, daß der Anstieg des Schlagvolumens nicht ausschließlich infolge der Frequenzveränderung zustandekommt. In den Fällen 1, 2, 4, 7 war die Frequenzerhöhung geringer als die Erhöhung des Herzminutenvolumens; in den Fällen 3, 5, 6, 9, 12, in denen sich eine Frequenzverminderung verzeichnen ließ, hätte das Herzminutenvolumen — falls zwischen Schlagvolumenerhöhung und Frequenzverminderung eine Proportionalität bestehen würde — entweder unverändert bleiben, oder aber in geringerem Maße abnehmen sollen; demgegenüber wurden auch in diesen Fällen erhöhte Herzminutenvolumenwerte registriert.

In den Fällen 10, 11 verminderten sich sowohl die Frequenz als auch das Herzminutenvolumen, die Quotienten der beiden Werte weisen jedoch darauf hin, daß sich das Zeitvolumen in geringerem Maße verminderte, als das anhand der Frequenzverminderung voraussichtlich war. Falls in diesen Fällen keine Erhöhung des Schlagvolumens zustandegekommen wäre, hätte die Herzminutenvolumenverminderung bedeutender sein sollen. Die zwischen Zeit-

volumen und Frequenzveränderung bestehenden Zusammenhänge haben wir in Tabelle IV zusammengestellt.

Tabelle IV

Veränderungen des Pulsfrequenz — Minutenvolumen-Quotienten

	Herzminutenvolumen: oder Zeitvolumen	Steigerung	Verminderung
Pulsfrequenz			
gesteigert		3	—
unverändert		2	—
vermindert		5	2

Besprechung

Die Bewertung der im Schrifttum bekanntgegebenen Untersuchungen erschwert die Tatsache, daß vor und nach der Wiederherstellung des Sinusrhythmus wesentlich verschiedene Umstände vorlagen. Die beiden Untersuchungsserien fanden in einem Abstand von einigen Tagen statt, die Patienten wurden nicht narkotisiert, was die Herzfunktion zweifellos beeinflußte. Auch die Herzkatheterisierung löst das erste Mal sicherlich eine andere Wirkung aus, als das zweite Mal. In den mit Chinidin behandelten Fällen ist außer den angeführten Faktoren auch der wohlbekannten myokarddepressiven Wirkung des Chinidins eine Bedeutung beizumessen.

Um den störenden Einfluß dieser bekannten und auch der zahlreichen unbekannten Faktoren auszuschalten führten wir unsere Untersuchungen in der bekanntgegebenen Weise durch. Es besteht kein Zweifel, daß für den, zwischen den Ergebnissen der beiden, in 20—30minutiger Narkose verfertigten Bestimmungen vorgefundenen Unterschied entweder die nach Wiederherstellung des Sinusrhythmus zur Geltung kommende Vorhofsystole oder die Wiederherstellungsprozedur selbst verantwortlich war. Die zweite Möglichkeit konnte anhand der bei der Kontrollgruppe (narkotisierte, mit Stromschlag behandelte Kranken) erzielten Resultate ausgeschlossen werden.

Unsere Untersuchungen führten somit zur Feststellung, daß die Vorhoffunktion die Gestaltung des Herzminutenvolumens — vornehmlich durch die Veränderung der Kammerfüllung — beeinflußt. Die bei Sinusrhythmus beobachtete Erhöhung des Schlagvolumens betrug im Verhältnis zu den während des Vorhofflimmerns registrierten Werten durchschnittlich 28%.

In Kenntnis dieser Angaben erhoben sich jedoch die Fragen, ob vom Gesichtspunkt des kranken Herzens die Steigerung des Zeit- und Schlag-

volumens günstig sind bzw. ob das Vorhofflimmern nicht etwa das Endresultat irgendeines Kompensationsmechanismus ist, der bezüglich der Herzfunktion im gegebenen Fall günstigere Verhältnisse sichert?

Die zuverläßliche Beantwortung dieser Fragen wäre nur in dem Fall möglich, wenn die Pathophysiologie der Vorhoffunktion in jeder Hinsicht geklärt wäre.

Die Ergebnisse der Serienuntersuchungen von BRAUNWALD und FRAHM [1], SARNOFF und Mitarb. [19, 20], MCINTOSH und Mitarb. [16] und anderer Verfasser [22, 8] können nicht einheitlich erklärt werden. Über die Frage, ob der Vorhof die Funktion einer Pumpe oder eines Reservoirs versorgt, wird auch heute noch viel diskutiert [7]. BRAUNWALD und FRAHM [1] beobachteten z. B., daß bei Kranken, bei denen der Vorhof »pumpenartig« arbeitete, das Auftreten eines Vorhofflimmerns die bedeutende Verschlechterung des Kreislaufs und den Anstieg des Mitteldrucks im linken Vorhof zur Folge hatte. Die zwischen dieser pathologischen Erscheinung und der Arbeitsdyspnoe sowie dem Herzasthma und dem Lungenödem bestehenden Zusammenhänge sind seit langem bekannt. In diesen Fällen scheint die Wiederherstellung des Sinusrhythmus unbedingt wünschenswert zu sein.

Zwecks Klärung der Fragen, welche Bedeutung den angeführten, statistisch signifikanten Veränderungen bezüglich der Leistungsfähigkeit des Herzens beizumessen ist bzw. in welchen Krankheitsbildern die Hervorrufung derselben sich für günstig erweist, hat unsere Arbeitsgruppe weitere Untersuchungen in Gang gesetzt.

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THE ROLE OF FUNCTIONAL FACTORS AND SPECIAL VASCULAR SPHINCTER APPARATUSES IN MYOCARDIAL INFARCTION

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In the ramifications of the left coronary artery, pad-like cellular structures bulging into the lumen have been demonstrated. They are similar to the structures found by Baráth *et al.* in the blood vessels of the gall bladder in chronic cholecystitis, as well as to the pad-like blood vessel sphincter apparatuses demonstrated in biopsy specimens of the human nasal mucosa. By changes in size due to an uptake of salts and fluids, these sphincter apparatuses can significantly influence flow relations in the terminal vascular system. In the pathogenesis of myocardial infarctions which are not associated with morphological changes of the coronary vessels, alterations in the function of the sphincter apparatuses may play a role. Such alterations may lead to ischaemia, anoxia, infarction accompanied by haemorrhage, and later to fibrosclerotic foci. Functional disturbances may result from abnormal reflexes, autonomic nervous affects, nutritive injuries, stress, and from excessive catecholamine effect. Acute infarction may develop in consequence of electric shock, physical over-exertion, epileptic seizures, burns, etc. The functional disturbances of the sphincter apparatuses may play an important role in the pathogenesis of myocardial infarction.

Among the causes of myocardial infarction, diseases associated with hypertension, as well as the diseases and occlusion of the coronary blood vessels are considered to be the commonest. The connection, however, is not always as close as usually believed. Albertini, Brunck and Papernitzky (1956), analyzing the 2-year autopsy material of all the pathological institutes of Switzerland, found that coronary sclerosis was present in 1208 of 7976 cases. However, while coronary thrombosis occurred in 15 per cent of the 1208 cases, the incidence of coronary infarction amounted to 62 per cent. The divergence between the relatively common myocardial infarction and the much smaller number of coronary thrombosis is remarkable. Pathological experience has, however, shown that myocardial infarction is quite frequent in such cases, in which the coronaries exhibit no morphological alterations. In such cases functional, neurohormonal, nutritive or other factors may be held responsible for the infarction, for example in connection with hypertension or as a result of terminal circulatory failure leading to ischaemia by some other mechanism.

Infarction, necrosis, and fibrosclerosis in various organs

In clinical and anatomical investigations, Baráth *et al.* (1955; 1958; 1959) demonstrated special groups of cells in the minutest blood vessels of many organs such as the gall bladder, the nasal and oral mucosa. These cells, which

have an important role in terminal circulation, form peculiar structures bulging pad-like into the lumen. The pads vary in size; they can take up water and salts or give them off, and as a result they swell or decrease in size. According to some authors, in certain organs these structures contain epitheloid cells with hormonal activity; according to SCHUMACHER (1938) certain groups produce acetylcholine. Some of these cellular conglomerates are in close correlation with the arteriovenous anastomoses (CLARA, 1956), and changes in their size may profoundly influence the terminal circulation. Their dysfunction may be due to neural, humoral or nutritive factors, and may give rise under pathological conditions to hypoxia, haemorrhage, infarction, necrosis and, ultimately, fibrosclerosis of non-inflammatory origin. According to RÉNYI *et al.* (1955) the pathogenesis of certain forms of chronic cholecystitis of non-bacterial origin may be explained in this way.

Apparatuses regulating terminal circulation and sphincter apparatuses in the coronary blood vessels

Those outlined above allow the conclusion that in the genesis of myocardial infarctions occurring without there being morphological changes in the vascular wall, functional causes may produce pathological changes in terminal flow. ZINCK (1939; 1940), WATZKA (1942), CONTI (1945; 1953) already described cellular structures in the ramifications of coronary blood vessels and in the arteriovenous anastomoses. These structures, composed of longitudinal smooth muscle cells and at sites of epitheloid conglomerates, may be considered to regulate blood flow. ZINCK assumed to them a regulating role in coronary blood flow; under pathological conditions they would exert variable and noxious effects. Studying fresh biopsy specimens of human nasal mucosa, BARÁTH *et al.* (1959; 1962) observed constriction and dilation, a sluice-like effect leading to congestion or changes in flow in response to various agents, for example to hypotensive drugs.

Thus, in the case of myocardial infarction in young people, exhibiting no morphological changes in the vascular wall, functional disorders of the vascular sphincter apparatuses may be responsible for the infarction. Myocardial infarction without blood vessel changes have been found following sudden death caused by electric shock, in stress, epilepsy, eclampsia, after physical overstrain, following a large dose of adrenaline, in cases of phaeochromocytoma, crush-syndrome, severe burns, in toxic conditions, etc. SÓS *et al.* (1965) have succeeded in inducing infarction by dietary means in the heart of rats, dogs and cocks; changes in the wall of the coronary vessels, hypertension and vasomotor disturbances also developed.

Terminal regulating apparatuses in the coronary vessels. The anatomical patterns

We have studied specimens of human heart muscle in order to determine whether the pad-like structures were present in the smallest and medium-sized coronary blood vessels of the area supplied by the left anterior coronary artery, where infarction is frequent.

The specimens of heart muscle were fixed in 7 per cent formalin, embedded in paraffin and sections 5 to 8 μ thick were cut. In such thick sections the elastic

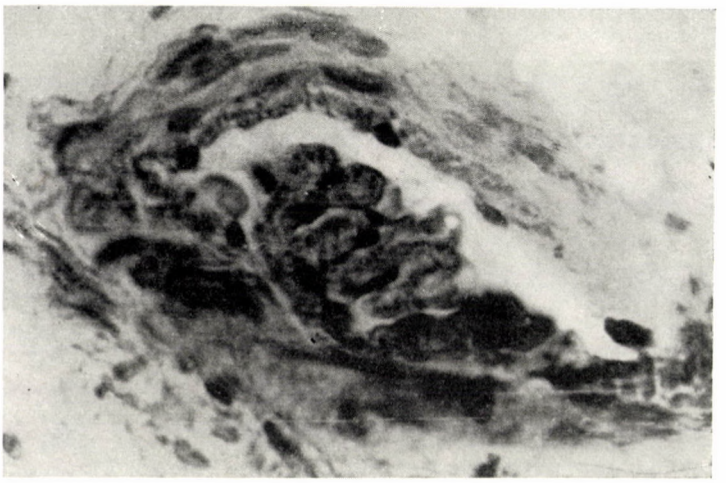

Fig. 1. Sphincter apparatus in the blood vessels of the nasal mucosa. They may show considerable variations in size under the effect of drugs. Biopsy specimen. TARJÁN, BÁLINT and BARÁTH, 1959

fibres appear in a bright colour; for staining, haematoxylin-eosin, resorcin fuchsin and van Gieson's dye were applied. Since the structures regulating terminal circulation must be normal anatomical ones, the cases in which at autopsy or on histological examination arteriosclerosis, or other blood vessel changes were detected, have been neglected.

Identification of the structures in question was made according to the description of CONTI. The structures are usually hemispheric in shape, sitting with their base on the internal elastic membrane, which retains its continuity in every case. The pad immediately under the endothelium is covered by a thin, so-called accessory elastic lamina. There may be one or two fine elastic fibres inside the pad. The pad between the two membranes is composed of longitudinally running muscle fibres which, if they contract, impede blood flow to a great extent. At the same time the circular mural muscles also contract and thereby complete occlusion of the vessel results. The figures show such coronary sphincter apparatuses. Their structure may vary in the various

organs, and even in the same organ. For example, those observed in the nasal mucosa hang into the lumen of the blood vessel, increase in diameter when they contract and occlude thereby the lumen *(Fig 1.)* Some smooth muscle elements stain light, these we call myoepithelial elements; according to some authors these are the ones capable of swelling.

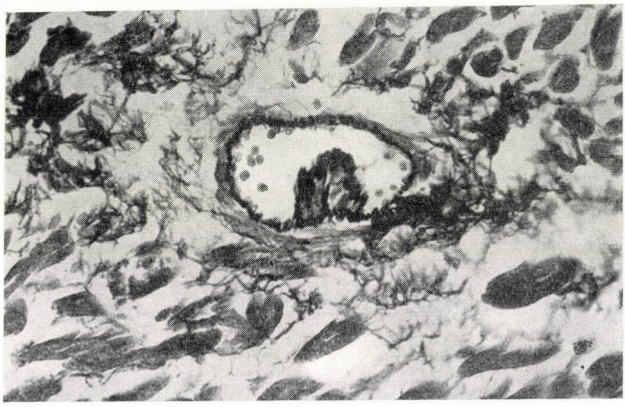

Fig. 2. Pad-like structure in a small artery of the left heart. The 31 years old female patient suffering from mitral stenosis died of heart failure. Haematoxylin-eosin

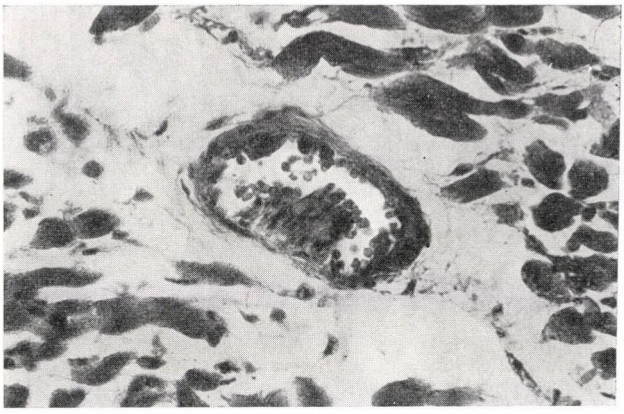

Fig. 3. Pad-like structure in left coronary artery

In *Fig. 2* and *Fig. 3* a sphincter apparatus is visible in a medium-sized artery of the left half of the heart of a female patient aged 31 years. *Fig. 4* shows another layer of the same pad. The elastic fibres appear in dark colour. Histologically, the heart muscle showed no pathological changes.

Fig. 4 shows a pad in a precapillary arteriole of a male aged 66 years who had died of aortic rupture. In the aorta slight traces of arteriosclerosis could only be demonstrated, the cause of the rupture was a necrosis of the

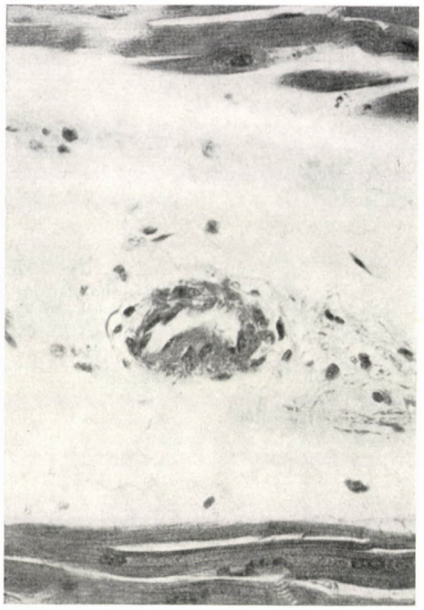

Fig. 4. Sphincter apparatus in a small artery of the left heart, from a male aged 66, who died of aortic rupture

media. Arteriosclerosis was ruled out on the basis that it affects the vascular wall diffusely and always damages the elastic fibres. Proliferation of the intima may occur in chronic inflammatory processes; this, however, extends inward from the internal elastic lamina and is not covered by an accessory elastic membrane. In rheumatic fever and necrotizing angiitis, fibrinoid necrosis is visible in the vascular wall; this was absent in the present cases. Likewise, cellular infiltration has been absent in every case.

Discussion

Pad-like formations on the intima of small blood vessels can be found in many organs. It is certain that they play a regulatory, directing, choking, closing role in terminal local flow. As already been mentioned, ZINCK described such structures and believed them to be more than simple parts of the arterio-venous anastomoses. The pads observed by us are by all certainty normal anatomical formations, as confirmed also by KISS. These contractile structures may play a significant role in the occlusion of vessels free from sclerosis. From an obstruction by the pads might result for example the myocardial infarction occurring during epileptic seizures.

The pad-like formations may play an important, though little known, role in circulatory regulation. Various noxious effects may lead to a hypoxaemic

condition, then to haemorrhagic infarction and necrosis. Such effects may be abnormal reflexes, autonomic nervous effects, stress (SELYE and BAJUSZ), hypoxia caused by catecholamines (RAAB) etc. According to RAAB the neurohumoral disturbance is often due to a lack of exercise, to an absence of the braking action of antiadrenergic factors. In response to adrenaline or to sympathetic stimulation, the sphincter apparatuses show in fact excessive swelling, with narrowing of the lumen (CLARA, 1956; BARÁTH and TEMESRÉKÁSI, 1958). We believe that an abnormal function of the sphincter apparatuses demonstrable in the area supplied by the left coronary artery plays a significant role in the development of myocardial infarctions not accompanied by morphological changes in the coronary blood vessels.

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UNTERSUCHUNGEN ÜBER DAS WESEN DES SENILEN EMPHYSEMS

Von

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Das senile Emphysem sollte als eine physiologische altersbedingte Erscheinung betrachtet werden, die auf dem Altern der elastischen Fasern und einer Vermehrung der argyrophilen und kollagenen Fasern beruht.

Das senile Emphysem ist eine der kennzeichnenden Veränderungen des Alterns beim Menschen, die so häufig vorkommt, daß Bürger die Veränderung »bis zu einem gewissen Grade als physiologisch« bezeichnete. Die Veränderung wurde durch mehrere Autoren, wie Hartung, Giese, Hieronymi usw. eingehend untersucht. Giese behauptete: ». . .man findet nur einen allmählichen Schwund der elastisch-muskulösen, weniger der kollagenen Fasersysteme, der mit einer zunehmenden Erschlaffung des Lungengewebes und einer allmählichen Erweiterung der Azini in das diffuse senile Emphysem übergeht«, und ferner: »An den elastischen Fasern sind histologisch bisher keine Veränderungen nachgewiesen worden, aus denen diese Funktionsminderung abgelesen werden kann.« Diese und ähnliche Behauptungen brachten uns zu dem Entschluß, uns mit dem Zustand der elastischen und kollagenen Fasern der senilen Lungen zu beschäftigen. Wir bearbeiteten 13 aus Männern und 37 aus Frauen stammende, also insgesamt 50 solche Lungen; der jüngste Fall war 65, der älteste 95 Jahre alt. Bei 8 der Fälle war nur Emphysem, bei 31 auch Ödem und hypostatische Pneumonie, bei 8 Lungenembolie und schließlich bei 3 spärliche Krebsmetastasierung zu beobachten. Histologisch wurden die von dem Lungenprozeß bzw. den Metastasen fernliegende Lungengebiete bearbeitet. Mit den Literaturangaben übereinstimmend fanden wir in keinem der Fälle Cor pulmonale. Diese Erscheinung wird durch Parsi und Geissler damit erklärt, daß »da ältere Menschen im allgemeinen keiner erhöhten körperlichen Belastung ausgesetzt sind, sich auch die an das Herz gestellten Anforderungen verringern«.

Die aus den Lungen verfertigten Schnitte wurden mit Hämatoxylin-Eosin, Orzein bzw. nach Van Gieson gefärbt. Schon die ersten Untersuchungen bewiesen, daß sich die einfache Orzeinfärbung zur Information über den Zustand der feineren elastischen Fasern nicht eignet, deswegen haben wir die Schnitte vor dem Färben mit Permanganat behandelt. Da in den üblichen

Schnitten von 5 μ die elastischen Fasern oft als zerbröckelt erschienen, bedienten wir uns mit Gefrierschnitten von 50 μ Dicke, die in Bergamottöl aufgehellt wurden. Der Verlauf der elastischen Fasern war so leicht zu verfolgen. Die argyrophilen Fasern wurden mit der Footschen Imprägnation nachgewiesen. Der Zustand der Fasern wurde durch die Anilin- bzw. Phenol-Reaktion laut ROMHÁNYI beurteilt. Zur Verdauung der elastischen Fasern wurde in unserem

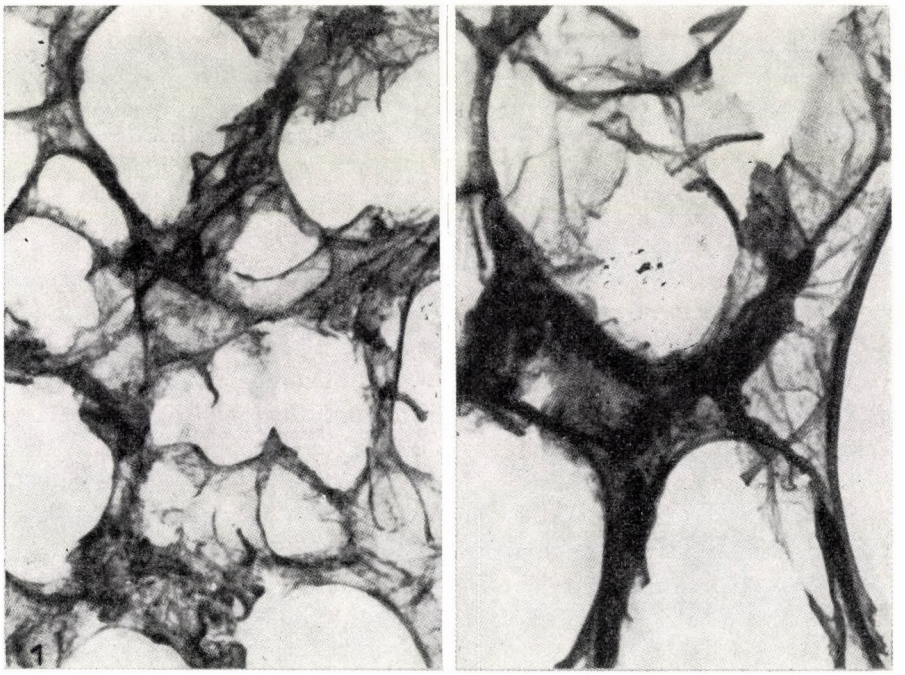

Abb. 1. Lunge, Gefrierschnitt von 50 μ, Orzeinfärbung. Links 13jähriges Kind, rechts 65jährige Frau. 50 ×

Institut isolierte Pankreas-Elastase benutzt. Die Doppelbrechung wurde mit Toluidinblau von verschiedenem pH untersucht. Laut HEIM und CSEH wurde auch die eigene Fluoreszenz der Fasern überprüft.

In den mit Orzein gefärbten 5 μ Schnitten (im weiteren als Dünnschnitte bezeichnet) erwiesen sich die elastischen Fasern als zerbröckelt. Demgegenüber war ihr Verlauf in den 50 μ Gefrierschnitten (im weiteren als Dickschnitt bezeichnet) gut sichtbar *(Abb. 1)*. In dem von einem 7jährigen Mädchen stammenden und mit Anilin behandelten Dünnschnitt waren keine elastische Fasern nachzuweisen, demgegenüber kamen solche mit schwach negativer Doppelbrechung in der Lunge einer 81jährigen Frau zum Vorschein. Im Dickschnitt ist die Anilinreaktion nicht verwertbar, weil die Doppelbrechung der

dünnen Fasern durch jene der dicken kollagenen Bündeln verdeckt wird. In Dickschnitten ist die negative Doppelbrechung mit der Phenolreaktion sowohl in der Alveolarwand wie auch in den Ductus alveolares gut sichtbar.

Die elastischen Fasern älterer Frauen wurden durch Elastase rascher verdaut als diejenigen von den Lungen jüngerer Personen bzw. Embryonen *(Abb. 2)*.

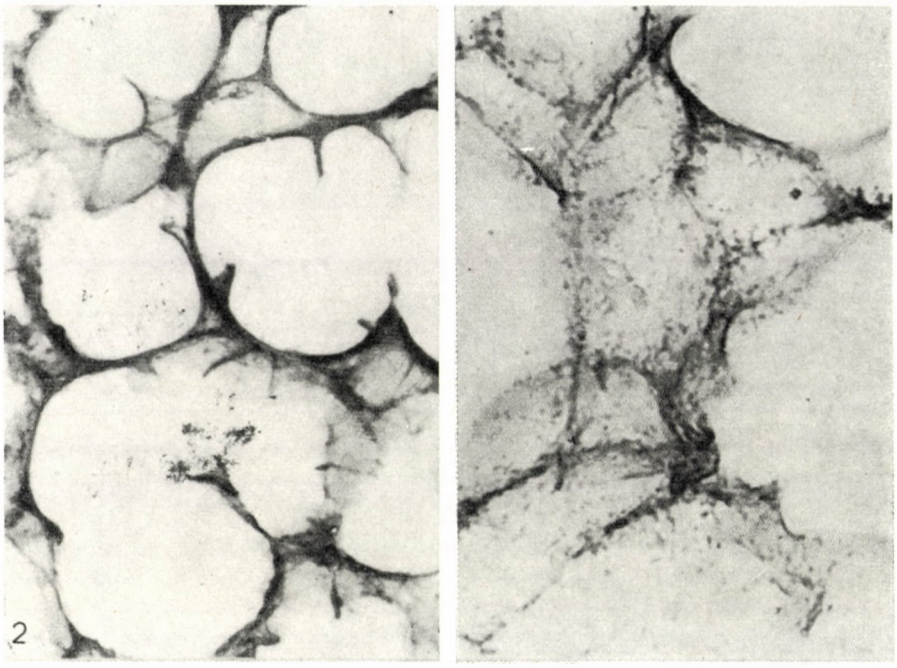

Abb. 2. Links 13jähriges Mädchen, rechts 65jährige Frau. Gefrierschnitt von 50 μ, Orzeinfärbung nach 6stündiger Elastaseverdauung. In der Lunge des jungen Mädchens sind die elastischen Fasern kaum verdaut. 50 ×

Während in fötalen Lungen entweder keine, oder nur wenige Foot-positive Fasern zu beobachten sind, konnten in der zellarmen Lunge von alten Frauen Foot-positive feinere und derbere Bündel in größerer Zahl vorgefunden werden. Beim senilen Emphysem erscheinen im Vergleich zu Säuglingslungen eine große Anzahl argyrophiler Fasern in feinen oder dickeren Bündeln *(Abb. 3)* ; ihre hochgradige Vermehrung führt dann zur Entstehung wellenförmiger oder gerade verlaufender Fasern *(Abb. 4)*. Die Verdickung der Fasern ist besonders im subpleuralen Gebiet auffallend. Hinsichtlich der senilen alveolaren Struktur können wir die Auffassung von HARTUNG vollkommen bestätigen: ». . .es ist also nicht richtig von einer Alveolenerweiterung zu sprechen. Die Alveolen verstreichen vielmehr und gehen in der Wand der

erweiterten Gänge auf, die zu länglichen ungegliederten Schläuchen umgeformt werden.« Die alveolare Struktur kann besonders nach Elastaseverdauung mit Footscher Imprägnation untersucht werden, da in dieser Weise die argyrophilen Fasern und dadurch die alveolare Struktur besser zum Vorschein kommt, als bei der einfachen Imprägnation; die Zahl der Nervenelemente unter den

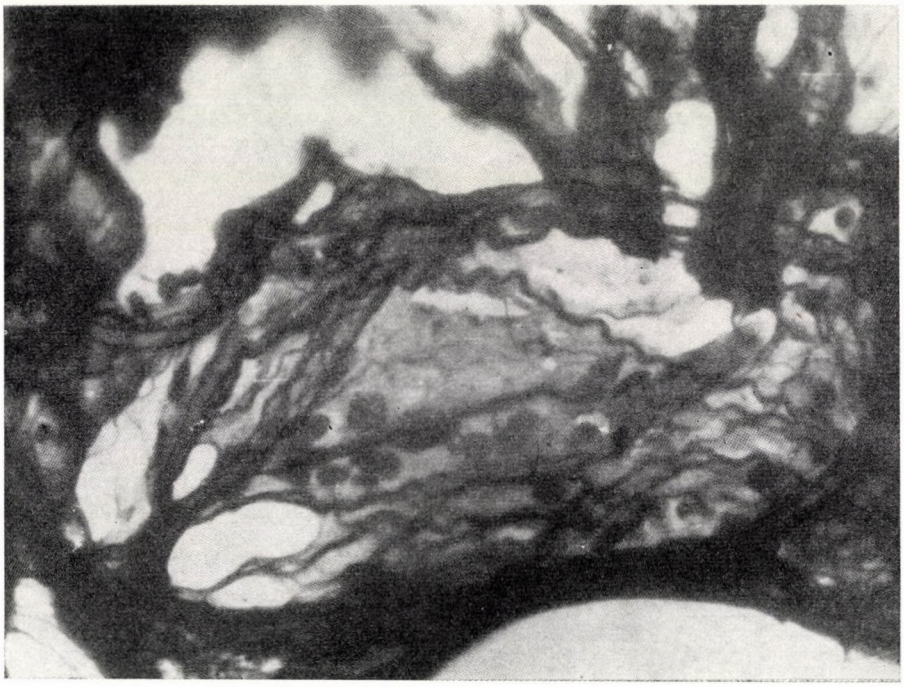

Abb. 3. Dickschnitt (50 μ) aus der Lunge einer Frau von 80 Jahren. Die Fasern bilden ein kompliziertes Netzwerk. Footsche Imprägnation. 320×

imprägnierten Strukturen kann jedoch erst durch weitere Untersuchungen bestimmt werden.

Weiterhin wurden im Polarisationsmikroskop mit Toluidinblau behandelte Dickschnitte von pH-Werten 4,4, 5,6 und 6 untersucht. Bei senilen Lungen (z. B. bei einer 65jährigen Frau) konnten wir bei pH 5,6 einen lebhaften Polarisationseffekt der elastischen Fasern beobachten. In der Alveolarwand alter Personen waren fast überall aus besenartig verzweigten dünnen elastischen Elementen zusammengesetzte elastische Fasern mit lebhafter eigener Fluoreszenz zu beobachten. In der jungen Lunge kommen nur in der Wand der Ductus alveolares und in der Gefäßwand vereinzelte elastische Fasern mit schwacher Fluoreszenz vor (S. *Abb. 5).*

Zusammenfassend dürfte also behauptet werden, daß bei dem senilen Emphysem sich die elastischen Fasern nicht zerbröckeln, sondern ihre Elastizität verlieren, und somit sich verlängern. Dementsprechend wird ihr Verlauf wellenförmig und ihre eigene Fluoreszenz verstärkt sich. Im Gegensatz zu der Säuglingslunge vermehren sich in der alten Lunge die argyrophilen Fasern

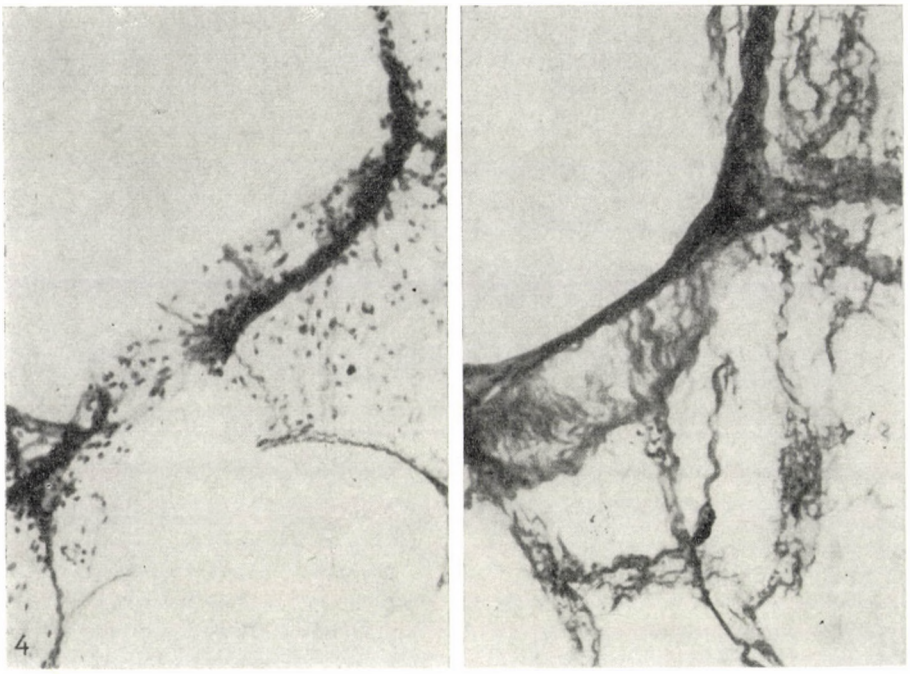

Abb. 4. 65jährige Frau, Gefrierschnitt von 50 μ, Footsche Imprägnation. Links ohne Verdauung, rechts nach 24stündiger Verdauung mit Elastase. Nach Verdauung sind die argyrophilen Fasern gut sichtbar. 128 ×

in der Alveolarwand, die Ductuli alveolares und ihre Wand verdickt sich. Zeichen der Umwandlung in Kollazin, Kollastin oder Elazin waren mit dem GIESEschen Verfahren nicht nachweisbar. Demgemäß dürfte wenigstens bei nicht vorgeschrittenem Emphysem mit Recht auf eine mit dem Altern in Zusammenhang stehende Funktionsabnahme geschlossen werden. Gleichzeitig vermehrt sich das kollagene Stützwerk der Wand der Alveoli und der Ductuli alveolares. Das senile Emphysem soll also als eine physiologische, altersbedingte Erscheinung betrachtet werden, die auf dem Altern der elastischen Fasern und einer Vermehrung der zum Aufrechterhalten der Elastizität der Lunge nicht genügenden argyrophilen und kollagenen Fasern beruht. Unsere morphologischen Untersuchungen sollen durch elektronenmikroskopische

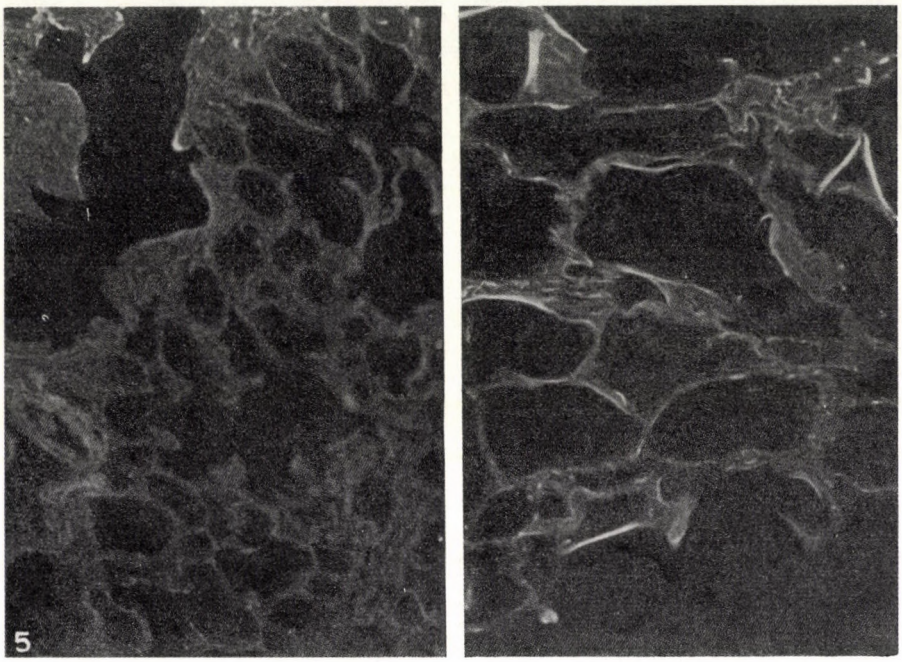

Abb. 5. Links 7jähriges Kind, rechts 81jährige Frau. Eigene Fluoreszenz der elastischen Fasern. Bei dem Kinde sind nur in der Wand der Ductus alveolares vereinzelte elastische Fasern sichtbar, die eine schwache Fluoreszenz aufweisen. 120 ×

Untersuchungen ergänzt werden, die aber eben bei senilen Lungen gewisse Schwierigkeiten bedeuten.

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CHANGES IN AV-CONDUCTION AFTER ATRIAL FIBRILLATION

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Conversion of established atrial fibrillation to sinus rhythm was closely followed by prolonged AV-conduction in 40 per cent of 119 patients. Normal conduction was attained in a fortnight in most cases. The change was rare after paroxysmal fibrillation and never occurred without organic heart disease, neither could it be demonstrated after transitory periods of fibrillation in animal experiments. Inhibition of AV-conduction, which is an essential factor of atrial fibrillation, may transiently persist after conversion to sinus rhythm when the conduction system has suffered a prolonged, though mostly reversible damage by the close sequence of atrial impulses.

In the genesis of atrial fibrillation two factors are prevalent, i.e. the high ratio of auricular impulses and the partial inhibition of AV-conduction (HOLZMANN [2]). According to the traditional view, even the timing of the two factors is very close. In the words of SCHERF [6] "as soon as fibrillation and tachycardia cease, AV-conduction becomes normal again". In contrast to this, we have collected a fairly large number of cases displaying a persistent prolongation of AV-conduction after conversion of atrial fibrillation to sinus rhythm. We could find no pertinent data in the literature, with the exception of KATZ's Electrocardiography [3] where Fig. 421 shows a tracing taken after conversion to sinus rhythm by quinidine, with an AV-interval of 0.21 sec. The first-degree block was ascribed to the drug, the inhibitory effect of which on conduction has been known for more than four decades (COHN and LÉVY [1], LEWIS et al. [4]). Investigations into the mechanism of this phenomenon have not been mede (PICK [5]) as, until recent years, it was rarely possible to rule out the effect of quinidine. Today, on the other hand, defibrillation by direct-current shock has enabled us to differentiate the drug effect from the conversion of atrial fibrillation.

Material and methods

The ECG has been studied in 119 subjects during atrial fibrillation and after conversion to sinus rhythm. As seen in Table I, most patients had rheumatic valvular disease (mitral, with the exception of a single subject), and about 25 per cent had coronary sclerosis. In 10 patients the paroxysms of fibrillation were interspaced by symptom-free intervals; in these patients the closest clinical and instrumental studies have failed to reveal any organic cardiovascular disease.

Before electrical defibrillation had become known, conversion to sinus rhythm had been attained by means of drugs, in the majority with digitalis and/or quinidine. In these cases

Table I

Distribution of cases under study

Diagnosis	No. of cases with established paroxysmal fibrillation	
Rheumatic valvular disease	57	9
Coronary sclerosis	16	17
Hyperthyroidism	2	3
Other heart disease	3	2
No organic change		10
Total	78	41

Table II

AV-conduction after conversion to sinus rhythm in 119 cases of atrial fibrillation

Type of fibrillation	Therapy	AV-conduction	
		prolonged	normal
	1. *Drugs*		
	(a) Digitalis	7	7
	(b) Quinidine	3	2
Established	(c) Digitalis + quinidine	4	8
	(d) Methimazole	1	1
		15	18
	2. *Electrical defibrillation*		
	(a) Digitalis	3	11
	(b) Quinidine	2	3
Established	(3) Digitalis + quinidine	1	4
	(d) No drug	9	12
		15	30
	3.		
	(a) Digitalis	2	20
	(b) Quinidine	1	2
Paroxysmal	(c) Digitalis + quinidine	1	10
	(d) No drug	1	4
		5	36
	Total	35	84

we correlated the prolonged and normal AV-intervals with the doses applied. In 2 cases, conversion to sinus rhythm was successful with Methimazole. Electrical defibrillation was effected by means of a Lown-type cardioverter at 100 to 400 W/sec.

As regards treatment, the patients fall into three groups (Table II). In cases of prolonged fibrillation the current method in the last year was electrical defibrillation. A rapid heart rate was lowered by means of digitalis prior to the procedure. At first, quinidine had also been used but was given up because of unfavourable reports. About 50 per cent of the patients had no previous medication.

In 36 out of 41 cases, fibrillation ceased on the intravenous injection of digitalis, the doses being successively increased until sinus rhythm was restored. In 5 cases fibrillation ceased spontaneously. Conversion to sinus rhythm was generally followed by preventive dosages of 0,20 to 0,60 g quinidine daily.

In 8 mongrel dogs of both sexes and various body weight, atrial fibrillation was induced after thoracotomy by means of a subepicardial injection of 2.5 mg acetylcholine chloride into the region of the sinus node, under chloralose anaesthesia and artificial respiration.

Results

Prolonged AV-conduction, from 0.22 to 0.32 sec. following upon the arrest of established atrial fibrillation appeared with an incidence of 43 per cent among the cases where sinus rhythm had been restored by direct-current shock alone, with an incidence of 25 per cent among those where drugs had been applied prior to the intervention and with an incidence of 38 per cent in the cases where exclusively drugs had been administered (Table III.). In one of the 2 patients with hyperthyroidism where established fibrillation had been controlled by Methimazole, AV-conduction was prolonged, while in the other patient it remained normal.

As shown by Table IV, the incidence of prolonged conduction among the patients who had been treated with digitalis and/or quinidine was lower than in the cases with no previous drug treatment.

Table III

Correlation of therapy with AV-conduction

Fibrillation	Therapy	AV-conduction			
		prolonged		normal	
		No. of cases	%	No. of cases	%
Established	Drugs	14	38	17	62
	Electrical defibrillation	9	43	12	57
	Electrical defibrillation + drugs	6	25	18	75
	Methimazole	1	0	1	0
	Total	30	38	48	62
Paroxysmal	Drugs	4	11	32	89
	Spontaneous	1	20	4	80
	Total	5	12	36	88

In paroxysmal fibrillation, whether its arrest had been spontaneous or induced by drugs, subsequent prolongation of AV-conduction only occurred in 11—20 per cent, respectively (Table III), and in none of the 10 cases where no organic heart disease had been found.

Table IV

Previous administration of digitalis and/or quinidine and AV-conduction after conversion of established atrial fibrillation to sinus rhythm

Drug	AV-conduction			
	prolonged		normal	
	No. of cases	%	No. of cases	%
Digitalis	10	36	18	64
Quinidine and/or digitalis	10	37	17	63
No drug	10	43	13	57
Total	30		48	

Twenty patients with prolonged AV-conduction were followed up for a period of 15 days after conversion to sinus rhythm. In 2 cases the result of therapy was transitory, in another, prolonged AV-conduction persisted during the whole period of observation, whereas in the remaining cases it successively decreased until the normal range was reached, though in a small number of cases it still accounted to more than 0.20 sec. on the 15th day. Quinidine was administered to all patients during the whole period of study (Table V).

Table V

Progress of prolonged AV-conduction under the influence of further quinidine dosage

Length of AV-conduction	Total	Conversion to sinus rhythm	
		Medicamentous	Electrical
Returned to less than 0.20 sec	11	3	8
Decreased (>20 sec.)	6	2	4
Unchanged	1	1	—
Fibrillation returned	2	—	2
Total	20		

Atrial fibrillation induced in 8 dogs in 16 instances ceased spontaneously after a duration of 30 to 45 minutes. AV-conduction in the moment of conversion to sinus rhythm was found normal throughout.

Discussion

Prolongation of AV-conduction subsequent to conversion of auricular fibrillation to sinus rhythm has been observed in 38 per cent of our material. The theory connecting this prolongation with a drug effect has been invalidated by our findings.

There was no evidence to suggest a rheumatic relapse in the cases displaying a prolonged AV-interval, nor was there in the individuals with coronary disease any cause to connect this alteration which spontaneously subsided in a fortnight, with the process.

In contrast to established atrial fibrillation, paroxysmal fibrillation entailed a prolonged AV-conduction in 12 per cent only. This is in agreement with our animal experiments where AV-conduction remained normal during 30 to 45 minute periods of fibrillation. Though this finding might be interpreted as being due to species differences, its close agreement with the clinical observations nevertheless justifies one to correlate the prolongation of AV-conduction with the duration of atrial fibrillation.

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EFFECT OF ANABOLIC HORMONE ON ALBUMIN AND GLOBULIN TURNOVER IN AGED PATIENTS

By

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A study of the effects of anabolic hormone preparations in aged patients revealed that

(i) delta-methyl testosterone and nor-androstenolone phenyl propionate were found to stimulate the daily production of both albumin and globulin.

(ii) According to direct turnover studies with ^{35}S-methionine, the anabolic hormones act in the anabolic phase of protein synthesis.

(iii) Anabolic hormones raise the mass distribution ratio of albumin and globulin in the extravascular pool, a finding indicative of an increased migration towards the tissues.

(iv) Anabolic hormones might induce a shifting of electrophoretic motility of certain protein fractions.

Earlier studies on the effect of the anabolic steroid hormone delta$_1$-methyl-testosterone (DMT) showed that while the serum total-protein level is not affected, albumin turnover is highly stimulated [1]. The study has raised further problems, viz. (i) whether anabolic hormones affected the metabolism of both albumin and globulin and whether the same effect asserted itself with both substances; (ii) whether the anabolic or the catabolic phase of protein metabolism was affected; (iii) whether the distribution of albumin and globulin in the extra- and intravascular pools was affected, and, finally (iv) whether any change due to the anabolic hormone effect might be detected in the serum protein fractions.

Material and methods

The patient material was selected according to the principles specified in the previous paper [1]. The mean age of the 13 patients under consideration was 69 years.

The first experimental series included gamma-globulin turnover studies with ^{131}I-labelled globulin in 3 patients subjected to DMT treatment. Human gamma-globulin (produced by the Institute for Serobacteriological Production and Research "Human", Budapest) was used.

Simultaneous turnover studies were performed to determine the rate of change induced by DMT in albumin and globulin metabolism. This was carried out as follows. Two patients were given ^{131}I-labelled gamma-globulin and other patients ^{51}Cr-labelled albumin intravenously: 50—150 μC, resp., were administered. The albumin was labelled according to GRAY and STERLING [2], the globulin according to VEALL, PEARSON and HANLEY [3]. Immune electrophoresis and determination of the biological half-life confirmed that labelling did not denature the proteins. Lugol's solution, administered two days before and during the whole

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experimental series was used to block the thyroid. Radioactivity in blood samples and collected urines was measured in a thallium-activated well-type NaI crystal *(Gamma* Optical Works, Budapest). The photo peaks of ^{131}I and ^{51}Cr being near to each other, a crystal of good resolution, i.e. not exceeding 8.5 per cent, was used.

Blood samples were collected 10 minutes after injecting the isotope and subsequently every other day for about a fortnight, from the cubital vein on the opposite side. This method allowed to estimate the intravasal and extravasal pools of globulin and albumin, and the amount protein produced daily.

Next, direct turnover studies were performed with ^{35}S-methionine to determine whether the anabolic or the catabolic phase of protein metabolism was affected by the hormone. These studies were also performed by self-control, so that the incorporation rate of ^{35}S-methionine into albumin was determined before and 3 months after DMT treatment. In the course of the examinations performed on two patients the radioactivity of plasma albumin fractionated according to Cohn was measured in infinite thickness in an argon gas flow detector *(Gamma* Optical Works, Budapest).

The values obtained in the first group of patients allowed to compute the extravasal and intravasal albumin and globulin distribution ratio. This was done according to the extrapolation method proposed by STERLING [4], and the equilibrium time method suggested by CAMPBELL *et al.* [5], MATTHEWS [6], and FREEMAN *et al.* [7].

Since the results obtained by the two methods were in perfect agreement, they will be presented according to the equilibrium time method only.

Paper electrophoresis was used for studying the effect on serum protein fractions.

DMT and norandrostenolone phenylpropionate (NPP) were administered by the method laid down in literature and specified in our previous paper [1]. The total dose was 630 mg of DMT and 175 mg of NPP.

Table I

Values of γ-globulins before and after DMT treatment

Patient, age			Plasma volume ml/kg	Intravascular globulin pool g/kg	K (degradation rate) %	Daily globulin production, mg/kg/day
S. J.	before	treatment	42	0.6	6.6	40
56	after		45	0.7	13.8	66
F. A.	before		38	0.87	6.0	35
82	after		40	0.95	11.0	62
V. P.	before		35	1.05	6.3	32
71	after		38	1.2	13.0	78

Results

As seen in Table I, DMT induced a slight increase in the intravascular gamma-globulin pool of all the three patients under consideration. Simultaneously, the daily production of gamma-globulin was also raised, together with the degradation rate (k). These data suggest — as concluded upon in our previous paper [1] — that, in addition to its effect on albumin metabolism, DMT has a similar effect on globulin synthesis.

This promising hypothesis necessitated a study of the DMT effect on the albumin and globulin turnover by a simultaneous estimation of radioactivity i.e. of the tracer administered.

As data in Table II clearly suggest, according to simultaneous indirect turnover studies with ^{131}I-labelled gamma-globulin and ^{51}Cr-labelled albumin,

Table II

Simultaneous values of γ-globulins and albumin before and after DMT treatment

Patient, age			Intravascular pool g/kg		Degradation rate %		Daily protein production, mg/kg/day	
			globulin	albumin	globulin	albumin	globulin	albumin
V. P.	before	treatment	1.05	1.0	6.3	9.9	32.0	42.0
71	after		1.20	1.25	13.0	13.6	78.0	80.0
A. F.	before		0.96	0.95	14.0	9.3	20.0	65.0
70	after		1.58	1.20	16.0	11.0	25.0	95.0

DMT induces a rise in the daily production of both albumin and gamma-globulin. Since the results were obtained in two patients only, it was impossible to obtain fully reliable data on the daily production of albumin and globulin; still, it was evident that the rise was more marked with albumin.

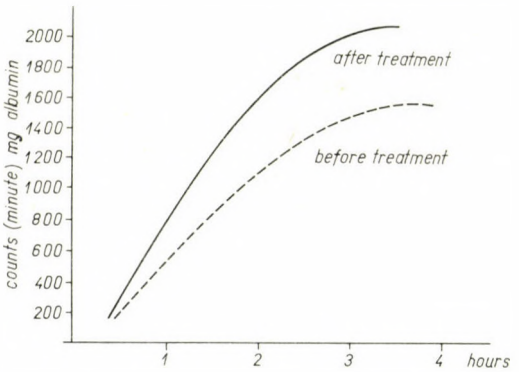

Fig. 1. Incorporation of ^{35}S-methionine into albumin before and after DMT treatment

Results of direct turnover studies with ^{35}S-methionine are shown in Fig. 1, expressed per mg of dry material from the albumin fraction isolated according to Cohn. Incorporation of labelled methionine into the albumin was increased by 30 to 35 per cent on DMT treatment. In both cases the incorporation peaks were reached about three hours after the injection of the amino acid. The half-life of albumin amounted to 24 days before treatment and fell to 18 and 16 days, respectively, afterwards (Fig. 2).

The mass distribution ratio of globulin and albumin — in constant transfer between the intravascular and extravascular pools — is shown in Fig. 3.

Following treatment, this ratio shifted towards the extravascular pool. Accordingly, the EV/IV ratio (r) for albumin increased from 1.3 to 1.6, that for globulin from 1.2 to 1.7. The rise was significant in both cases.

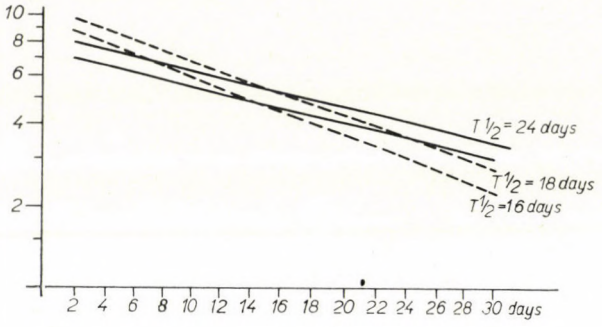

Fig. 2. Semilogarithmic plot showing relationship of specific activity of plasma albumin and interval following the administration of ^{35}S-methionine

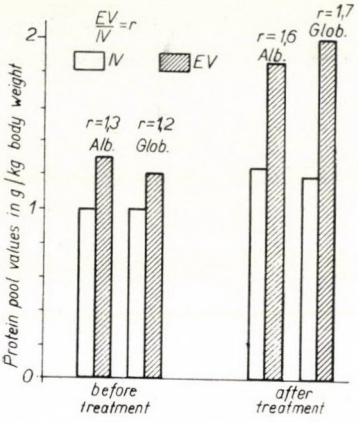

Fig 3. Changes of extravascular/intravascular (EV/IV) mass ratios after treatment

The changes induced by DMT in the serum protein level and in the ratio of the protein fractions are shown in Table III. While the albumin mass decreased significantly in three patients and somewhat less significantly in two others, a significant increase in globulin mass took place in all the patients.

Table III

Serum proteins before and after treatment

Patient, age		Total protein g per 100 ml	Albumin	Globulin fractions %				
				α_1	α_2	β_1	β_2	γ
Sz. I.	before treatment	6.3	39.2	9.6	13.2	8.9	4.4	24.7
76	after	7.4	29.4	10.4	13.0	13.7		33.5
K. F.	before treatment	6.3	36.6	3.9	10.7	12.2	6.4	30.2
72	after	7.0	34.7	13.4	12.2	12.2		27.5
N. J.	before treatment	7.4	50.3	6.7	9.0	8.9	4.5	20.6
73	after	8.4	45.6	11.4	13.4	10.0		19.8
M. J.	before treatment	7.4	59.0	5.0	6.5	11.5		18.0
63	after	7.4	34.1	12.9	11.4	16.8		24.8
J. J.	before treatment	5.5	35.4	8.3	8.7	17.3	5.7	24.6
82	after	6.7	28.0	10.8	16.5	13.6		33.5

In four patients this increase fell to the alpha-1, in three to the alpha-2, and in further three to the gamma globulin fraction.

Upon the effect of DMT, the beta-1 and beta-2 fractions became homogeneous and instead of migrating separately — as before treatment — they continued to migrate together.

Discussion

Our earlier experiments have shown that anabolic hormones stimulate the albumin turnover. In the present study an attempt has been made to elucidate some details of the hormones' action mechanism. It is well known that in addition to a relative decrease in albumin synthesis, an increase of the globulin mass is characteristic of the protein pattern in aged subjects. If the anabolic hormone were affecting the metabolism of albumin only, this would necessarily lead to a normalization of the protein pattern in aged subjects. Accordingly, it seemed interesting to study whether the hormone exerted an effect on the globulin fractions, too. The results suggest that anabolic hormones are stimulating the synthesis of both albumin and globulin. As confirmed by direct turnover studies, anabolic hormones act in the anabolic phase.

If compared with the daily albumin production characteristic of young and healthy individuals, amounting to 145 mg/kg body weight/day [8] and with that of globulin, amounting to about 35 mg/kg body-weight/day [9] our results have shown that the anabolic hormones fail to normalize the A/G ratio. Since these hormones stimulate the production of both albumin and globulin, the A/G ratio in old people fails to become similar to that of young individuals.

The simultaneous determination of albumin and globulin metabolic processes by double isotope labelling has been made difficult by the fact that the photo peaks of ^{51}Cr and ^{131}I are near to each other (0.32 MeV and 0.364 MeV, respectively). However, as proved by ADAMS *et al.* [12, 13] and ÖBRINK and ULFENDAHL [14], double isotope determination might still be performed, provided the detecting crystal has a solving capacity of less than 10 per cent. Some authors failed to obtain reliable results with estimating only the values falling between 0.64 and 0.72 MeV in the energy range of labelled iodine. Since only about 12 per cent of radioactive disintegrations are included in this energy range, the statistical accuracy of the method is greatly reduced.

Our calculations were done according to ADAMS [13].

Labelling of proteins with cationic chromium isotopes is highly efficient and, if carefully done, the tagging procedure does not denature the proteins, as has been confirmed by immunological experiments [16]. Isotope linkage is stable and no appreciable disintegration occurs during several weeks.

Data concerning the action mechanism of anabolic hormones are uniform in emphasizing that upon the effect of these hormones body-weight is increased and the nitrogen balance becomes positive [10, 11]. When determining the extravascular and intravascular pools of albumin and globulin, DMT was found to increase the distribution ratio of both proteins in the extravascular pool. This suggests that one of the essential effects of the anabolic hormones consists in directing proteins towards the tissues. Thus, even if these hormones fail to alter the protein pattern characteristic of advanced age, indirectly, — by increasing the synthesis of albumin and globulin — they still promote their utilization.

In addition to this effect, anabolic hormones interfere even more deeply with protein metabolism. This hypothesis is supported by our finding that NPP changed the migration speed of beta-1 and beta-2 globulins. Considering that NPP did not induce essential changes either in the total protein pattern or in the single protein fractions, while both the serum protein level and the ratio of the single protein fractions were changed, it might be supposed that the effect of the anabolic hormones depended in its chemical structure.

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BLOOD CHEMISTRY STUDIES IN RENAL FAILURE

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1. An oxidimetric method has been worked out for the quantitative determination of organic materials other than protein, carbohydrate and carbamide in blood serum.

2. Plasma sodium, potassium, chloride, bicarbonate, as well as the oxidizable residue and NPN were determined in every case, emphasis being laid on the correlations between potassium, NPN, and oxidizable residue.

3. In chronic nephritis the bicarbonate level is generally reduced, though, occasionally, normal values may be found. The levels of potassium, NPN and the oxidizable residue are generally high. As the oxidizable residue consists for the greatest part of organic acids, it has a large share in the reduction of bicarbonates.

4. In tubular lesions associated with obstructive jaundice or hepatitis, serum potassium remains normal until it comes to extreme oliguria, whereas the levels of NPN and oxidizable residue increase in spite of the polyuria.

5. A similar pattern may be found in tubular lesions of septic nature.

6. There is no close correlation between the values for NPN and oxidizable residue.

7. Potassium-losing renal failure may be accompanied by severe chloracidosis with greatly increased NPN, but normal values for oxidizable residue.

Increased levels of organic acids were demonstrated in uraemic human serum by Csapó and Kerpel-Fronius thirty years ago [1]. While subsequent investigators arrived at the same results by determinations of other electrolytes involved in acid-base balance, i.e. sodium, potassium, bicarbonate, chlorides, in other words by indirect inference from increased levels of residual acid radicals (phosphates, sulphates, organic acids, total proteins), our findings were arrived at by direct alkalimetric titrations. Accurate knowledge of the amount of organic acids requires, in the case of indirect methods, the determination of the individual acid radicals.

Apart from the quantitative interrelation of anorganic ions and increased carbamide levels it is the organic fraction which has the greatest significance in the clinical features of renal failure, as it represents the majority of toxic substances involved in uraemia.

Since for alkalimetric titration 5.0 ml of serum is required, too much to be suited for serial studies, it has been attempted to work out a procedure simpler and yet more accurate than alkalimetry with minimum amounts of serum to be used. We have modified for this purpose our method developed for the determination of the organic non-protein and non-carbohydrate con-

stituents of the CSF, to make it suitable for the quantitative study of the oxidizable residue of serum. We are going to describe our method and the results obtained in cases of renal failure.

Method

Reagents. 2 per cent $ZnSO_4$; 2 per cent $Ba(OH)_2$ (the amount required for the neutralization of 1.0 ml $ZnSO_4$ is determined on each occasion using phenolphthalein as indicator); *n*/10 $KMnO_4$; *n*/10 oxalic acid; 10 per cent NaOH; 4 *n* H_2SO_4; SOMOGYI—SCHAFFER's copper reagent; 2.5 per cent solution of KI containing 2.5 per cent potassium oxalate; 2.5 per cent solution of KI; *n*/100 ceric sulphate; *n*/200 sodium thiosulphate; 1 per cent starch solution.

Deproteinization

To 1.5 ml serum are added 5 ml of a 2 per cent solution of $ZnSO_4$ and the amount of $Ba(OH)_2$ required for its neutralization. The mixture is made up to 30 ml with distilled water, allowed to stand for half an hour, centrifuged and filtered through Whatman No. 1 filter paper.

Hot oxidation

To 5.0 ml of the deproteinized filtrate, 5.0 ml *n*/10 $KMnO_4$ and 2 ml 10 per cent NaOH are added in a 160 × 25 mm test tube, kept in a boiling water bath for 30 minutes, then acidified with 4 *n* H_2SO_4, the permanganate excess reduced with 5.0 ml *n*/10 oxalic acid, and after clearing, titrated with freshly prepared *n*/50 permanganate, against a blank solution containing instead of serum the same amount of distilled water.

Calculation

The difference of ml *n*/50 permanganate required for the titration of the blank and of the serum filtrate multiplied by 80 gives the total oxidation value. From this the amount of permanganate required for the oxidation of sugar is subtracted. Oxidation of each g sugar requires 1.33 ml *n*/10 permanganate. The mg per 100 ml sugar value multiplied by 1.33 must be subtracted from the total oxidation value. This corresponds to the mEq value of the oxidizable residue per 1 litre serum. Carbamide undergoes no oxidation.

Sugar determination by the SOMOGYI—SHAFFER's *method*

SOMOGYI and SCHAFFER's copper reagent is prepared from anhydrous Na_2CO_3; ROCHELLE salt; 10 per cent solution of crystalline $CuSO_4$; $NaHCO_3$; KI; *n*/10 KIO_3. The reagents should be dissolved in the right order. 25 g $NaHCO_3$ and 25 g ROCHELLE salt are dissolved in 500 ml cold distilled water with continuous stirring. When solution is complete, 75 ml 10 per cent $CuSO_4$ is layered under the fluid with gentle stirring, then 1.0 g KI and 20 g $NaHCO_3$ are added. After solution, 50 ml KIO_3 is added, made up to 1 l with distilled water, and passed through a filter paper of good quality.

Technique

4.0 ml deproteinized filtrate (0.2 ml serum) is put into a 160 × 25 mm test tube. 5.0 ml copper reagent is added, then the mixture is kept in a boiling water bath for 15 minutes, cooled in cold water for 4 minutes, and acidified with 1.25 ml 4 *n* H_2SO_4. To this is added 2.0 ml 2.5 per cent KI with 2.5 per cent potassium oxalate and titrated with starch as indicator with *n*/200 thiosulphate, against a blank of serum-free filtrate.

Calculation

The amount of thiosulphate used for blank titration minus the amount used for titration of the filtrate is multiplied by 56.5. This product corresponds to the serum sugar level in mg per 100 ml. If the blood sugar level considerably exceeds 200 mg (as in the case of intravenous drip infusions), then 2.0 ml filtrate is sufficient, in which case the factor is 113.

Blood sugar determinations have also been carried out from the original sera by the method of HAGEDORN and JENSEN. Though this method yields slightly higher figures than that of SOMOGYI and SCHAFFER, we have found it satisfactory.

Cold oxidation

5 ml deproteinized filtrate is put into each of two 160×25 mm glass tubes. With each tube a blank of serum-free material is set up. 2.0 ml *n*/100 ceric sulphate is pipetted into each tube and the mixture is allowed to stand at 21°C. After 10 minutes 0.5 ml 2.5 per cent KI is put into the first tube and the corresponding blank, and titrated with *n*/200 thiosulphate using starch as indicator. This is repeated with the other two tubes which have been allowed to stand for 4 hours at 21°C.

Calculation

The difference between the amount of thiosulphate used for titration of the blank and the amount used for titration of the test material is multiplied by 200; the result gives the amount in mEq of ceric sulphate necessary for the oxidation of 1 litre protein-free serum. At 21°C, oxidation of sugar is negligible.

For the estimation of oxidizable substances in the CSF, the method is modified in that instead of 1.5 ml serum 3.0 ml CSF is used; in the case of hot oxidation the factor is 40, and of cold oxidation, 100. Determination of sugar in CSF requires 5.0 ml filtrate (0.5 ml CSF), the factor being 23 in this case.

Results

First of all the hot and cold oxidation values were established in 30 healthy children and adults. The former ranged between 8 and 13 mEq, the residue oxidizable in cold between 2 and 3 mEq at 10 minutes and 8 and 12 mEq at 4 hours. Cold oxidation values showed a further rise but these figures were not more informative than those obtained at 4 hours.

After establishing the normal range, patients (most of them adults) displaying primary or secondary renal failure were studied. Plasma sodium, potassium, chloride and standard bicarbonate values were determined. The calcium level was invariably accepted as 5.0, the magnesium as 2.0 mEq. The difference between the total kations and the sum of standard bicarbonate and chlorides corresponds to the so-called residual acid radicals which include phosphates, sulphates, organic acids and proteins. The oxidizable residue was estimated in serum, not in plasma. The values obtained with ceric sulphate at 21°C at 10 minutes and 4 hours as well as values for NPN are also tabulated. When serum was left over, phosphate and creatinine were also determined. Creatinine was found to belong to the less oxidizable substances. We were, however, chiefly concerned with potassium, oxidation values, and non-protein nitrogen.

The patients listed in Table I can be divided into three groups: 1. primary renal affections (Cases Nos. 1 to 9); 2. renal failure consecutive to parenchymatous or obstructive jaundice (Cases Nos. 9 to 18); 3. miscellaneous group.

In the first group, sodium and chloride values were not characteristic, and showed no distinct parallelism. Standard bicarbonate, the decrease of which usually points to metabolic acidosis and its rise to metabolic alkalosis, was generally low, though normal values were also encountered. The acid radical residue, normally between 24 and 28 mEq, was invariably high in the renal cases. Though this rise may have been due to an increase in phosphates,

Table I

	Na mEq/l	K mEq/l	Cl mEq/l
1 K. J. Chronic nephritis	132.5	5.0	95
2 S. J. Chronic nephritis	150.0	7.9	122
3 T. J. Chronic nephritis	149.0	5.75	98
4 Sz. P. Chronic nephritis	141.5	3.8	96
5 V. Gy. Chronic nephritis	126.0	6.3	81
6 F. S. Chronic nephritis	132.0	4.2	91
7 O. A. Subacute nephritis	137.0	4.5	100
8 D. J. Lipid nephrosis	143.0	5.4	90
9 G. L. Pyelonephritis	133.0	7.7	100
10 S. M. Obstructive jaundice	151.0	5.5	103
11 P. I. Obstructive jaundice	140.0	3.2	97
12 L. J. Obstructive jaundice	145.0	3.5	67
13 P. S. Obstructive jaundice	133.0	4.0	86.5
14 H. J. Obstructive jaundice	134.0	6.3	83
15 S. I. Infectious hepatitis. Cholaemic nephropathy	139.0	5.0	84
16 K.Zs. Infectious hepatitis. Cholaemic nephropathy	127.0	4.8	88
17 M.Gy. Infectious hepatitis. Cholaemic nephropathy	130.0	4.2	84
18 B. I. Infectious hepatitis. Cholaemic nephropathy	137.0	4.0	101
19 S. D. Malignant oedema. Septicaemia	142.5	3.9	93
20 T. L. Purulent meningitis	141.0	5.0	80
21 K. Gy. Salmonella gastroenteritis	147.0	3.6	102
22 M. P. Leptospirosis	135.0	4.5	93
23 B. É. Peritonitis, Appendicitis	131.0	5.3	84
24 Sz. L. Septic abortion	137.0	7.5	90
25 K. M. Hypernephroma	147.0	4.0	98
26 B. J. Falciparum malaria	140.5	6.5	97
27 L. Ph. Falciparum malaria	139.0	4.9	93

sulphates, and proteins, this alone does not account for the high values found in our cases. A large part must be ascribed to organic acids, an assumption which was supported by earlier alkalimetric studies of CSAPÓ and KERPEL-FRONIUS, as well as by the present investigations which showed high values for the oxidizable residue. The latter obviously comprises organic substances of non-acid character too. Here lies one of the advantages of oxidimetry over alkalimetry. The values of oxidizable residue may call our attention to some possible inaccuracy in the technique of determining the residual acid

Standard bicarbonate mEq/l	Residual acid radicals mEq/l	Hot oxidation mEq/l	Cold oxidation		NPN mg per 100 ml	Phosphate mg per 100 ml	Creatinine mg per 100 ml
			10 min.	4 hours			
			mEq/ml				
7.5	42	267	9.6	24.2	208	—	14.0
6.1	37	277	11.6	20.8	243	6.6	11.6
24.0	40	248	4.0	14.8	216	—	—
15.8	40	211	6.8	15.6	162	5.6	7.6
9.0	49	322	10.0	24.4	310	—	—
20.1	32	159	2.8	11.2	85	5.3	3.6
13.8	35	275	6.8	16.4	212	—	—
21.0	44	352	—	—	260	—	—
10.0	38	268	9.2	17.2	215	—	—
21.0	40	231	5.6	17.6	165	—	—
19.0	34	246	8.0	21.4	172	5.8	10.0
40.5	48	220	3.2	14.0	102	—	—
16.0	41	225	5.8	12.4	256	8.3	6.5
11.0	53	318	11.2	23.6	338	4.8	8.1
18.0	49	262	8.4	18.0	247	2.6	5.8
13.0	38	346	7.6	24.0	214	9.4	—
18.0	39	253	6.0	22.0	182	4.8	—
13.0	35	193	6.0	18.8	203	8.0	6.1
12.3	48	302	—	—	255	—	—
23.0	50	253	12.8	34.2	128	—	—
16.4	39	279	8.4	20.0	257	6.7	11.8
17.0	36	171	3.6	14.0	190	—	—
23.0	36	266	6.4	20.6	102	—	—
10.0	51	274	6.0	14.8	300	10.9	—
25.0	35	232	2.0	14.0	87	—	—
14.3	43	256	5.4	18.2	105	—	—
17.4	41	175	6.0	12.8	241	7.7	—

radicals calculated from the values of sodium, potassium, calcium, magnesium, bicarbonate, and chloride.

In our cases the increase in organic acids was largely responsible for the reduction of standard bicarbonate. Owing to certain correlations of sodium and chlorides, there may occasionally occur normal or even increased bicarbonate levels in spite of an increase in organic acids (Case No. 12). Normal or excessive bicarbonate values in the range of metabolic alkalosis may mask a substantial rise in organic acids which is by no means indifferent to the

organism, this fraction being responsible for the largest part of uraemic toxins. In short, bicarbonate values alone do not reflect the true condition of acid-base balance and may even be misleading.

Our results are in agreement with the fact that in advanced stages of chronic nephritis hyperkalaemia may occur. The high NPN levels were invariably associated with an increase in oxidizable residue. Occasionally, the serum potassium levels may remain within the normal range until oliguria ensues.

Oxidability of protein-free serum was determined by BODA [3] in uraemic animals by the ceric sulphate method at 21°C. Excessive values were found at 10 minutes. This is in agreement with our findings in clinical cases where 2—4 fold values were noted at 10 minutes. The elevation was still distinct at 4 hours though less excessive than found at high temperatures. Presumably, in chronic nephritis the rise in the NPN level is the earliest sign, while retention of the residual substances oxidizable at low and high temperatures follows only later.

The second group comprised cases of jaundice due to hepatitis, biliary obstruction or tumour, complicated with renal failure of tubular origin. The results were in certain respects different from those obtained in chronic nephritis. The sodium values were variable, the chlorides were reduced in nearly every case. The bicarbonate level varied within a wide range with an equal incidence of reduced, normal or excessive high values. With the exception of one case, serum potassium was within normal limits. The acid radical residue was invariably high. When, however, the oxidizable residue is correlated with the NPN in nephritis on the one hand and in cholaemic nephropathy on the other, in some cases of the latter type the rise in the oxidizable residue was higher than in the NPN. What may have been the cause of this discrepancy? While the early stages of renal failure are marked by a retention of normal waste products excessive production of organic materials of acid and non-acid character ensues only at later stages. In nephropathy associated with cholaemia, control of oxidation by the liver is disturbed, this probably accounts for the overproduction of pathologic metabolites in addition to simple retention. Obviously, the NPN values may also be high, depending on the severity of renal damage. The oxidizable residue, or, more exactly, its correlation with the NPN, gives better information about the degree of metabolic disturbances than either potassium and bicarbonate or NPN alone. This correlation shows whether the oxidative or rather the excretory functions are upset in a given case.

The third group is a mixed one. In these cases, except for No. 25 with hypernephroma, tubular insufficiency and nephritis with anuria developed as a consequence of severe infections. The potassium level reached a pathological high value only in one case (No 24, septic abortion). The decrease of bicarbonate is not an indispensable peculiarity of the pathological picture in renal failure. The correlation between the oxidizable residue and the NPN

depends on the fact, whether the disturbance in metabolic processes or in the renal function is prevalent. The potassium would increase to pathological level only in cases with anuria. Cold oxidation values give a less true information of renal failure than those of hot oxidation.

Two cases of tropical malaria are very illustrative (Cases Nos. 26 and 27). In the first (26) one, the blood samples taken in a state of high pyrexia showed a moderate rise in NPN together with excessive high values for oxidizable residue. In the other case, the samples were taken on the sixth day of apyrexia, renal failure consequent to tubular lesion having developed in the meantime. Here, the rise in NPN was prevalent whereas the oxidizable residue exceeded the normal only slightly, its increase having been due to renal failure and not to a toxic disturbance of the oxidative processes.

Table II

	Na mEq/l	K mEq/l	Cl mEq/l	Standard bicarbonate mEq/l	Residual acid radicals mEq/l	Hot oxidation mEq/l	NPN mg per 100 ml
CS. J. *E. coli* septicaemia, liver abscess							
12. XII. 1963	132.0	3.8	92	15.6	35	221	70
13. XII.	132.0	4.0	93	12.7	37	335	126
14. XII.	139.0	4.0	87	21.5	42	332	189
16. XII.	123.0	3.9	81	16.0	37	249	225
18. XII.	127.0	4.7	85	21.0	33	197	207
20. XII.	137.0	3.7	94	26.0	28	157	106
27. XII.	160.0	3.2	116	25.5	28	131	71
29. XII.	161.0	3.0	119	22.0	30	125	72
3. I. 1964	148.0	4.3	114	16.0	29	174	72
8. I.	147.0	4.3	106	15.0	38	278	118

Table II shows the data of a 30-year-old female patient suffering from liver abscess and *E. coli* septicaemia. The urinary output was 1 to 2 litres daily, therefore hyperpotassaemia did not develop, in spite of excessive NPN values. The sodium, chloride, bicarbonate levels were greatly influenced by the intravenous drip. In the initial stage the oxidative disturbance due to bacterial toxins was prevalent, whereas in the second stage, when the oxidative disorder had subsided, a tubular lesion, marked by a persistent elevation of NPN, developed. The oxidizable residue was not increased when the NPN was still 72 mg per 100 ml. The figures give a true reflection of the toxic state prevailing in the first stage, in opposition to the uraemia ensuing a few days later. The patient succumbed to circulatory failure.

Table III

	Na mEq/l	K mEq/l	Ca mEq/l	Standard bicarbonate mEq/l	Residual acid radicals mEq/l	Hot oxidation mEq/l	NPN mg per 100 ml
			S. G. Gastroenteritis				
6. II. 1965.	135.0	2.8	42	56	47	263	77
8. II.	129.0	3.5	62	36	41	191	109
9. II.	132.0	2.0	74	34	33	181	102
10. II.	137.0	3.3	89	30	28	97	70
12. II.	138.0	5.0	104	21	25	123	52
13. II.	134.0	5.2	102	19	25	111	47
15. II.	134.0	4.1	100	21	24	113	47

Table III presents data for a 52-year-old male patient suffering from acute gastroenteritis; he was dehydrated and comatose at admission and displayed exceptionally low values for chloride and a consecutive rise in plasma bicarbonate. While the NPN was slightly elevated, the oxidizable residue showed a considerable increase, a pattern made up of toxic oxidative disturbance and consecutive dehydration. Copious amounts of saline and potassium were administered. Adequate diuresis ensued, chloride and bicarbonate were restored to normal. The successful control of dehydration and the clearing up of the confused mental state were folloved by a rapid fall of the oxidizable residue while NPN values remained slightly increased for a time. The extrarenal disorder led to a tubular failure of moderate severity, still present when the oxidizable residue had returned to normal values.

Table IV presents a case of potassium-losing renal failure in a 16-year-old male with congenital ectopia of the bladder, having made implantation of the

Table IV

	Na mEq/l	K mEq/l	Cl mEq/l	Standard bicarbonate	Residual acid radicals mEq/l	Hot oxidation mEq/l	Cold oxidation 10 min. mEq/l	Cold oxidation 4 hours mEq/l	NPN mg per 100 ml
				P. J. Hypopotassaemia					
9. XI. 1964	142.0	1.9	119	6.2	26	122	3.6	10.8	155
10. XI.	140.0	1.9	107	18.0	24	126	2.0	9.2	111
13. XI.	140.0	2.8	105	20.0	25	84	2.0	8.4	116
17. XI.	142.0	3.7	108	18.0	27	103	1.8	8.4	27

ureters into the sigmoid necessary. He had been admitted earlier on several occasions for hypopotassaemic paralysis, having neglected to take the prescribed maintenance doses of potassium. Then he was again admitted, with total muscular paralysis. Blood sodium was normal, chloride level was excessively high, there was a corresponding reduction of bicarbonate and severe acidosis. This had been found on earlier occasions too. The potassium value was 1.9 mEq. The NPN was high, the residual acid radicals, the oxidizable residue as well as the cold oxidation values were within the normal range. The decrease in bicarbonate was ascribable to the increase in chloride. No retention of organic acids was present. The patient was rehydrated with 5 per cent glucose with high amounts of potassium, with the result that on the next day, though still hypopotassaemic, he sat up in bed and moved his extremities. With the normalization of the chloride value, the bicarbonate level was restored to normal. Though the residual acid radicals, as well as the hot and cold oxidation values remained normal, the NPN level was still 116 mg per 100 ml on the fourth day, when diuresis was abundant, and fell rapidly to normal values.

We have no reason to ascribe the increased NPN values to pyelonephritis, since in that case the oxidizable residue would also have been high, as in patient No. 9 (Table I). Moreover, in any severe case of pyelonephritis with uraemia the tendency of both the clinical state and the NPN to return to normal is much slower.

According to the investigations of KERPEL-FRONIUS *et al.* [4], persistent hypopotassaemia leads to structural changes in the distal tubuli. In the cases under study, chloracidosis of variable severity was presumably an additional factor. In tubular damage of this kind, as opposed to nephropathies of cholaemic or bacteriotoxic origin, retention is confined to anorganic radicals and NPN while excretion of organic acids and other organic compounds remains unaffected.

There seems to be a difference between the tubular lesions according to the causative factor, e.g. potassium loss or bacterial toxins. In our case, tubular failure was soon brought under control by restitution of potassium losses and the patient, while still slightly hyperchloraemic, was discharged on a maintenance dose of 6.0 g potassium bicarbonate and 3.0 g sodium bicarbonate.

Discussion

It is questionable what kind of organic substances take part in the increase of the oxidation values. As we have pointed out in another study, the rise of cold oxidation values at 10 minutes is due to a retention of uric, pyruvic and citric acids, while for the increased hot and cold oxidation values found at 4 hours, in addition to the former substances, retention

of amino-acids, lactic acid and of various unidentified organic substances is responsible.

According to the current view, in uraemia the blood plasma contains increased amounts of organic acids, apart from retained carbamide. This is calculated from the elevation of residual acid radicals in plasma, but has not been ascertained by direct quantitative determinations owing to methodological difficulties. Reliable quantitative determination of organic acids is not possible if the amount of phosphates, sulphates, and proteins, moreover the character of the latter substances, are left out of calculation. On the other hand, indirect methods yield unreliable values, owing to various inherent sources of error. It is the direct method that gives the promptest and most reliable quantitative information about the abnormally increased substances of acid and partly non-acid character other than carbamide. It can be applied to serial studies and helps to check the figures obtained by indirect methods.

Accumulation of oxidizable residue may result either from retention owing to renal failure, as in chronic nephritis or anuria, or from excessive production in consequence of inadequate oxidation due to bacteriotoxic or chemical influence. In certain conditions, e.g. in tubular lesion of bacteriotoxic origin or in the hepatorenal syndrome, both factors may be involved. There is no close correlation between the amount of oxidizable residue and of NPN. In retention caused by renal failure both values are high, but elevation of NPN appears earlier. When, however, excessive production is prevalent, the increase in NPN is slight, whereas the oxidizable residue shows high values. The ratio of NPN and oxidizable residue gives useful information, whether the oxidative or the excretory processes are disturbed. The potassium-losing tubular lesions have the common feature of being associated with high NPN levels while leaving the amount of oxidizable residue unaffected. The tubular lesions thus show a different behaviour according to their origin.

Standard bicarbonate, which depends on a variety of factors, gives no reliable information about the disorder, namely whether excretory or oxidative processes are deranged. Normal or even excessive bicarbonate levels are by no means incompatible with oxidative or excretory failure. This may be best recognized by correlating the values of NPN and of the oxidizable residue.

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ADRENOCORTICAL AND PITUITARY FUNCTION FOLLOWING LONG-TERM PREDNISOLONE TREATMENT

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Adrenocortical and pituitary function has been studied following prolonged prednisolone treatment in 40 cases of rheumatoid arthritis, by the ACTH and metopirone tests. The ability of the adrenal cortex to react was diminished or ceased following intermittent prednisolone administration over 2 ½ years. The normal pituitary response was usually absent after one year of steroid treatment. After a total dose of 3000 mg of prednisolone had been administered, neither the adrenals nor the hypophysis reacted. During protracted steroid treatment during and following stress, prophylactic cortisone administration is necessary for at least half a year.

The glycocorticoids by suppressing ACTH secretion, cause atrophy of the adrenal cortex, mainly in the two inner zones. Consequently, the patient treated for long periods with corticosteroids develops a state of hypadrenalism and when exposed to stress is not capable to mobilize sufficient quantities of glycocorticoids. Thus, in connection with surgery, infections, diarrhoea, etc., circulatory failure may develop [2, 3, 4, 7, etc.]. As a preventive measure, ACTH treatment has been recommended during and after protracted corticoid treatment. In response to the ACTH, the atrophied cortex regains its normal function. In our experience [5] 3 to 4 doses of 40 units of Zn ACTH each suffice to produce this result. This, however, does not yet solve the problem, it being unknown what the reactivity of the pituitary will be following protracted corticoid treatment. This is the essence of the problem because, if exposed to stress, the patient requires an increased ACTH secretion and no matter how adequately the adrenal cortex is functioning, the absence of increased ACTH secretion during stress will lead to life-endangering collapse in the same way as adrenocortical atrophy does. No protection against this is afforded by the administration of ACTH following protracted corticosteroid treatment. This is clearly shown by clinical experience; severe, or even fatal collapse has been described to occur after the administration of ACTH following protracted steroid treatment [8]. We, too, have made similar observations. One of our patients had been taking steroids for 3 years; after this treatment was discontinued, 40 U of Zn ACTH were administered daily for 4 days. Two days after the last ACTH injection vomiting, hypotension and excessive weakness appeared. These symptoms were promptly alleviated by cortisone. HOLUB *et al.* observed the same hyaline changes in the basophilic cells of the

hypophysis as CROOKE found in Cushing's disease. From this it was concluded that the changes of the basophilic cells were induced by the glycocorticoids. According to KYLE *et al.*, when the blood cortisol level is high, ACTH secretion remains insufficient for a while. In such cases if ACTH treatment is discontinued, the adrenal is not capable of maintaining a normal hormone secretion, and adrenocortical activity decreases immediately when ACTH administration is discontinued. HOLUB *et al.*, as well as CARREON *et al.* found that ACTH synthesis and secretion decrease following protracted steroid treatment. According to HOLUB *et al.*, however, the pituitary ACTH-storing capacity decreases only after massive doses of steroid. SAVAGE reported that the ACTH response to metopirone diminishes after one year of steroid treatment already while MELBY found a diminished response to pyrogenic vaccine. On the other hand, AMATRUDA is of the opinion that ACTH secretion remains normal even after protracted steroid treatment. In view of the contradictory data, it seemed interesting to study the problem.

Materials and methods

Pituitary and adrenal cortical function has been studied in 40 patients with rheumatoid arthritis treated for long periods with 5 to 10 mg of prednisolone daily. This treatment was suspended 2 to 5 days before the test. To estimate adrenal function, the 24 hour urine was pooled on three consecutive days. On the 2nd and 3rd days, 40 units of Zn ACTH were injected intramuscularly. Blood samples were taken before and 24 hours after the first ACTH injection. Pituitary ACTH reserve was studied at least one week later. The 24 hour urines were pooled on three consecutive days. On these days the patients ingested from 8 o'clock a. m. till 8 o'clock p. m. 500 mg of metopirone every two hours, i.e. a total dose of 3 g. In 4 cases the tests were repeated after prednisolone treatment had been suspended for 3 to 6 months. The result was considered normal when under the effect of ACTH the urinary ketogenic steroid content increased by at least 9 mg, and, in response to metopirone by at least 8 mg, by NORYMBERSKI's method, and in response to ACTH the plasma hydrocortisone level, as determined by VECSEI's paper chromatographic method, increased by at least 3 μg per 100 ml. The measure of the increase was judged from the highest steroid content of the urine samples obtained on the second and third days. The response was considered positive in the case of an increase in urine or plasma. The reliability of urine collection was controlled by creatinine determination. As a control group, 38 subjects not treated with prednisolone and displaying normal adrenal and pituitary functions were tested with metopirone. In patients sensitive to ACTH the metopirone test alone was carried out. To study ACTH sensitivity, 1 : 1000 and 1 : 10 000 dilutions of the drug were injected intradermally; in positive cases, erythema appeared in 2 to 4 hours.

Results

Table I contains the data for the prednisolone treated patients, in the order of the treatment's duration.

ACTH test. Twenty patients had been taking prednisolone for 2 to 30 months; among these, 17 showed a satisfactory response to ACTH of the adrenal cortex. Twenty patients had been treated with steroid for more than 30 months, of these in response to ACTH the urinary ketogenic steroid output increased in 2 cases and the plasma hydrocortisone increased in 3 cases. In 7 patients the test could not be carried out in view of their sensitivity to ACTH.

Table I

No.	Sex	Age	Duration of treatment (months)	Prednisolone		Response after ACTH		Response after metopirone urine ketog. st. mg/24h	Result		
				total mg	daily dose mg	urine ketog. st. mg/24h	plasma cortisol µg per 100 ml		after ACTH		After metopirone urine
									urine	plasma	
1.	♀	65	2	500	8.3			5.5—57.8			+
2.	♀	65	3	120	1.3	1.9— 4.5	14.0—20.8	16.1— 5.0	⌀	+	⌀
3.	♀	57	4	240	2.0	3.7— 3.9	37.4—13.5	5.1—11.6	⌀	⌀	⌀
4.	♀	57	5	500	3.3	3.2—29.9	13.3—18.0	5.9—14.1	+	+	+
5.	♀	30	7	900	4.3	20.3—31.3	2.2— 3.5	11.5—22.3	+	⌀	+
6.	♀	59	10	1 800	6.0			5.5—22.5			+
7.	♀	74	11	270	0.8	2.7—15.8	17.8— 6.9	5.8—22.3	+	⌀	+
8.	♀	42	12	800	2.2	4.3—45.0	3.0—12.0	3.4—25.0	+	+	+
9.	♀	51	14	1 200	2.9	14.2— 7.5	8.7—11.4	9.7—16.9	⌀	⌀	⌀
10.	♀	43	16	850	2.0	8.9—27.2	12.0—18.7	13.6—14.8	+	+	⌀
11.	♂	59	17	1 100	2.1	6.2—20.1		6.6— 9.9	+		⌀
12.	♀	36	17	2 400	4.7	5.0— 6.0	9.4—17.0	14.3—11.3	⌀	+	⌀
13.	♂	34	18	400	0.7	38.4—19.1	9.2— 1.6	1.9— 6.4	⌀	⌀	⌀
14.	♀	52	21	2 600	4.1	28.6—83.5	2.2—15.7	29.2—21.7	+	+	⌀
15.	♀	78	23	1 600	2.3			21.8—12.3			⌀
16.	♂	53	25	1 600	2.1	6.5—61.3	9.0—12.0	7.0—16.9	+	+	⌀
17.	♀	64	26	1 000	1.3	19.7—28.1	10.0—10.0	10.6—21.5	+	⌀	+
18.	♀	53	27	2 100	2.6	5.7—15.8	17.5—16.0	11.2— 5.6	+	⌀	⌀
19.	♀	71	27	2 800	3.5	10.0—19.0	8.1— 9.2	6.6—10.3	+	⌀	⌀
20.	♀	62	30	1 900	2.1	9.0—44.6	27.2—33.3	16.4—20.8	+	+	⌀
21.	♀	74	33	800	0.8	2.0— 2.9	15.7— 2.8	13.4—14.1	⌀	⌀	⌀
22.	♀	71	33	1 500	1.5	11.3—25.0	25.0—12.2	4.7—12.1	+	⌀	⌀
23.	♀	75	33	3 000	3.0	5.0— 8.3	12.7—10.7	3.4— 2.9	⌀	⌀	⌀
24.	♀	80	34	2 800	2.7	14.0—12.5	17.5—38.0	11.2—10.9	⌀	+	⌀
25.	♀	68	35	800	0,8	3.0— 3.3	14.1—21.0	4.5— 4.6	⌀	+	⌀
26.	♀	48	35	1 400	1.3	9.9— 8.8	14.6—11.7	16.3— 8.0	⌀	⌀	⌀
27.	♀	67	36	4 000	3.9	10.8—19.7	15.0— 8.5	4.9— 8.0	⌀	⌀	⌀
28.	♀	51	36	1 200	1.1	8.1— 5.9	17.6—25.0	6.0—12.9	⌀	+	⌀
29.	♂	70	39	3 800	3.2	12.3—10.0	10.8— 7.2	25.2— 9.8	⌀	⌀	⌀
30.	♀	59	45	10 000	7.4			7.0— 5.5			⌀
31.	♀	45	45	3 500	2.6			6.6— 8.8			⌀
32.	♀	43	46	10 000	7.2	6.6—12.0	15.0— 8.5	12.5—11.8	⌀	⌀	⌀
33.	♀	56	46	11 000	8.0			5.4—12.5			⌀
34.	♀	56	46	2 200	1.6	7.9—16.1	7.5— 3.5	6.9—22.3	+	⌀	+
35.	♀	71	55	3 000	1.8			4.4— 4.0			⌀
36.	♀	28	59	7 000	4.0			4.8 — 5.0			⌀
37.	♀	58	59	9 000	5.1	9.3— 9.3	15.7—16.5	7.8— 7.9	⌀	⌀	⌀
38.	♀	39	56	4 000	2.0			2.1— 4.7			⌀
39.	♀	48	67	2 200	1.1			1.8— 7.9			⌀
40.	♂	50	68	12 000	6.6	3.2— 3.8	10.1—10.8	2.6— 3.6	⌀	⌀	⌀

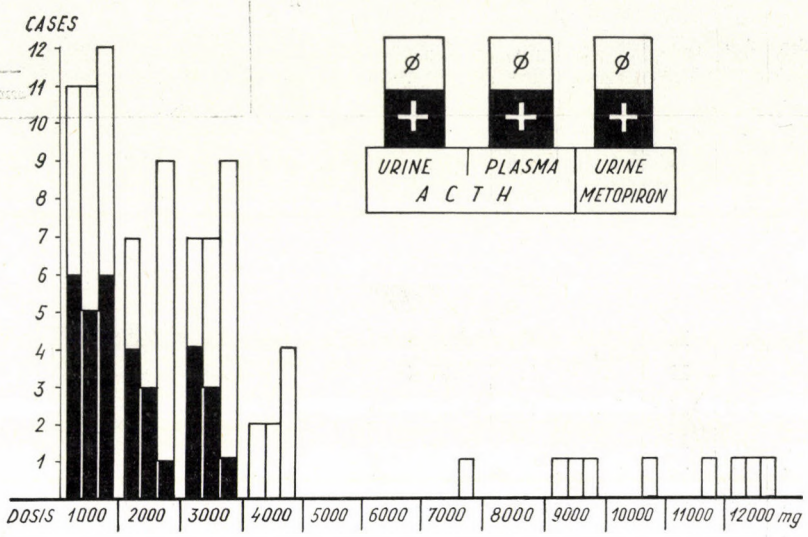

Fig. 1. Effect of the total dose of prednisolone on adrenocortical and pituitary hormone production

Table II

Control cases

Metopirone test

Urinary ketogenic steroids mg/24h

1.	14.0—28.2	+	20.	15.6—42.5	+
2.	12.7—21.2	+	21.	17.1—35.9	+
3.	15.8—59.6	+	22.	9.2—27.2	+
4.	7.1—13.2	∅	23.	12.0—27.4	+
5.	22.4—31.5	+	24.	10.6—33.4	+
6.	16.4—36.1	+	25.	9.8—15.4	∅
7.	18.3—33.8	+	26.	6.0—63.8	+
8.	30.7—62.4	+	27.	5.5—39.2	+
9.	6.3—18.6	+	28.	5.6—19.0	+
10.	16.0—22.8	∅	29.	14.8—12.1	∅
11.	9.5—37.6	+	30.	5.1—14.7	+
12.	10.8—34.4	+	31.	5.2—13.2	+
13.	27.2—52.4	+	32.	14.5—27.3	+
14.	5.2—28.4	+	33.	10.3—25.6	+
15.	11.1—46.5	+	34.	7.9—16.6	+
16.	15.8—23.8	+	35.	9.3—23.2	+
17.	7.7—23.3	+	36.	9.9—37.7	+
18.	9.7—24.5	+	37.	10.1—18.2	+
19.	14.0—28.0	+	38.	15.2—34.4	+

This seems to indicate that after 2 ½ years of treatment with 5 to 10 mg of prednisolone daily the reactivity of the adrenal cortex is abolished, even if administration has been intermittent as was usual with these patients. This is in agreement with our earlier results [5] in that the adrenal failed to respond to ACTH after 1 ½ years of corticoid treatment. In those cases corticoids were administered continuously and the doses were higher, and that is why adrenal insufficiency developed sooner.

Metopirone test. Of eight patients who had been treated with prednisolone for 2 to 12 months, six showed a good response to metopirone. Among the 32 patients who had had prednisolone for more than 12 months, only 2 showed a satisfactory response (Nos. 17 and 34; who had received a total dose of 1000 and 1200 mg, respectively).

In the control groups of 38 patients 37 showed an increase; this was slight in 3 of them. In one case there was no increase *(Fig. 1* and *Table II).*

Discussion

According to LIDDLE, metopirone inhibits the secretion of cortisol by the adrenal cortex. This is followed by increased ACTH secretion, which then increases the secretion of other hormones by the adrenals. These are converted by the method of NORYMBERSKI to ketogenic steroids, the urinary output of which increases if the adrenal corticoid secretion and pituitary ACTH secretion are intact. Thus, if the adrenal cortex is intact, the metopirone test allows conclusions as to pituitary ACTH secretion. If corticoid output increases in response to metopirone, adrenal cortical and pituitary ACTH activities are normal. If it does not increase, the defect may be either in the adrenals or in the pituitary. This can be decided by the ACTH test. If an increase ensues upon the administration of ACTH, but not upon that of metopirone, pituitary function is impaired. It does not occur, of course, that in the absence of an increase in response to ACTH there should be one in response to metopirone, because if the adrenal cannot react to exogenous ACTH, it will not be capable of reacting to the endogenous ACTH mobilized by metopirone, either. The control of our method is that no such case has occurred. The changes in urine and plasma were in the same direction or there occurred no change following ACTH administration in 17 cases, in 7 cases there was an increase in urinary output, but none in plasma, and in 5 cases there was an increase in plasma only. In these last 5 cases the reaction was considered positive although one might suggest that 24 hours after the injection of Zn ACTH the higher plasma level was due not to the ACTH, but occurred spontaneously or was induced by some kind of a stress.

According to the above results it takes about 30 months for low doses of prednisolone to cause such a hypofunction of the adrenal cortex that it

does not respond to ACTH, while pituitary ACTH secretion decreases after one year already. This is why in 12 of the cases the adrenals still reacted to ACTH when no pituitary response was elicited by metopirone. In the patients who had had total dose of at least 3000 g of prednisolone both the adrenal cortex and the pituitary showed a diminished or no reactivity. In the cases of TREADWELL *et al.*, 4600 mg was the smallest total dose after which the pituitary did not react to metopirone.

In 2 cases the pituitary failed to react to metopirone after treatment of less than 10 months' duration; and in one of these cases the adrenal cortex failed to react to ACTH. In the latter patient the initial values, too, were low; thus, a hypoadrenalism due to some other cause might have been present. However, it may happen that metopirone elicits no rise in healthy subject (KAPLAN); this was the case in 4 patients of the control group.

Sensitivity to ACTH seemed to be increasingly frequent in patients treated with prednisolone for longer periods (Cases Nos. 30, 31, 33, 35, 36, 38 and 39). It appears as if hypoadrenalism would increase the tendency to allergy.

TREADWELL *et al.* have studied adrenal and pituitary activity in 41 arthritic patients treated with prednisolone whom they gave 40 U of Zn ACTH daily for 4 days, in order to normalize adrenal cortical function before carrying out the metopirone test. On the fourth day, urinary ketogenic steroid output was increased in every patient; this was only natural in the light of the experience that the hypoadrenalism subsequent to cortisone treatment is abolished by four doses of 40 U of Zn ACTH each. In the same patients, pituitary function was studied with metopirone; the results agreed well with our experience in that there was an increase after 1 to 15 months of treatment, variable results after 16 to 31 months of treatment (8 patients reacted, 8 failed to do so), while among the patients treated longer than 31 months only 2 reacted to metopirone.

In *Table III* we have compared our results with those obtained by TREADWELL *et al.* There was a divergence in the group treated for 16 to 31 months, where the said authors observed a positive result more often than

Table III

Effect of the duration of treatment on the result of the metopirone test

	1–15 months		16–31 months		longer than 32 months		Total	
	∅	+	∅	+	∅	+	∅	+
TREADWELL *et al.*, 41 cases	1	4	8	8	18	2	27	14
Present series 40 cases	3	6	10	1	19	1	32	8

we did. This may have been due to the fact that they applied 4.5 g of metopirone instead of the 3 g applied by us, a dose to which our control cases always responded normally. The effect of protracted treatment is indicated by the fact that in the cases treated for less than 40 months the mean initial ketogenic steroid value was 10.5 mg/24 hours, while in the patients treated for longer periods it was only 5.9 mg, failing to reach the lower limit of normal, 8 mg. The mean value for the patients treated for the same length of time was 5.5 mg in our material and 5.9 mg in that of TREADWELL *et al.* The mean initial value in our control group was 11.7 mg *(Table IV)*. Mean urinary output in

Table IV

Mean urinary ketogenic steroid levels before metopirone administration

	1—40 months treatment	More than 40 months treatment	Control cases
TREADWELL *et al.*	7.4	5.5	—
Present series	10.5	5.9	12.3

response to metopirone was also much lower in the treated group: 13.2 mg, than in the controls (30.3 mg) *(Table V)*. The marked decrease in pituitary-adrenal function is clearly indicated by the data in *Table IV* and *Table V*.

Table V

Mean urinary ketogenic steroid levels after metopirone administration
mg/24 hours

Cases treated with prednisolone		Control cases
TREADWELL *et al.*	15.6	—
Present series	13.2	31.3

From the above the conclusion has been drawn that protracted glycocorticoid treatment leads to a decrease not only in adrenal cortisol secretion, but also in pituitary activity. As a result, in the case of stress no ACTH will be mobilized. ACTH administration following protracted prednisolone treatment is no safeguard against this. Following steroid treatment for more than 1 year one must always reckon with a collapse in the case of stress due to surgery, injury, infection, etc. In the case of such events if the patient had had prednisolone treatment for more than one year, the dose should therefore be increased temporarily, and after treatment prophylactic administration of corticosteroid is necessary for about half a year. According to some informative

tests, the restoration of pituitary function takes about half a year after prednisolone treatment has ceased.

It seems therefore important that patients subjected to long-term prednisolone treatment should carry on them a warning note and to reach an international agreement in this sense.

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NEUE MÖGLICHKEIT ZUR VERHINDERUNG DER HORNHAUTVASKULARISATION

Von

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II. AUGENKLINIK (DIREKTOR: PROF. DR. T. NÓNAY)
DER MEDIZINISCHEN UNIVERSITÄT BUDAPEST

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Pantothensäure und Zystein, die in der Koenzym-A-Produktion teilnehmen und den oxydativen Stoffwechsel der Hornhaut fördern, hemmen die mit Milchsäure ausgelöste Hornhautvaskularisation bei Kaninchen. Am wirksamsten erwies sich die 3%ige Zysteinsalbe. An den mit diesem Präparat behandelten Augen entstand eine signifikant spärlichere Hornhautvaskularisation, und auch der Zustand der Kornea besserte sich rascher als an der Kontrollseite. Im Gegensatz zu den bisherigen therapeutischen Verfahren fördert diese Behandlung — nebst Verhinderung der Hornhautvaskularisation — auch die Regenerationsprozesse der Kornea.

Die Hornhautvaskularisation ist im allgemeinen schädlich, da sie die Durchsichtigkeit der Kornea beeinträchtigt, die Antigenimmunität derselben aufhebt und Fettablagerung in der Kornea ermöglicht [4]. Eben deshalb ist die Prognose der Keratoplastik bei vorangehend vaskularisierter Hornhaut schlecht. In diesen Fällen wird die übergepflanzte Scheibe infolge allergischer Reaktionen häufig trübe, außerdem kann es an der Wirt-Spender-Grenzoberfläche zur Lipoidablagerung kommen [15]. Die Vaskularisation der überpflanzten Scheibe ist ebenfalls schädlich; besonders um den 10. postoperativen Tag führt sie zur Scheibentrübung [1].

Die zur Verhinderung der Vaskularisation dienenden gegenwärtig gebrauchlichen Verfahren (Kortikosteroidbehandlung, Irradiation durch Röntgen- bzw. beta-Strahlen) ergeben nicht immer befriedigende Ergebnisse und entsprechen nicht in jeder Hinsicht dem »nil nocere« Prinzip, da sie auch die Regenerationsprozesse der Kornea hindern. Die neuesten Kenntnisse, die uns über die Gefäßeinsprossung zur Verfügung stehen, ermöglichen dagegen die Bearbeitung einer kausalen, die schädlichen Spätfolgen ausschaltenden Methode.

Die Gefäße wachsen stets in Gewebe mit hypoxischem Stoffwechsel und mit schlechtem venösem Abfluß ein, wo die im Laufe der anäroben Glykolyse produzierte Milchsäure angehäuft wird. Anhand unserer vorangehenden Experimente halten wir es für wahrscheinlich, daß der vasoformative Faktor die Milchsäure ist [9]. Die Hornhautvaskularisation läßt sich in sämtlichen Fällen auf die Verminderung des oxydativen Stoffwechsels der Kornea zurückführen, woraus folgt, daß im Interesse der Verhinderung der kornealen Vaskularisation

eine Steigerung des oxydativen Stoffwechsels von ausschlaggebender Bedeutung wäre.

Diese Steigerung des oxydativen Stoffwechsels kann durch Sauerstoffüberschuß nicht erreicht werden, da für die Verminderung des oxydativen Stoffwechsels der Kornea im allgemeinen nicht der Sauerstoffmangel, sondern die infolge der beeinträchtigten Sauerstoffverwertung zustandekommende Schädigung des Epithels bzw. des oxydativen Enzymsystems verantwortlich ist. Dieser Umstand erklärt auch die Erfolglosigkeit der von ASHTON und COOK [2] sowie MICHAELSON, HERZ und KERTESZ [16] durchgeführten Kaninchenversuche, in denen die experimentell (mittels Alloxan bzw. Elektrokoagulation) herbeigeführte Hornhautvaskularisation durch Hyperoxie nicht vermindert werden konnte.

Des weiteren wollen wir einige, sich zur Steigerung des oxydativen Stoffwechsels der Kornea eignende Methoden anführen. Nach TOMIZAWA und KUDO [19] sowie THIEL und WACKER [18] fördert die Pantothensäure — ein Bestandteil des im oxydativen Stoffwechsel wichtigen Koenzym-A — die Regenerationsprozesse der Kornea. Die Untersuchungen von FUNATSU und MOGI [7] ergaben, daß die subkonjunktival injizierte Pantothensäure auch die Hornhautatmung beschleunigt. Einige Verfasser wiesen darauf hin, daß das Riboflavin die Heilung der oberflächlichen Keratitiden fördert [11, 5]. DEBERARDINIS und BONAVOLONTÀ [3] nahmen an, daß die die Kornearegeneration fördernde Wirkung einiger Aminosäuren — Arginin, Histidin [3], Zystin [17, 6] und des in der Koenzym-A-Bildung teilnehmenden Zysteins [3, 8] — mit dem Umstand zu erklären ist, daß diese Verbindungen den oxydativen Stoffwechsel der Kornea steigern. Nach KURACHI und MATSUDA [13] fördern auch K- und Mg-aspartat die Atmung der Hornhaut.

In vorliegender Arbeit wollen wir über Tierexperimente berichten, in denen die Einwirkung der oxydativen Stoffwechselsteigerung auf die durch intrakorneale Milchsäure-Injektion herbeigeführte Korneavaskularisation untersucht wurde.

Versuchsmaterial und Methodik

Vierzig, 2,5 kg wiegende erwachsene Kaninchen wurden in 4 Gruppen eingeteilt. Zwecks Herbeiführung der Korneavaskularisation wurde in beide Augen der Tiere mit Hilfe einer in die Nähe des Hornhautzentrums eingestochene und interlamellar in Richtung des Limbus geführten Injektionsnadel (Nr. 20) 0,05 ml 0,1%ige Milchsäurelösung injiziert. Auf Wirkung der Injektion entstand im allgemeinen dem Limbus entlang eine Schwellung von 4 mm Durchmesser, die an einem 3—4 mm langem Abschnitt mit dem Limbus in Verbindung stand. Nach 3 Tagen wurde, möglichst durch den ersten Stichkanal die Milchsäureinjizierung wiederholt.

Beide Augen der Kaninchen wurden von der ersten Injektion gerechnet 10 Tage hindurch behandelt, das eine Auge wurde mit Medikamenten und das Kontrollauge mit dem Grundstoff der angewandten Augentropfen bzw. Salbe behandelt.

I. Gruppe (10 Tiere): In der Zeitperiode von 6—22 Uhr wurde das rechte Auge stündlich mit Solutio ophthalmica isotonica und das linke mit 1%iger Bernsteinsäurelösung behandelt. Zur Anwendung des letzterwähnten Mittels veranlaßte uns die Annahme, daß die Bern-

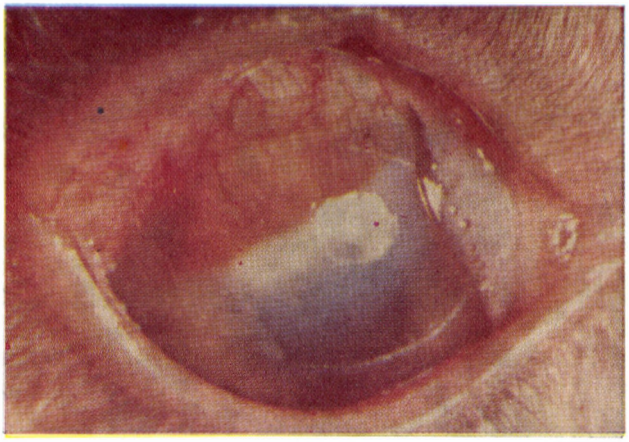

Abb. 1

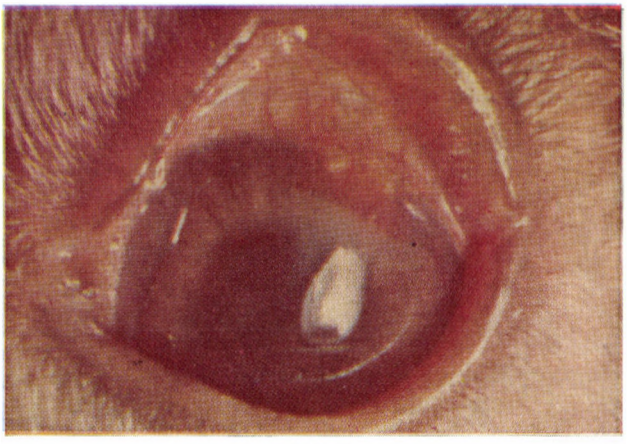

Abb. 2

steinsäure durch Steigerung der Oxydation im Zitrat-Zyklus den oxydativen Stoffwechsel der Hornhaut beschleunigt.

II. Gruppe (9 Tiere) (das 10. Tier ging am 2. Tag der Experimente ein): Die Augenbehandlungen erfolgten täglich 3mal und zwar wurde rechts 10%ige Streptomycinsalbe und links 10%ige, 3% Na-Pantothenat enthaltende Streptomycinsalbe angewandt.

III. Gruppe (10 Tiere): Zwischen 6—22 Uhr wurde das rechte Auge stündlich mit Solutio ophthalmica isotonica und das linke mit 1%iger Zysteinlösung behandelt. Die Zysteinlösung wurde täglich 2mal frisch hergestellt.

IV. Gruppe: Bei den, zu dieser Gruppe gehörenden Tieren wurde ein Blindversuch vorgenommen. In der Apotheke der Medizinischen Universität Budapest ließen wir zweierlei Salben [3%ige Zysteinsalbe und KAHÁN—KORITSÁNSZKYsche Salbe] verfertigen, die — unseren Anweisungen entsprechend — in mit I bzw. II bezeichnete Tiegel verpackt wurden. Das rechte Auge der 10 Versuchstiere wurde mit Salbe I und das linke mit Salbe II täglich 5mal behandelt. Die Salben wurden jeden zweiten Tag frisch hergestellt.

Bei sämtlichen Versuchstieren wurden täglich folgende Verhältnisse untersucht: Maximalentfernung des zentralen Randes der Hornhautvaskularisation vom Limbus, Intensität der Vaskularisation und Zustand der Kornea. In einigen Versuchen wurde auch die Breite des Limbusabschnitts abgemessen, von dem die Vaskularisation ausging. Der arithmetische Durchschnitt und die Streuung der bei den identisch behandelten Augen entstandenen Vaskularisation wurde berechnet. Das *Student*sche-t-Verfahren diente zur Feststellung der Signifikanz der Differenz zwischen den beiden Seiten.

Ergebnisse

Die experimentellen Angaben sind in Tabelle I dargestellt.

I. Gruppe: Zwischen der in den mit 1%iger Bernsteinsäure-Tropfen behandelten und in den Kontrollaugen entstandenen Vaskularisation bzw. Korneazustand war nur ein geringer Unterschied festzustellen.

II. Gruppe: In den mit 3%iger Na-Pantothenatsalbe behandelten Augen war eine geringere Vaskularisation zu beobachten als in den Kontrollaugen. Am 10. Tag war das Vaskularisationsgebiet in 6 von 9 Fällen kleiner, in 3 Fällen war die Gefäßbildung von identischem Ausmaß. Der zwischen den beiden Seiten bestehende Unterschied war zwar geringfügig, aber selbst am 4., 6. und 10. Tag signifikant ($p < 0{,}01$, $p < 0{,}01$, $p < 0{,}05$). An der mit Pantothenatsalbe behandelten Seite war die Vaskularisation in 2 Fällen bedeutend spärlicher und in 5 Fällen ging sie von einem schmaleren Gebiet aus, als an der anderen Seite. In 4 Fällen verlief die Verminderung des Hornhautödems in den mit Pantothenatsalbe behandelten Augen wesentlich rascher. In den übrigen Fällen waren in dieser Hinsicht keine Unterschiede festzustellen.

III. Gruppe: In den mit 1%iger Zystein-Tropfen behandelten Augen kam eine wesentlich kürzere Gefäßbildung zustande. Am 10. Tag war die Länge der Vaskularisation durchschnittlich um 19,9% kürzer als an der Kontrollseite. Bei 7 von 10 Kaninchen war die Vaskularisation an der mit Zystein behandelten Seite kürzer und spärlicher: in 3 Fällen war ein der Kontrollseite entsprechendes Bild zu erhalten. Die zwischen den beiden Seiten beobachtbare Abweichung war am 8. und 10. Tag signifikant ($p < 0{,}05$).

Bei der IV. Gruppe (Blindversuch) entstand in den mit der Salbe II behandelten linken Augen eine wesentlich mildere Vaskularisation. Am 10. Tag war die Gefäßbildung in 8 von 10 Fällen an der linken Seite kürzer und durch-

Tabelle I

Behandlungsergebnisse

Nr.	Zahl der Tiere	Seite	10tägige Behandlung nach der ersten intrakornealen Milchsäureinjektion (0,1%, 0,05 ml)	Beginn der Vaskularisation (Tage)	4. Tag
I.	10	rechte	Sol. ophthalmologica isotonica. Bei Tag stündlich	2—4	0,49 ± 0,40
		linke	1%ige Bernsäurelösung. Bei Tag stündlich	2—5	0,44 ± 0,39
II.	9	rechte	10%ige Streptomycinsalbe. 3mal täglich	2—4	0,36 ± 0,17
		linke	5%ige Na-Pantothenat + 10%ige Streptomycinsalbe. 3mal täglich	2—4	0,27 ± 0,15*
III.	10	rechte	Sol. ophthalmologica isotonica. Bei Tag stündlich	2—4	0,53 ± 0,40
		linke	1%ige Zysteintropfen. Bei Tag stündlich	2—4	0,53 ± 0,43
IV.	10	rechte	Ung. ophthalmicum Kahán—Koritsánszky. 5mal täglich	2—4	0,21 ± 0,07
		linke	3%ige Zysteinsalbe täglich	3—4	0,14 ± 0,06*

* = $0{,}001 < p < 0{,}01$, ** = $0{,}01 < p < 0{,}02$, *** = $0{,}02 < p < 0{,}05$, **** = $p = 0{,}05$

schnittlich um 19,6% geringer; der Unterschied war selbst am 4., 8. und 10. Tag signifikant ($p < 0{,}01$, $p < 0{,}05$ und $p = 0{,}05$). In 9 dieser Fälle ging die Vaskularisation von einem wesentlich (im Durchschnitt um 24,4%) schmaleren Limbusabschnitt aus, als in den kontralateralen Augen; der Unterschied war signifikant ($p < 0{,}02$). In 6 Fällen war im Verhältnis zu den Kontrollaugen eine bedeutend spärlichere Pannusbildung zu beobachten, in 7 Fällen entwickelte sich die Trübung der Kornea rascher zurück. Abb. 1 veranschaulicht das rechte Auge eines Versuchskaninchens, auf Abb. 2 ist das mit Salbe II. behandelte linke Auge desselben Kaninchens sichtbar. Die Aufnahmen wurden am 10. Behandlungstag verfertigt. Nach Beendigung der Experimente teilte die Apotheke mit, daß der mit No I bezeichnete Tiegel KAHÁN—KORITSÁNSZKYsche Salbe und der mit No II bezeichnete 3%ige Zysteinsalbe enthielt.

Besprechung

Unsere vorangehenden Versuche ergaben [10], daß intrakorneal injizierte Milchsäurelösung bei Kaninchen frühe und ausgeprägte Hornhautvaskularisation verursacht. Vorliegende Experimente haben es bewiesen, daß Pantothensäure und Zystein — die nach einigen Verfassern die Regenerations-

Entfernung der Vaskularisation vom Limbus (mm). Arithmetischer Durchschnitt und Standarddeviation			Länge der Vaskularisation im Limbus (mm). Arithmetischer Durchschnitt und Standard-deviation
6. Tag	8. Tag	10. Tag	
1,15 ± 0,33	1,79 ± 0,23	2,49 ± 0,46	—
1,06 ± 0,36	1,70 ± 0,98	2,39 ± 0,65	—
1,22 ± 0,35	1,96 ± 0,26	2,57 ± 0,43	8,55 ± 3,06
1,06 ± 0,32*	1,78 ± 0,35	2,37 ± 0,38***	7,39 ± 1,92
1,17 ± 0,46	1,98 ± 0,62	2,61 ± 0,66	—
1,02 ± 0,41	1,68 ± 0,48***	2,09 ± 0,77***	—
1,08 ± 0,27	1,82 ± 0,35	2,55 ± 0,38	6,35 ± 1,60
0,91 ± 0,35	1,59 ± 0,31***	2,05 ± 0,68****	4,80 ± 1,32**

prozesse der Kornea fördern und den oxydativen Stoffwechsel der Kornea steigern — die durch Milchsäureinjizierung ausgelöste Gefäßbildung der Kaninchenhornhaut zu verhindern vermögen. Bei unseren Versuchen hat sich zu diesem Zweck die 3%ige Zysteinsalbe am besten bewährt.

Die Korneavaskularisation hemmende Wirkung des Zysteins und des Na-Pantothenats ist aller Wahrscheinlichkeit nach mit dem Umstand zu erklären, daß diese Verbindungen den oxydativen Stoffwechsel der Hornhaut steigern und die Milchsäureverwertung bzw. die Epithelregeneration beschleunigen. Die Besserung des oxydativen Stoffwechsels trug auch dazu bei, daß die Regenerationsprozesse die anärobe Glykolyse d. h. die Milchsäureproduktion in geringerem Maße steigerten. In diesem Prozeß ist annehmbar auch der rascheren Regeneration des geschädigten oxydativen Enzymsystems eine Bedeutung beizumessen.

Im Gegensatz zu den bisherigen therapeutischen Verfahren übt die 3%ige Zysteinsalbe eine doppelte Wirkung aus: Hemmung der Korneavaskularisation und Förderung der Regenerationsprozesse. Zur Verhinderung schwerer Vaskularisationsprozesse wird sich die Zysteintherapie allein voraussichtlich nicht eignen, in diesen Fällen empfiehlt sich deshalb die mit anderen Aminosäuren bzw. Pantothensäure kombinierte Behandlung. Anschließend sei betont,

daß im bei Korneaverletzungen bzw. nach Keratoplatik ein durch Dauerverband herbeigeführter, geringfügiger Oxygenmangel den Zustand noch weiter verschlimmert. Das Abbinden der Augenlider hindert die Sauerstoffaufnahme der Augen, was nach KAUFMAN, CAPELLA und ROBBINS [12] die Regenerationsprozesse der Kornea ungünstig beeinflußt, nach LANGHAM [14] den Anstieg der Milchsäurekonzentration verursacht und nach unseren vorangehenden Experimenten [10] die Hornhautvaskularisation fördert. Diese Angaben sprechen dafür, daß nach Korneaverletzungen und Keratoplastik der Augenverband möglichst rasch entfernt werden soll. In Anbetracht, daß im Laufe der Keratoplastik die übergepflanzte Scheibe mit Nähten fixiert wird, kann die frühe Verbandentfernung keineswegs nachteilig sein.

Unsere Experimente ergaben, daß die zur Verhinderung der Korneavaskularisation dienende Kausaltherapie auf der Besserung des oxydativen Stoffwechsels, d. h. auf der Förderung der Epithelisation und der Regeneration des geschädigten oxydativen Enzymsystems beruht. Diese Therapie ermöglicht die Vermeidung einiger Hornhautüberpflanzungen und eine Besserung der Operationsergebnisse.

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THE SPERMATOGRAM OF PATIENTS WITH CRYPTORCHIDISM

A FOLLOW-UP STUDY

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It is impossible to decide by physical examination whether a patient with cryptorchidism belongs to the fertile or infertile group. Therefore every patient has to be treated, first with human chorionic gonadotrophine (HCG). The optimal time to start this treatment is before the 10th year of life because after this time irreversible defects (secondary atrophy of the germinal epithelium) occur. Some authors start hormone treatment at the beginning of the 6th year of life (MAIER, HECKER and BRAREN). According to our opinion, the best time is between the 8th and 10th year of life. A dose of 1000 to 1500 I.U. of HCG should be administered twice weekly, the total dose should be 12,000 to 15,000 I.U. If descensus is not attained after this first course of treatment it is necessary to start a second cure 6—8 weeks later with the same hormone dosage. In case of a negative result, funiculo-orchidolysis (OBERNIEDERMAYR and MAIER) should be performed immediately after hormone treatment. Orchidopexy is not advisable, it being often followed by testicular atrophy (MAIER).

It is impossible to decide the optimal time of herniotomy in childhood. Sometimes the course of the disease fixes the term of surgery. One should abstain from performing herniotomy in early childhood.

It is impossible to predetermine the therapeutical effect, a cosmetically satisfactory effect is no proof of fertility.

These studies were conducted in cooperation with Dr. O. Steeno, Department of Medicine of Löwen University, Belgium.

The most important part of medical treatment is to follow-up the patient in regard to the cause of his disease and to the treatment prescribed by the physician. So every treatment for undescended testis must come to a negative final result as long as the physician does not follow-up the patient for many years.

In the following we shall report on follow-up studies of patients previously treated for undescended testis.

According to the literature the incidence of cryptorchidism in the newborn is between 1 and 10 per cent with a general rate of 4 to 5 per cent (SCORER; BIERICH). A great part of the retained testes descend spontaneously into the scrotum; in adult men the percentage of cryptorchidism is 0.23 to 0.5 per cent (GILBERT and HAMILTON; CARROLL; BIERICH).

We looked into the files of our 5000 andrologic cases to find all the patients with a delayed descensus testis or those who had been treated with hormones or surgically in order to ensure a descensus. Cases operated upon for inguinal hernia in early childhood were included in the material as we

believe that the term "herniotomy" in early childhood often means an undescended testis. In addition it must be mentioned that in former years the testes were often damaged after a herniotomy in childhood.

A total of 264 cases were examined. The majority consisted of males who reported because of childlessness. The minority consisted of patients who had been treated by us with hormones for undescended testis and who have

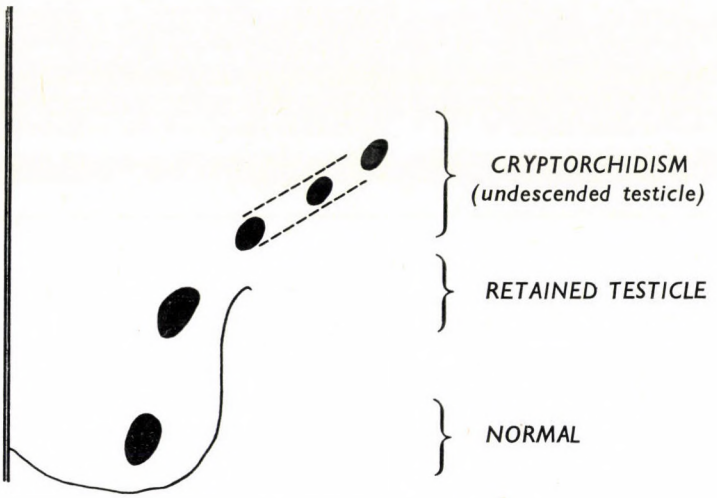

Fig. 1. The position of the testicle

been followed-up for many years. We did a physical examination with special regard to the genitalia and took a spermatogram (see SCHIRREN — 1961). We abstained from taking a spermatogram in the case of young unmarried males without sexual experience, it being unnecessary to require masturbation if the spermatogram has no therapeutic implications.

Results are demonstrated in the Tables, all of which contain

(i) The age of the patients and the duration of treatment, in groups of 0—10, 11—14, 15—20 and >20 years.

(ii) Clinical examination with special regard to testicular volume in order to compare the cosmetical result with the clinical facts.

(iii) Spermatograms according to diagnoses.

In 5 cases the testis came down spontaneously at the age of 10—14 years. The cosmetical results was satisfactory while the spermatogram showed normospermia in one case only and severe oligospermia in 4 cases. In the latter the histological picture was poor (Table I).

21 patients in the age group <20 years were treated with HCG in divided doses of 1000 I.U. twice weekly; the total dosage was 6000—8000 I.U. In 14 cases the testis was of normal size bilaterally, 1 patient had only one normal

Table I

Delayed spontaneous descensus

Age (investigation) (years)	Age (descensus) (years)	Volume of testicle	Sperm count	Motility high (moderate) immotile
29	12	normal	<3	— — 100
35	14	normal	<3	50 20 30
30	10	normal	300	60 20 20
31	14	normal	<3	— — 100
29	10	normal	<3	50 40 10

Testicular biopsy

Normal (occlusion)	—
Sertoli cells only	—
Tubular insufficiency	2
Tubular degeneration	2

Table II

Result of hormonal treatment

Age (therapy)	0—10	11—14	15—20 years	Total
Cases (total)	7	12	2	21
Clinical result				
testicular volume normal	6	7	1	14
testicular volume abnormal bilaterally		5	1	6
testicular volume abnormal on one side	1			1
Spermatogram				
impossible	1	3		4
normospermia	1	2		3
hypozoospermia				—
oligospermia	3	3		6
oligospermia, severe	2	1	1	4
azoo-, aspermia	1	2	1	4
Testicular biopsy				
tubular insufficiency	1			1

testis. Among these 15 cosmetically satisfactory cases only 3 were normospermic, in 6 cases there was oligospermia and in 8 cases (47.5 per cent) absolute infertility. As regards the effect on fertility, it was irrelevant at what age the patients were treated as all 3 patients with normospermia had been treated at the age of 11—14 years (Table II). Fifty per cent of the 6 patients with

Table III

Cryptorchidism
(one-sided)

Cases (total)	23	
Clinical result		
testicular volume unilaterally normal	14	60.9%
testicular volume unilaterally abnormal	9	39.1%
Spermatogram		
impossible	1	
normospermia	2	
hypozoospermia	1	
oligospermia	5	
oligospermia, severe	2	54.0%
azoo-, aspermia	10	
hypokinesis	2	
Testicular biopsy (contralateral)		
normal occlusion	—	
Sertoli cells only	2	
tubular insufficiency	1	
tubular degeneration	1	

Table IV

Surgery for undescended testicle
(one-sided)

Age (operation)	0—10	11—14	15—20	>20 y.	Total
Clinical result					
testicular volume normal bilaterally		1			1
testicular volume abnormal bilaterally	2	1			3
testicular volume normal unilaterally	6	4	4		14
Spermatogram					
impossible	1	1			2
normospermia	2	2	1		5
hypozoospermia					—
oligospermia	3	1			4
oligospermia, severe			1		1
azoo-, aspermia	2	1	2		5
hypokinesis		1			1
Testicular biopsy					
tubular insufficiency	3	—	1		4
cases (total)	8	6	4		18

oligospermia were treated at the age of 0—10 years and the other fifty per cent at 11—14 years. The motility rate of spermatozoa was extraordinarily high and was considered normal. These six patients might still have a small chance to become fertile. In 4 of the 21 patients a spermatogram could not be performed. All this means that a cosmetically satisfactory result of hormone treatment does not necessarily mean normospermia or fertility.

In the cases of one-sided cryptorchidism in 60.9 per cent the normally descended contralateral testis was of normal size, while in 39.1 per cent it was below normal. Only in 2 cases were normospermic. In 12 cases (52.1 per cent) there was absolute infertility. 6 patients showed oligospermia or hypozoospermia (Table V). On 2 patients out of 4 biopsy of the normally descended testis revealed Sertoli cells only. In the cases of bilateral cryptorchidism a normal spermatogram could not be expected. Sertoli cells only were found in 2 cases. Although only 9 cases were examined by us, we feel justified in stating that in bilateral cryptorchidism fertility cannot be expected. Results after surgical treatment were equally poor. In Tables IV and V 43 cases are shown, of which 18 were subjected to unilateral surgery. The contralateral testis was normally descended. Table IV demonstrates that in more than 3/4 of the cases

Table V

Surgery for undescended testicle
(bilateral)

Age (operation)	0—10	11—14	15—20	>20 y.	Total
Cases (total)	13	19	1	2	35
Clinical result					
testicular volume normal bilaterally	2	4			6
testicular volume abnormal bilaterally	9	13		2	24
testicular volume normal unilaterally	2	2	1		5
Spermatogram					
impossible	2	1			3
normospermia		1			1
oligospermia	1	2			3
oligospermia, severe	4	4			8
azoo-, aspermia	5	11	1	2	19
hypokinesis	1				1
Testicular biopsy					
normal (occlusion)	2				2
Sertoli cells only	1		1	1	3
tubular insufficiency		1			1
tubular degeneration	1			1	2

only the normally descended testis had a normal size. The spermatogram of this group showed a disturbance of fertility in 65.2 per cent of which 37.5 per cent was absolute infertility. If we take into account that the normally descended contralateral testis was of normal size, then the poor spermatograms of this group allow the conclusion that both testes had been damaged and not only the undescended one or one could not explain the high percentage of fertility disturbance. Normospermia was found in 5 cases (27.7 per cent). In all the 4 cases biopsy of the normally descended testis revealed a tubular insufficiency.

In Table V we see the final results of 45 cases of bilateral surgical treatment. In 29 cases (82.9 per cent) the volume of the testis was below normal. Among these 35 cases only one was normospermic. In 31 cases (96.9 per cent) a disturbance of fertility and in 84.4 per cent an absolute infertility was demonstrated. Of 8 testicular biopsies two revealed occlusion of the deferential duct with a histologically normal testis, 3 times there were Sertoli cells only, once a tubular insufficiency, and twice tubular degeneration.

Table VIII demonstrates the number of patients with "retained testes" — that means testes which were palpable just at the exit of the inguinal canal. According to the patients, their testes had never shown a tendency to come down further into the scrotum. It was of interest whether certain relationships existed between the high localisation and the spermatogram. Table VIII fails to reveal such a relationship. The percentage of oligospermia in this group (31.25 per cent) equalled the incidence of oligospermia (30 per cent) in the total number of 5000 andrologic patients.

There are 136 cases with herniotomy, 100 unilateral and 36 bilateral. In these cases the differentiation into certain groups of age is of importance. Among the 100 cases with unilateral herniotomy (Table VI) in 51 per cent were both testicles normal in size, while in 31 per cent only the contralateral testis was of normal size. This means that in 49 per cent the testis which had been operated upon was abnormally small. In 18 per cent the contralateral non-operated testis was also small. These 18 cases were probably operated upon for undescended testis or for this and hernia. This is supported by the following data. In the age group 0—10 years there were 21 cases and in the age group 11—14 years 4 cases with a small testis which makes a total of 25 cases (25 per cent). Among the 100 cases 32 per cent were normospermic, 62 per cent had a disturbance of fertility with 28 per cent absolute infertility. Among the 57 patients with disturbances of fertility 23 patients belonged to the 0—10 year and 5 to the 11—14 year age group. Even if it is after the 20th year of age that herniotomy is performed, disturbances of fertility amount to 20 per cent. This proves that unilateral herniotomy cannot be the cause of the subfertility. The 12 biopsies of the contralateral testis showed an occlusion of the deferential duct in 2 cases, Sertoli cells only in 4 cases, tubular insuffi-

Table VI

Surgery for inguinal hernia
(one-sided)

Age (operation)	0—10	11—14	15—20	20 y.	Total
Cases (total)	41	7	18	34	100
Clinical result					
testicular volume normal bilaterally	20	3	6	22	51
testicular volume abnormal bilaterally	7	3	2	6	18
testicular volume abnormal unilaterally	14	1	10	6	31
Spermatogram					
impossible	1	1	1	1	4
normospermia	14	1	7	10	32
hypozoospermia	3		1	3	7
oligospermia	12	2	2	7	23
oligospermia, severe	1	1		6	8
azoo-, aspermia	8	2	3	6	19
hypokinesis	2		4	1	7
Testicular biopsy					
normal (occlusion)				2	2
Sertoli cells only	3			1	4
tubular insufficiency	1	1		2	4
tubular degeneration		1		1	2

ciency in 4, and tubular degeneration in 2 cases. In the 36 cases of bilateral herniotomy (Table VII), in 14 patients surgery had been performed at the age of 0—10 years and in 6 patients at 11—14 years. In 17 of these cases both testes were of normal size and 6 were operated upon after the age of 15 years. In 15 cases both testes were extremely small, in 8 cases only 1 testis was small. The spermatogram revealed normospermia in 8 cases, a disturbance of fertility in 25 cases of which 16 cases showed an absolute infertility. Normospermia was found especially in those patients who had been operated upon after puberty; the disturbances of fertility were found especially in those patients who had been operated on before the onset of puberty. In 1 case biopsy revealed occlusion of the deferential duct.

The histological results of the biopsies show that in the majority of these cases we have to expect a poor excretory function of the testis. It has been mentioned that in 13 patients only Sertoli cells could be observed; because of the small diameter of tubuli and lack of any kind of spermatogenesis we have to consider these cases as congenital ones. Figs. 2 to 6 show the results of testicular biopsies in 5 different patients with cryptorchidism between the

Table VII

Surgery for inguinal hernia
(bilateral)

Age (operation)	0–10	11–14	15–20	>20 y.	Total
Cases (total)	14	6	6	10	36
Clinical result					
testicular volume normal bilaterally	4	3	2	4	13
testicular volume abnormal bilaterally	7	1	3	4	15
testicular volume abnormal unilaterally	3	2	1	2	8
Spermatogram					
impossible	1		1		2
normospermia	1	1	2	4	8
hypozoospermia	1				1
oligospermia	5	2	1	1	9
oligospermia, severe	2	3		1	6
azoo-, aspermia	4		2	4	10
Testicular biopsy					
normal (occlusion)	1				1
Sertoli cells only	1				1
tubular insufficiency			1		1
tubular degeneration	1				1

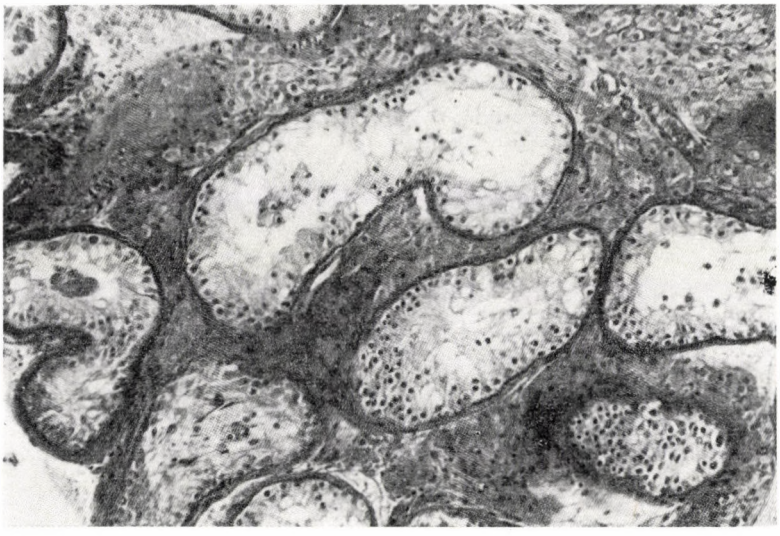

Fig. 2. Cryptorchidism. Seminiferous tubules with normal or decreased diameter and sclerosis of the tubular wall. Inside the tubules Sertoli cells only and some spermatogonia, spermatocytes I.° and sporadic mitoses. No sperms. Interstitium extensive; Leydig cells increased (biopsy at age of 42 years). ×240

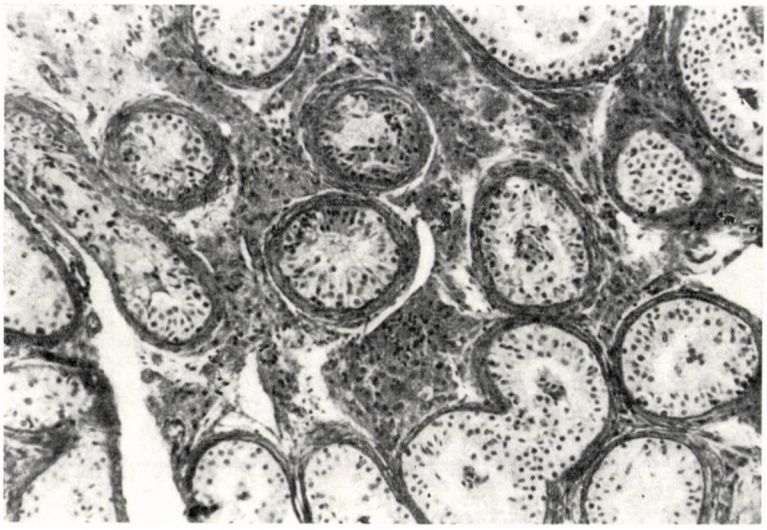

Fig. 3. Cryptorchidism. Many seminiferous tubules with decreased diameter and sclerosis of the tubular wall. Inside the tubules Sertoli cells only and spermatogonia. No spermatogenesis. Interstitium extensive; Leydig cells increased (biopsy at age of 22 years). ×240

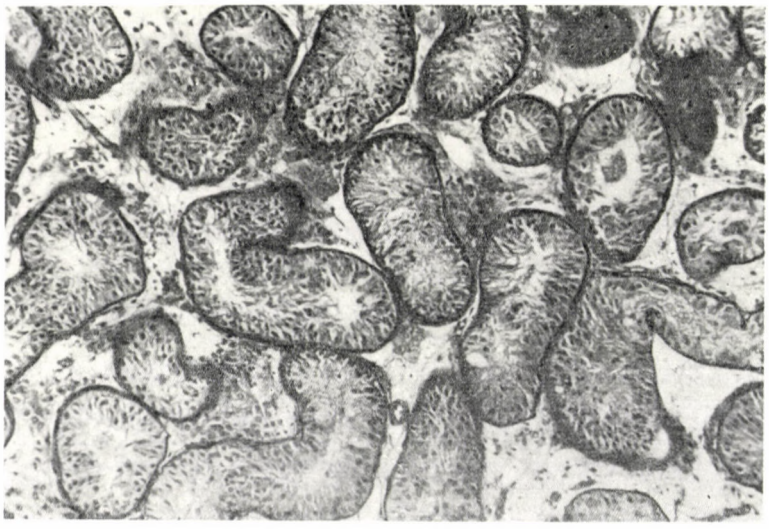

Fig. 4. Cryptorchidism. Seminiferous tubules with decreased diameter and sclerosis of the tubular wall. Inside the tubuli Sertoli cells only. Interstitium extensive; only some Leydig cells (biopsy at age of 15 years). ×240

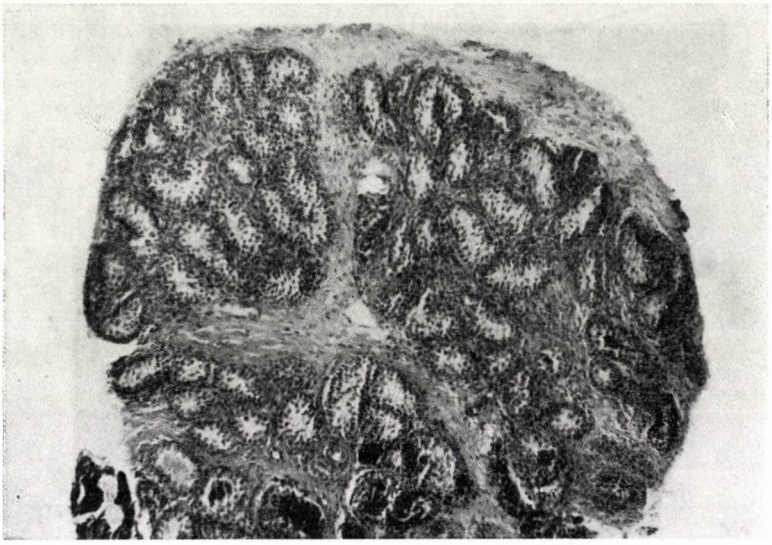

Fig. 5. Cryptorchidism. Seminiferous tubules with normal diameter, without sclerosis of the tubular wall. Connective tissue between the tubules. Immature testicular cells inside the tubules. No Leydig cells (biopsy at age of 13 years). ×240

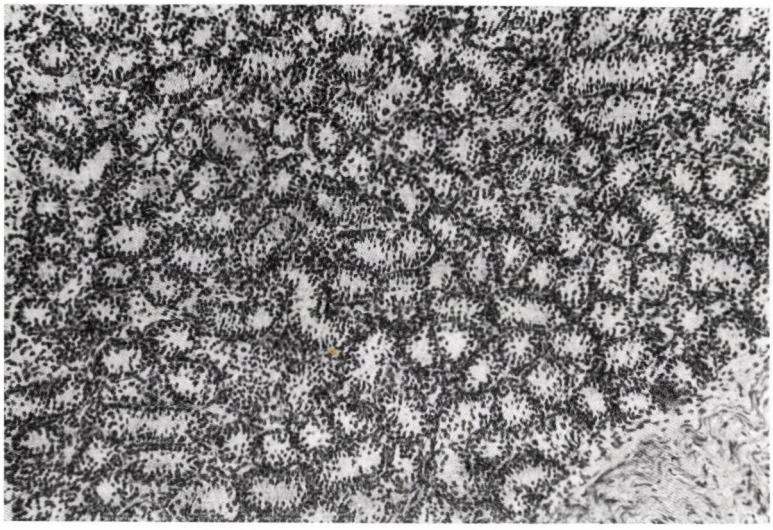

Fig. 6. Cryptorchidism (pendulum-like testis). Seminiferous tubules with normal diameter, without sclerosis of the tubular wall. Inside the tubules immature testicular cells. No Leydig cells. (Biopsy at age of 10 years.) ×240

ages of 10 years to 42 years. These figures show a relationship between the age of the patient and the testicular findings with diminished diameter, sclerosis of the tubular wall and the lack of any kind of spermatozoa. It seems that if the testis is located near the abdomen or in the inguinal canal for a longer period, the tubular wall undergoes a progressive sclerosis; afterwards the tubuli are dispersed and connective tissue or Leydig cells are found between the seminiferous tubules. Inside the tubules there are only Sertoli cells or a poor initial state of spermatogenesis without spermatozoa. In the 5 patients with a histologically normal testicle surgery had been performed for bilateral descensus (Table VIII) or unilateral (Table VI) or bilateral hernia (Table VII).

We do not intend to discuss the incidence of malignant growth of the undescended testicle. In contrast to THURZÓ and PINTER, HAUSFELD and SCHRANDT, many authors (OBERNIEDERMAYR and MAIER; OSTROWSKI; CARROLL; BIERICH) found no significance in the malignancy of cryptorchid testes. In our opinion surgical removal of the testis should not be done as we found no malignancy of the cryptorchid testis in our patients. In spite of this, cryptorchidism may be a common symptom of several embryologic abnormities, which may differ just in the possibility of tumour development (THURZÓ and PINTER).

Conclusions

In 2 of our groups (1 and 4) there were less than 10 cases; therefore statistical conclusions cannot be drawn.

In all groups the disturbance of fertility amounted to 60 to 80 per cent; a normospermia was found in 20 to 40 per cent only (Table VIII). If both testes

Table VIII

Synopsis of fertility-disturbances
(264 cases)

Diagnosis	Cases	Fertility-disturbance (%)	Infertile (%)
Delayed spontaneous descensus	5	80	80
Hormonal treatment (HCG)	21	82.6	47.2
Cryptorchidism (one-sided)	23	76.5	54.0
Cryptorchidism (bilateral)	9	100	100
Surgery for undescended testis (unilateral)	18	65.2	37.5
Surgery for undescended testis (bilateral)	35	96.9	84.4
Retained testicle (bilateral)	17	31.25	18.75
Surgery for inguinal hernia	100	62.13	28.53
Surgery for inguinal hernia (bilateral)	36	77	47

were retained, the incidence of fertility disturbance was 15 to 30 per cent more than in unilateral cases. In our material, hormone treatment, surgery, or the expectance of spontaneous descensus had all the same therapeutical effect. The incidence of absolute infertility was the same in all groups. The high percentage of fertility disturbances in cases with herniotomy in early childhood demonstrates the danger of this kind of surgery. We believe, however, that in many of these cases a combination of herniotomy and descensus or a pure descensus surgery may help. The group "retained testes" was added as control. Here the incidence of fertility disturbance was about 30 per cent, with absolute infertility in about 18 per cent. The testicles descended at the right time, and then no abnormalities could be demonstrated. The 60—70 per cent incidence of fertility disturbance in cases with unilateral cryptorchidism or unilateral herniotomy may prove our theory that in these cases there is a primary maldevelopment of both testicles.

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EFFECT OF ANAESTHESIA ON SURVIVAL AND RENAL FUNCTION OF DOGS AFTER SEVERE HAEMORRHAGE

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Oligaemic shock has been induced in dogs by bleeding to a systemic blood pressure of 50 mm Hg maintained for 90 minutes, then the whole blood was retransfused.

1. Of the animals bled under chloralose anaesthesia a greater number survived the immediate (48 hour) effects of injury than of the non-anaesthetized animals.

2. Six out of 16 animals surviving the first 48 hours succumbed to acute renal failure between the 2nd and 8th days. By a suitable adjustment of blood loss it is thus possible to induce acute renal failure in the dog. Ten animals surviving the first 14 days may be regarded as definite survivors.

3. On the evidence of acute experiments carried out within the first 30 hours after blood loss or 14 days later, respectively, RBF decreased parallel with a rise in renal resistance and a considerable reduction of GFR and E_{PAH} in the first days after injury, while on the 14th day no impairment of renal function was demonstrable.

4. The correlation of renal O_2 consumption to RBF and to tubular sodium reabsorption was comparable in the control and the bled animals.

The outcome of injury, haemorrhage or surgical intervention is variable in the human subject. Immediate death or, conversely, complete recovery may ensue, or, as a further possibility, the syndrome of shock-kidney may develop, making its appearance in the first days after injury with the full-blown picture of acute renal failure and often leading to death through an oligo-anuric and azotaemic phase, in a few days or weeks. In contrast, the laboratory animal either succumbs to, or recovers from, the injury. It has not as yet been possible to induce acute renal failure by bleeding or injury in animal experiments. PHILLIPS, DOLE, HAMILTON, EMERSON, ARCHIBALD and VAN SLYKE [14] ascribed this to two possible factors, (i) a slighter sensitivity of the animal kidney; (ii) and/or a higher sensitivity of the animal organism to injuries to which it is, for this very reason, bound to succumb before renal failure ensues (SMITH [20]).

As we have reported in several papers in the course of recent years, we were able to induce acute renal failure in dogs by temporary clamping of the renal artery. With certain restrictions this may serve as a model experiment for acute renal failure in man. It emerged from these studies that, the duration of renal ischaemia being equal, the functional and structural changes are less marked if (i) the intervention is carried out under deep anaesthesia and (ii) if

surgical or pharmacological denervation of the ischaemic kidney has been carried out (BÁLINT, FEKETE and TARABA [4, 5]; BÁLINT, CHÂTEL, FEKETE and FORGÁCS [2]). The "protective" influence of general anaesthesia on post-ischaemic renal failure has been demonstrated by SHEEHAN and DAVIS as well [19].

The present experiments were designed to clear the questions, (i) whether general anaesthesia had any influence on the mortality of dogs after bleeding; and (ii) whether it is possible to induce renal failure in anaesthetized and non-anaesthetized dogs by bleeding.

The dogs were bled by the standard method of WIGGERS and WERLE [24]. The point of this procedure is to maintain arterial blood pressure on a given level of hypotension through a given period by removing and returning suitable amounts of blood. According to WIGGERS et al. [23] when blood pressure has been maintained in dogs at 50 mm Hg for 90 minutes and at 30 mm Hg for further 45 minutes, 100 per cent of the animals die in irreversible shock within a few hours after reinfusion of the whole blood. "Standard injury" was considered a systemic hypotension of 50 mmHg maintained for 90 minutes, after which period the whole amount of blood withdrawn was returned by the arterial route. The animals were then kept under observation for a period of 14 days, or until spontaneous death ensued. In the course of the investigations, the parameters of renal function were determined in acute experiments (referred to below).

Methods

Forty-six mongrel dogs of both sexes were used. In the first group (24 animals) deep anaesthesia was induced by the intravenous injection of 0.10 g/kg of chloralose (anaesthetized group), while in the second group (22 animals) bilateral inguinal procaine anaesthesia was carried out (unanaesthetized group). The femoral artery was exposed on both sides in each animal for the purposes of bleeding and reinfusion, as well as of blood sampling and blood pressure reading.

The animals were bled from the femoral artery into a heparinized container at a rate of 50 ml/min. until the arterial blood pressure had fallen to about 50 mm Hg. In some animals the initial withdrawal was sufficient to maintain arterial pressure on the desired level for 90 minutes, while in others a spontaneous rise of blood pressure made further bleeding necessary. The volume of total blood loss per kg body weight is referred to as "total bleeding volume".

At 90 minutes the whole bleeding volume was returned and the wound was closed. In some of the animals, a falling tendency of systemic blood pressure after the initial bleeding required partial return of blood before the term of 90 minutes.

NPN was determined in each dog every second day until the 14th day or spontaneous death (CLEGHORN and JENDRASSIK [10]).

An acute experiment was carried out in 21 cases on a day of arbitrary choice. The techniques applied have been described in detail (BÁLINT, FEKETE, and TARABA [5]). The parameters determined were, arterial blood pressure (mm Hg), cardiac output (l/min/m^2 body surface); renal blood flow (RBF; ml/min/100 g kidney); glomerular filtration rate (GFR; ml/min/100 g kidney); extraction ratio of PAH; arterio-venous O_2-difference, and the corresponding O_2-consumption by the kidneys. Total peripheral resistance (TPR per kg body weight), renal resistance (R_{ren} per kg kidney weight), and renal fraction of cardiac output (RF in per cent) were calculated from the corresponding data. Statistical evaluation was carried out by the usual procedures (FISHER [11]).

Results

Table I shows the mortality of 34 dogs subjected to haemorrhage. Eighteen animals (53 per cent) succumbed the very day of bleeding or next day, with irreversible oligaemic shock. (The 12 animals sacrificed in acute experiments within the first 48 post-bleeding hours were not evaluated from the aspect of mortality.)

Table I

Mortality of dogs after bleeding

Bleeding	Died on the day of bleeding	Died on					Survived the 14th day
		1st day	2nd day	3rd day	6th day	8th day	
Under chloralose anaesthesia (18 dogs)	1	4	1 (130)	1 (188) 1 (152)	1 (216)	1 (220) 1 (270)	7
In the unanaesthetized state (16 dogs)	5	8	—	—	—	—	3

Figures in brackets show NPN values in mg per 100 ml.

It emerges from the comparison of mortality in the "anaesthetized" and "unanaesthetized" groups that 13 of the 16 non-anaesthetized animals died as against 5 of the 18 anaesthetized animals. By the aid of the χ^2-test which showed a highly significant difference ($P < 0.001$), the protection conferred by general anaesthesia on the animals against irreversible shock has been established beyond any doubt.

The three animals of the unanaesthetized group surviving the first 48 hours survived the 14th day as well. In the anaesthetized group, however, 13 animals survived the first 48 hours, but only 7 of them were alive on the 14th day, 5 died between the 2nd and 8th days; one animal showed excessive azotaemia on the third day when it was subjected to the acute experiment.

Fig. 1 shows the NPN values observed in the experiments evaluable from the aspect of spontaneous death rate. In the animals which died within the first 48 posthaemorrhagic hours, NPN did not exceed 100 mg per 100 ml, and in those which were alive on the 14th day, NPN remained in the normal range. In one of the surviving animals, transient azotaemia developed, reaching its peak of 224 mg per 100 ml on the 6th day and persisting at about 60 mg per 100 ml on the 14th day.

Six animals in the anaesthetized group died with acute renal failure between the 2nd and 8th days. (This group includes an animal sacrificed for the purpose of an acute experiment on the 3rd day, with a NPN level of 188 mg per 100 ml.) The kidneys of these animals displayed extensive necrotic areas

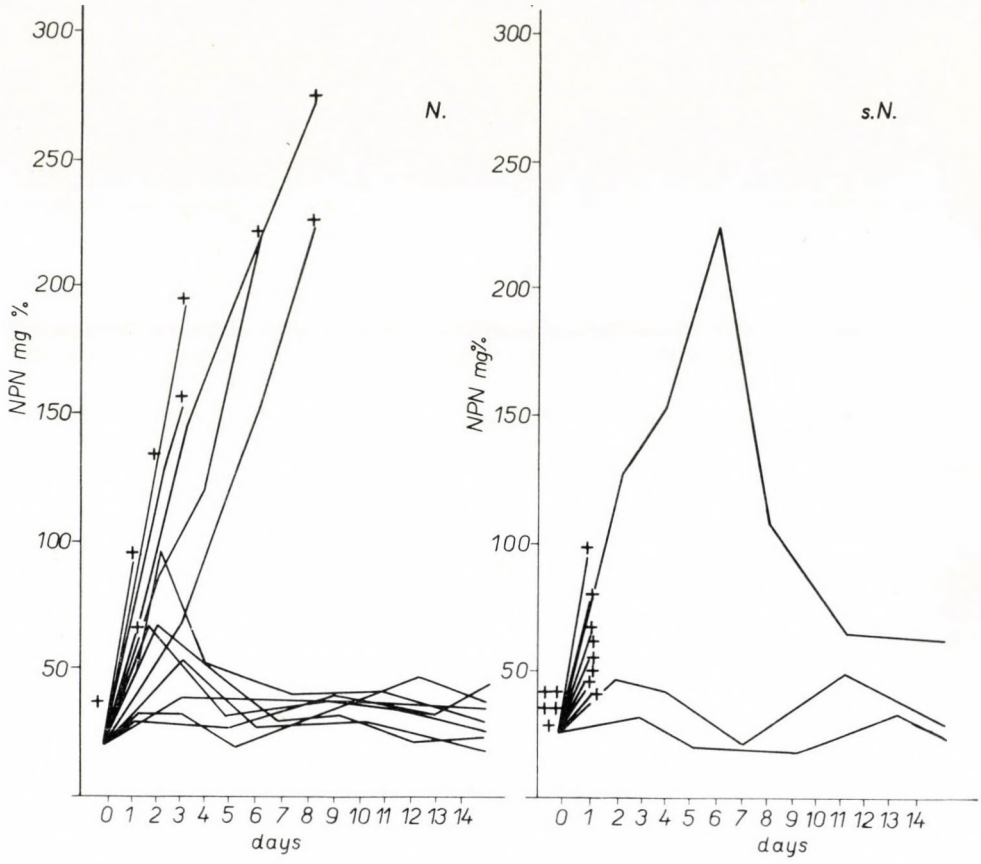

Fig 1. NPN values in the days after bleeding
N = anaesthetized group
s.N.= non-anaesthetized group

involving the renal cortex to a variable extent. No serious changes were detectable in the kidneys of the animals which died in the first 48 hours or survived (including those used in acute experiments).

Total blood loss in the anaesthetized group was 50.0 ± 2.3 ml per kg, in the unanaesthetized group, 36.5 ± 1.9 ml per kg; the difference was significant ($P < 0.001$). In other words, in the conscious group considerably less blood had to be withdrawn to decrease blood pressure to 50 mm Hg and maintain it there than in the anaesthetized group. On the other hand, within the two groups there was no difference in bleeding volume between surviving animals and those which died in shock, or between the animals which died of renal failure and the other animals of the anaesthetized group.

The acute experiments are presented and evaluated in two separate groups. Table II shows data obtained immediately after bleeding and on the next day (including those of the animal sacrificed on the 3rd day after bleed-

Table II

Circulatory and renal functions in the first days after bleeding

No.	Systemic blood pressure mm Hg	Cardiac output l/min	TPR	RBF ml/min	R_{ren}	RF %	GFR ml/min	V ml/min	E_{PAH}
Bleeding under chloralose anaesthesia Experiment one hour after retransfusion									
74/63	108	1.85	51.9	240	2.68	16.5	21	0.79	0.39
75/63	110	3.19	47.8	340	1.92	12.2	31	0.53	0.68
12/64	90	1.33	117.0	94	5.90	12.3	0	0	0.20
Experiment one day after bleeding									
21/64	99	5.26	23.3	285	2.10	10.2	41	0.47	0.77
56/64	80	3.04	38.8	319	1.51	13.7	23	0.11	0.65
57/64	103	3.92	37.7	579	1.07	17.3	42	0.89	0.89
Experiment three days after bleeding									
28/64	75	3.31	29.2	264	1.70	12.9	7	0.15	0.05
Bleeding in the unanaesthetized state Experiment one hour after retransfusion									
73/63	98	2.44	53.1	284	2.07	13.1	0	0	0.17
15/64	79	3.17	36.2	154	3.08	7.8	10	0.04	0.29
Experiment one day after bleeding									
17/64	95	3.23	40.0	185	2.28	8.1	30	0.25	0.66
22/64	119	2.33	69.1	262	2.72	16.8	5	0.21	0.66
37/64	90	1.97	133.0	247	2.28	17.5	6	0.11	0.17
$\bar{x}$	94	2.88	48.7	266	2.50	13.3	19	0.30	0.45
$s_{\bar{x}}$	±4	±0.32	±4.6	±35	±0.34	±1.0	±5	±0.08	±0.08
Control									
$\bar{x}$	122 (146)	3.97	66.2	467	1.69	14.8	59	0.95	0.68
$s_{\bar{x}}$	±2 (6)	±0.39	±5.5	±11	±0.05	±1.1	±3	±0.11	±0.01

ing). As compared with the controls, the difference in relation to RBF, GFR and E_{PAH} was significant. In the bled animals, the average figures for RBF were 57 per cent, for GFR 32 per cent, for E_{PAH} 66 per cent, of the corresponding mean control values. (The latter are reproduced from the papers of BÁLINT and FORGÁCS [7] and BÁLINT and CHÂTEL [1].)

Table III shows results found in the surviving animals on the 14th day after bleeding. Comparing the mean values to the controls, none of them showed any significant difference.

Table III

Circulatory and renal functions in dogs surviving the 14th day after bleeding

No.	Systemic blood pressure mm Hg	Cardiac output l/min	TPR	RBF ml/min	R_{ren}	RF %	GFR ml/min	V ml/min	E_{PAH}
Bleeding under chloralose anaesthesia									
81/63	121	4.80	35.3	473	1.59	11.3	34	0.54	0.86
4/64	99	1.92	74.6	177	3.38	13.4	18	0.19	0.52
10/64	117	2.68	59.2	535	1.31	21.0	39	0.84	0.75
48/64	106	3.98	33.5	558	1.14	18.3	34	0.44	0.39
49/64	103	3.58	34.6	416	1.53	12.6	32	0.23	0.67
54/64	123	2.69	63.3	415	1.78	14.8	50	0.38	0.68
55/64	123	2.15	71.3	336	2.20	18.6	48	0.25	0.78
Bleeding in the unanaesthetized state									
3/64	103	—	—	247	2.49	—	45	1.21	0.67
5/64	125	2.27	73.7	470	1.61	24.2	51	1.54	0.79
$\bar{x}$	114	3.00	50.6	403	1.92	17.0	49	0.63	0.66
$s_{\bar{x}}$	±2	±0.36	±3.6	±45	±0.21	±1.7	±4	±0.16	±0.05
Control									
$\bar{x}$	122 (146)	3.97	66.2	467	1.69	14.8	59	0.95	0.68
$s_{\bar{x}}$	±2	±0.39	±5.5	±11	±0.05	±1.1	±3	±0.11	±0.01

Fig. 2 shows the relationships between renal O_2-consumption, RBF and Na-reabsorption in the acute experiments. The data shown in Tables II and III figure separately (circles and dots) but in plotting the line of regression the totality of data has been taken into account. The line of regression derived from the control experiments (continuous line) shows no deviation from the corresponding relationships demonstrable in the animals after bleeding. On the basis of this evidence, bleeding did not affect renal basal and suprabasal O_2 consumption (BÁLINT and FORGÁCS [6]).

Discussion

According to WIGGERS [23] the procedure of bleeding used in the present study (50 mm Hg blood pressure for 90 minutes) carries a mortality of around 40 per cent. Similar figures were recorded in our material, where 53 per cent of the dogs died in the first 24—30 hours after retransfusion of blood. The standard procedure includes barbital, amytal or chloralose anaesthesia (WERLE, COSBY and WIGGERS [22]).

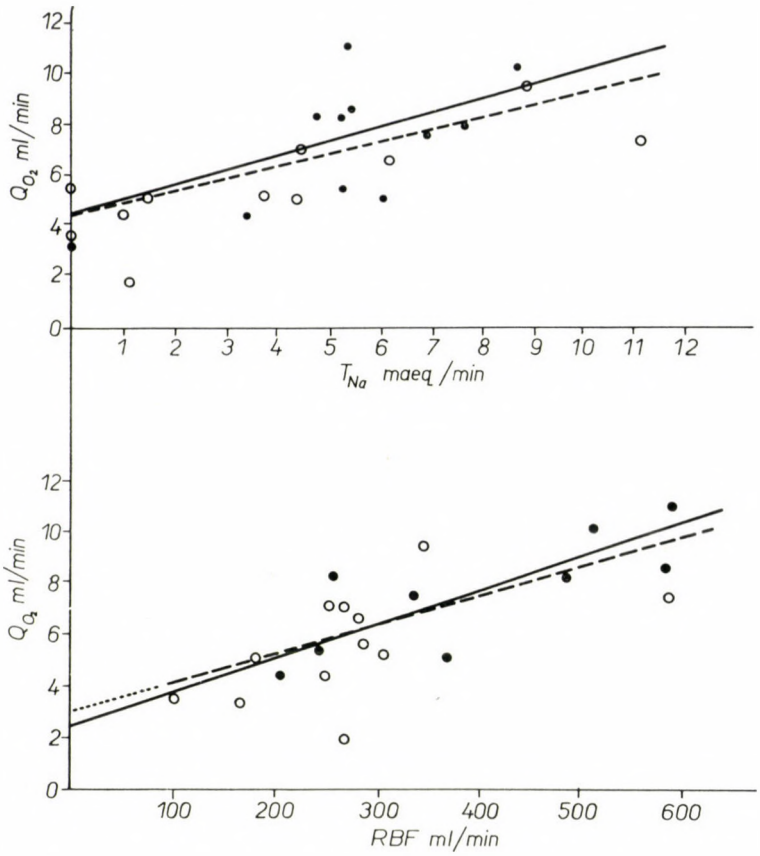

Fig. 2. Renal O_2 consumption correlated to tubular sodium reabsorption (upper figure), and renal blood flow (lower figure)
Circles: values obtained in the first days after bleeding (data of Table II)
Dots: values obtained in surviving dogs (data of Table III)
Broken line: line of regression in test group
Continuous line: line of regression in control group

In the present material, mortality of the animals bled under anaesthesia was significantly lower than of those bled in the unanaesthetized state. According to WIGGERS, INGRAHAM and DILLE [25], their standard bleeding method is associated with a lower mortality and a less severe condition of the animals when carried out under barbiturate anaesthesia instead of local anaesthesia, i.e. in the conscious state. This is at variance with the results of INGRAHAM, GOLDBERG, ROEMHILD and WIGGERS [12] who found no difference in mortality of dogs bled in the unanaesthetized state or under pentobarbital anaesthesia. According to WALCOTT [21], it requires slightly larger bleeding-volumes to induce shock in the non-anaesthetized dog.

According to our data, total bleeding volume is greater in the anaesthetized than in the non-anaesthetized animal. In other words, mortality in the

anaesthetized group is lower in spite of the greater bleeding volume. Since we have no reason to suppose that there has been any difference in total blood volume in the two groups prior to bleeding, it necessarily follows that the total blood volume left after bleeding must have been considerably less in the anaesthetized group. It is therefore obvious that the mechanism maintaining blood pressure were better adapted in this group, since it required a considerably greater blood loss to maintain systemic blood pressure at 50 mm Hg. Consequently, the greater bleeding volume is not inconsistent with the lower mortality; on the contrary, it is a sign of a better adaptation enabling the circulation to cope with a greater blood loss.

To bridge over the seeming discrepancy between the present findings and other reports, we must assume that chloralose affords more protection against shock than the barbiturates used by WIGGERS et al.

In opposition to the current view we have been able to show that the consequences of bleeding may be the same in the laboratory animal as in man. Some animals die in irreversible shock released by the injury, some survive without further harm, and there are animals which survive the injury but develop acute renal failure in the course of the first days. Out of 16 animals (from the anaesthetized and unanaesthetized groups) surviving the blood loss, 5 died in uraemia, one further animal sacrificed on the 3rd day in an acute experiment would also have probably died, and one animal in the unanaesthetized group recovered after transient severe azotaemia. Out of the 13 anaesthetized dogs surviving the immediate consequences of injury, 6 developed renal failure, inevitably fatal in 5 animals and presumably so in one. In one animal of the 3 unanaesthetized survivors, transitory severe azotaemia was demonstrable.

It is obvious on these grounds that the immediate life-saving effect of chloralose anaesthesia provides a greater number of animals with the chance of developing acute renal failure, though the present material was too small to decide conclusively whether among the actual survivors, those of the anaesthetized or of the non-anaesthetized group would be affected in a greater number with acute renal failure.

Table II shows blood pressure, cardiac output and renal functions in the course of the acute experiment in the first 24 hours after retransfusion (with the exception of animal No. 28/64, where the experiment took place on the 3rd day following haemorrhage). In agreement with other authors, a reduction of systemic blood pressure, cardiac output and RBF was demonstrable along with an increase in renal resistance. The reduction of cardiac output is comparable with that of RBF, as reflected numerically by the absence of any significant change in the renal fraction of cardiac output (SELKURT [15], BÁLINT, FEKETE and STURCZ [3], BÁLINT, KISS and STURCZ [9], BÁLINT, FORGÁCS and PALÁSTI [8], etc.). The GFR is considerably reduced, or, to express it in the

current terms, the filtration fraction decreases, presumably in consequence of constriction of the afferent arterioles. The decrease in E_{PAH} is indicative of impaired tubular function owing to bleeding.

The data for circulation and renal functions on the 14th day of survival (Table III) showed no significant deviation from the control values. A survival of 14 days thus allows full restoration of the circulatory and renal functions.

KRAMER [13] who also failed to find data concerning post-haemorrhagic or post-traumatic renal failure in dogs which would correspond to the human type, carried out acute experiments in the first five hours after retransfusion and observed an increased renal resistance, an extreme reduction of GFR, and a distinct impairment of the concentrating capacity as shown by the low osmolarity ratio of urine and plasma (U_{osm}/P_{osm}). SELKURT [16, 17] as well as SELKURT and ELPERS [18] were able to confirm these findings. As shown in Tables II and III, the average value for U_{osm}/P_{osm} found in the present studies was 1.54, which, however, does not significantly differs from the corresponding figures of similarly treated normal dogs. The massive fluid infusions involved by the experiments seem to interfere with a true assessment of concentrating capacity.

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Page 114, Fig. 2: instead of haematoxylin-eosin: resorcin-fuchsin, Van Gieson.

Page 116, in the second line from below, instead of Dr József Veres: Dr János Veres

ACTA MEDICA

ТОМ XXII — ВЫП. 2

РЕЗЮМЕ

ПОВЕДЕНИЕ СЫВОРОТОЧНЫХ ЛИПОИДОВ И СЫВОРОТОЧНЫХ У СОБАК, ПОЛУЧИВШИХ МЕТИЛЦЕЛЛЮЛОЗУ И ЭНДОТОКСИН, А ТАКЖЕ У БОЛЬНЫХ ДИСПРОТЕИНЕМИЕЙ

Ш. БЕНКЕ, Л. ВАРГА, М. ЧАНАДИ, Ф. ДУРСТ, Т. ТИБОЛДИ и Д. МЕДДЬЕШИ

В продолжении своих прежних работ авторы изучали на собаках действие метилцеллюлозы и бактериального эндотоксина липополисахаридного характера. Установлено, что оба вещества вызывают, как при изолированной, так и при совместной даче, моноцитоз, и, помимо этого, эндотоксин оказывает также анемизирующее действие. Дача очищенного эндотоксина не понижает воспаления сосудов и числа гранулов в легких, вызванных дачей метилцеллюлозы, как это наблюдалось в прежних экспериментах авторов в случае дачи фильтрата бактериальной культуры **E. coli**. Эндотоксин не вызывал во внутренних органах гистологически доказуемых изменений. Метилцеллюлоза накоплялась в элементах РЭС печени и, главным образом, селезенки, а в клубочках почек она отлагалась в виде шаровидных масс. В почках животных, получивших метилцеллюлозу и эндотоксин совместно, накопление метилцеллюлозы было значительно меньшим. Оба вещества вызывали подобные физико-химические изменения фракций сывороточных протеинов и липопротеинов. После одновременной дачи двух веществ количественные отклонения сывороточных белков и липопротеинов уменьшились. В случае болезней, сопряженных диспротеинемией у 15 из 26 больных наблюдалась характерная аномалия липопротеинов. На бумажных электрофореграммах это видно из появления трех протеиновых фракций, а на иммунноэлектрофореграммах — из расплывчатости полосы преципитации.

ДЕЙСТВИЕ СИСТОЛЫ ПРЕДСЕРДИЙ НА УДАРНЫЙ ОБЪЕМ

А. НАСЛАДИ и Д. ГОТТЗЕГЕН

Авторы исследовали у 12 больных до и после синусового ритма, восстановленного электрокардиоверсией, изменения минутного объема и систолического объема. После восстановления синусового ритма оба показателя значительно нарастали, систолический объем в среднем на 28%. У дальнейших 6 больных, у которых электрокардиоверсией не удалось восстановить синусовый ритм, не было отмечено нарастания минутного объема и систолического объема.

РОЛЬ ФУНКЦИОНАЛЬНЫХ ФАКТОРОВ И СПЕЦИАЛЬНЫХ ЗАМЫКАТЕЛЬНЫХ АППАРАТОВ КРОВЯНОСНЫХ СОСУДОВ В ВОЗНИКНОВЕНИИ ИНФАРКТА СЕРДЦА

Й. БАРАТ, Я. ВЕРЕШ и М. ЧЁРГЕИ

Авторы выявили в разветвлениях левой венечной артерии подушкообразные клеточные образования, проникающие в просвет сосуда. Они напоминают образования, обнаруженные Баратом и сотрудниками в сосудах стенки желчного пузыря в случае хронического холецистита, а также подушкообразные запирательные аппараты, выявленные в кусках сосудов слизистой носа, взятых у человека **in vivo**. Эти запирательные аппараты могут — путем изменения своих размеров, поглощения воды и солей — оказать значительное влияние на условия кровообращения в терминальной сосудистой системе. В возникновении инфаркта сердца, при котором нельзя выявить морфологических изменений

коронарных сосудов, по-видимому, участвуют патологические нарушения функции этих образований. Они могут создать ишемию, аноксемию, приводить к возникновению инфаркта, сопряженного кровотечением, и они вызывают позже развитие фиброзклеротических очагов. Патологическое нарушение функции может возникнуть в силу ненормальных рефлексов, воздействий со стороны вегетативной нервной системы, расстройств питания, действий, стресса чрезмерного действия катехоламина. Острый инфаркт может обуславливаться ударом электрического тока, при перенапряжении, эпилептическом приступе, ожогах и т. д. Согласно авторам патологические функциональные расстройства так называемых запирательных аппаратов играют важную роль в возникновении инфаркта сердца.

ИССЛЕДОВАНИЯ СТАРЧЕСКОЙ ЭМФИЗЕМЫ ЛЕГКИХ

Л. ХАРАНГИ, К. СЕМЕНЕИ, Е. ФЮРЕДИ, К. ТЕННЕР, Е. КОЧАР и Ж. САБО

Старческую эмфизему легких следует рассматривать как физиологическое явление, вызванное старением эластических волокон и размножением аргирофильных и коллагенных волокон.

ИЗМЕНЕНИЯ АТРИОВЕНТРИКУЛЯРНОЙ ПРОВОДИМОСТИ ПОСЛЕ ТРЕПЕТАНИЯ ПРЕДСЕРДИЙ

П. КАЛМАН, И. ДЬЯРФАШ и Д. ГОТТЗЕГЕН

Непосредственно после прекращения трепетания предсердий на электрокардиограмме приблизительно в 40% случаев обнаруживается удлиненное время атриовентрикулярной проводимости. В преобладающем большинстве случаев время проводимости в пределах двух недель нормализуется. После пароксизмального трепетания предсердий указанное удлинение времени передачи импульсов наблюдается редко, а у лиц с интактным сердцем никогда не встречается. У животных — путем экспериментального создания кратковременного трепетания предсердий — удлинения времени проводимости получить не удалось. Преходящее торможение проводимости, непременный фактор трепетания предсердий, наблюдается после прекращения последнего тогда, если система проводимости претерпевает вследствие большой частоты импульсов, продолжающейся в течение длительного времени, — как правило обратимое — нарушение.

ИССЛЕДОВАНИЕ ДЕЙСТВИЯ АНАБОЛИЧЕСКИХ ГОРМОНОВ НА АЛЬБУМИНОВЫЙ И ГЛОБУЛИНОВЫЙ ОБМЕН У ЛИЦ ПРЕКЛОННОГО ВОЗРАСТА

Л. КОЧАР, Ш. ВИРАГ, М. ВАШ и Л. АЧ

При исследовании действия препаратов анаболических гормонов у лиц преклонного возраста было установлено:

1. Препараты анаболических гормонов (Неробол и Нероболил) стимулируют выработку альбумина и глобулина, выраженную в мг/кг/день.
2. На основании результатов прямого исследования обмена веществ при помощи метионина, меченого S^{35}, анаболические гормоны оказывают свое действие в анаболической фазе синтеза белков.
3. Анаболические гормоны повышают процентное количество альбумина и глобулина во внеклеточном пространстве, что, повидимому, доказывает повышенное перемещение в направление тканей.
4. Анаболические гормоны могут вызвать еще более тонкие изменения, как например, повышение электрофоретического перемещения некоторых белковых фракций.

ИССЛЕДОВАНИЕ ХИМИЗМА КРОВИ ПРИ НЕДОСТАТОЧНОСТИ ПОЧЕК

Й. ЧАПО, Г. НЬЕРГЕШ и Й. БУДАИ

1. Авторы разработали оксиметрический метод для определения органических веществ в крови за исключением белков, углеводов и карбамида.

2. Они во всех случаях определяли в плазме содержание натрия, калия, стандартного бикарбоната и хлора, а в сыворотке содержание окисляемого остатка и остаточного азота. Наибольшее значение приписывается соотношению между калием, остаточным азотом и окисляемым остатком.

3. При хроническом нефрите содержание бикарбоната в общем понижается, но в виде исключения оно может быть также нормальным. Содержание калия, как правило, повышается, точно так же как и окисляемый остаток, вместе с остаточным азотом. Большая часть окисляемого остатка, являясь органической кислотой, участвует в понижении количества бикарбоната.

4. В случае поражения канальцев, возникающего в связи с обструкционной желтухой и гепатитом, содержание калия остается нормальным до тех пор, пока не появляется крайне сильная олигурия. Остаточный азот и окисляемый остаток значительно нарастают даже в случае выраженной полиурии.

5. При поражении канальцев в связи с септико-токсическими случаями картина крови подобна картине, наблюдаемой при обструкционной желтухе и гепатите.

6. В отношении остаточного азота и окисляемого остатка не во всех случаях наблюдается параллельные изменения.

7. При гипокалемической недостаточности почек может развиваться тяжелый хлорацидоз, остаточный азот сильно повышается, однако, величина окисляемого остатка остается нормальной.

ФУНКЦИЯ ГИПОФИЗА И КОРЫ НАДПОЧЕЧНИКОВ ПОСЛЕ ДЛИТЕЛЬНОГО ЛЕЧЕНИЯ ПРЕДНИЗОЛОНОМ

Э. ГОТ, Г. ГЁРГЕНЬИ, Й. ФЁВЕНЬИ и Э. САНТО

При помощи пробы АКТГ и метопироном исследовалась функция гипофиза и коры надпочечников у 40 больных с ревматоидным артритом, получивших длительное лечение преднизолоном. После интермиттирующего лечения преднизолоном в течение шести месяцев реактивность надпочечников уменьшилась или прекратилась. Нормальная реакция гипофиза на дачу метопирона уже после лечения стероидами в течение года в большинстве случаев отсутствовала. После принятия общего количества 3000 мг преднизолона ни надпочечники ни гипофиз не реагировали на указанные пробы. При длительном лечении стероидами в случае стресса и после стресса по крайней мере в течение шести месяцев необходима профилактическая дача кортизона.

НОВЫЙ МЕТОД ДЛЯ ПРЕДОТВРАЩЕНИЯ ВАСКУЛЯРИЗАЦИИ РОГОВИЦЫ

Д. ИМРЕ и Й. БЕГИ

Пантотеновая кислота и цистеин, улучшающие окислительный обмен веществ роговицы, и участвующие в синтезе коэнзима А, препятствуют васкуляризации роговицы кроликов, вызванной молочной кислотой. Эффективнее всего оказалась 3%-ная мазь цистеина. После нанесения этой мази пять раз в день на роговице возникновение васкуляризации было достоверно меньшее и состояние роговицы улучшалось быстрее, чем на контрольной стороне. В противоположность терапевтическим методам, применявшимся раньше для предотвращения васкуляризации роговицы, этот способ лечения, кроме предотвращения васкуляризации роговицы, способствует также процессам регенарации.

СПЕРМАТОГРАММА БОЛЬНЫХ КРИПТОРХИЗМОМ

Ц. ШИРРЕН

При помощи физического обследования нельзя установить, относится ли больной с крипторхизмом к плодовитой или бесплодной группе. Поэтому каждого пациента приходится лечить человеческим хорионгонадотрофическим гормоном (ХГГ). Лечение следует начинать до достижения десятилетнего возраста, так как после этого срока происходят необратимые изменения (вторичная атрофия зародышевого эпителия). Некоторые авторы начинают лечение гормонами в пятилетнем возрасте (Майер; Хеккер и Баррен). Авторы настоящей статьи придерживаются того мнения, что оптимальным сроком является возраст от 8 до 10 лет. Они рекомендуют давать 1000 до 1500 ед. гормона два раза в неделю, при общем количестве 12 000 до 15 000 ед. Если в результате первого курса лечения не достигается опущения яичек, то по истечении 6—8 недель необходимо повторять курс при прежней дозировке. Если и второй курс лечения остается безрезультатным, то немедленно после гормонотерапии следует провести фуникулярный орхидолиз (Обернидермайр и Майер). Проведение орхидопексии не рекомендуется, так как после этого вмешательства часто наблюдается атрофия яичек.

Оптимального срока проведения грыжесечения в детском возрасте нельзя определить. Иногда срок операции определяется течением болезни. У детей раннего возраста по возможности не следует провести грыжесечение.

Предопределить терапевтический эффект не представляется возможным, удовлетворительный косметический результат еще не является доказанием плодородности.

Исследования проводились при сотрудничестве с доктором О. Стеено, медицинский факультет университета г. Левен, Бельгия.

ДЕЙСТВИЕ НАРКОЗА НА ВЫЖИВАЕМОСТЬ СОБАК И ФУНКЦИЮ ИХ ПОЧЕК ПОСЛЕ ОБЕСКРОВЛИВАНИЯ

П. БАЛИНТ, А. ФЕКЕТЕ, И. ТАРАБА и М. ВИШИ

Авторы вызвали у собак путем кровопускания олигемический шок: на протяжение 90 мин артериальное давление понизилось до 50 мм ртутного столба, после чего полное количество снятой крови реинфундировалось. Установлено, что

1. Если провести кровопускание под хлоралозным наркозом, то доля животных, не погибающих от непосредственных последствий травмы (до второго дня после кровопускания) больше, чем в случае обескровливания бодрствующих животных.

2. Шесть из 16 животных, еще живших на второй день после обескровливания, погибли на 2—8-ой день после травмы при явлениях острой недостаточности почек. Следовательно, соответствующей дозировкой обескровливания можно у собак вызвать патологический процесс острой недостаточности почек. 10 животных жили еще на 15-ый день после кровопускания. Этих собак мы считали окончательно выжившими.

3. В одной группе собак в пределах 30 часов или спустя 14 дней после кровопускания авторы провели острый опыт. Они установили, что непосредственно после травмы количество крови, протекающей через почки, понижается и сопротивление в почках повышается. Клубочковая фильтрация или выделение ПАГК в значительной мере понижаются. При проведении исследований спустя 14 дней после травмы наблюдается нарушение функции почек.

4. Соотношение между потреблением кислорода почками, количеством крови, протекающей через почки и канальцевой реабсорбцией натрия в норме и после кровопускания отклонений не было выявлено.

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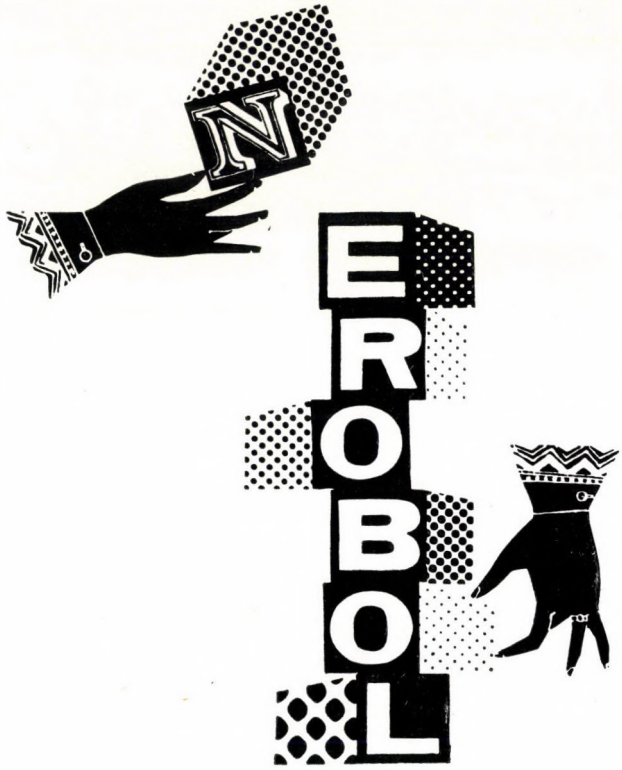

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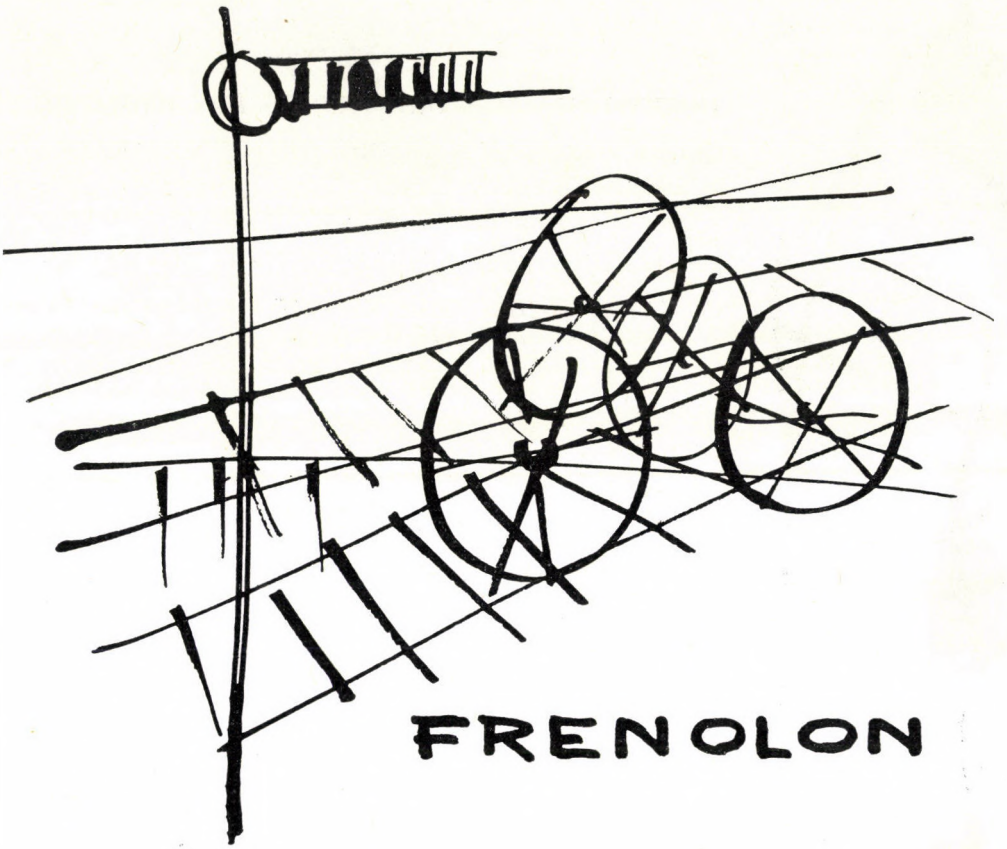

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THE SYNDROME OF HAEMORRHAGIC THROMBOCYTHAEMIA

THROMBOCYTOPATHY VERSUS COAGULOPATHY AFFECTING PRIMARY HAEMOSTASIS

By

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(Received February 17, 1965)

Coagulation factors have been investigated in two, and platelet functions in four cases of haemorrhagic thrombocythaemia. The platelet count exceeded one million in all the four cases, bleeding time was prolonged in three, and in the fourth it was at the upper limit of normal. In one case, the thromboplastin generation test was indicative of an abnormality in the phase of thromboplastin formation, more specially of the deficiency in factor XI (PTA). On the evidence of that test, the platelet component has been found to exhibit an anticoagulant effect at high platelet concentrations. According to the investigations of the *in vivo* adhesive platelet count described by BORCHGREVINK, formation of the platelet plug involved an excessive number of platelets.

Though on the basis of its clinical features haemorrhagic thrombocythaemia has a distinct place within the myeloproliferative syndromes, it is no clear-cut entity from the pathogenetic aspect. The diagnostic criteria of the syndrome have been summarized.

According to the current concept, the part played by the platelets in haemorrhagic phenomena asserts itself through the clotting system. Data derived from cases recorded in the literature and from the present observations suggest, however, that in haemorrhagic thrombocythaemia the primary haemostasis, more exactly its second step, the phase of irreversible platelet aggregation (viscous metamorphosis and platelet contraction) is affected, and this is the reason why no firm platelet plug impermeable to blood is formed. Though the closer mechanism of the process is obscure, it may be presumed that for some reason the effect of the thrombin required by the process fails to assert itself. The clotting disturbance associated with haemorrhagic thrombocythaemia may be further impaired by a defect in plasma clotting which plays a decisive part in secondary haemostasis.

It is now generally recognized that a tendency to haemorrhage is far from being incompatible with thrombocytosis and may even be one of its prominent features. This is the very case in the rare syndrome known as haemorrhagic thrombocythaemia (HT). The cause of this seeming discrepancy is not yet fully understood though the syndrome has been cleared up from many other aspects. On the basis of numerous observations accumulated since its first description thirty-five years ago, HT must be placed among the myeloproliferative syndromes, next to polycythaemia vera and fairly close to chronic granulocytic leukaemia and the so-called primary (agnogenic) myeloid metaplasia. Its features distinctive from those of the related syndromes include a joint tendency to haemorrhage and thrombosis, persistent thrombocytosis associated in some cases with splenomegaly, leucocytosis, mild hypo-

chromic anaemia or again with slight erythrocytosis, particularly during the haemorrhage-free intervals and medullary signs of enhanced thrombocytopoiesis. The syndrome is furthermore characterized by a protracted course and a prevalence in middle-aged patients. It has been found to respond to certain therapeutic agents, particularly busulphan (Myleran) and radio-phosphorus.

The *tendency to haemorrhage* has remained the most puzzling feature of HT. Results of thromboplastin generation test obtained by most of the investigators would seem to suggest a disturbed production of thromboplastin. Opinions diverge, however, as to the nature and extent of this disturbance. A possible disturbance in the clotting function of platelets is generally assumed while some authors ascribe an anticoagulant effect to the platelets at the thrombocythaemic level. There are also other theories. While some authors leave the whole question open until further observations or more suitable methods of investigation are forthcoming, the majority ascribe the bleeding tendency to a coagulopathy and look upon HT as a "haemophilia-like syndrome" (SPAET [1]). The question, however, is by no means settled.

The incidence of HT is low, in spite of the increasing attention it has been receiving. From the cases reported between 1930 when it was first described by EPSTEIN and KRETZ [2] and 1955, HARDISTY and WOLFF [3] accepted 18 cases as authentic and to these added five observations of their own. OZER et al. [4] accepted 21 out of 48 recorded cases after very thorough sifting, describing in the same paper further six cases. In the same year, i.e. 1960, GUNZ [5] reviewed 50 cases from the literature with five of his own observations in a comprehensive study on the main aspects of the syndrome. We have no knowledge of any recent review with evaluable data as regards incidence, but in our estimation the number of recorded cases recognized as HT by all standards should not be far below a hundred. With the unpublished cases, there must be, obviously, still more.

LEHOCZKY et al. [6] in 1963 were the first Hungarian authors to describe HT. Another case of "essential thrombocythaemia" was reported in the same year by NAGY and LEÖVEY [7]. Then BARTA [8] gave a comprehensive review of the various conditions associated with thrombocytosis. The syndrome figures in KELEMEN's monograph [9] and in 1964, FÖLDVÁRY et al. [10] reported two cases.

In the past years we have observed haemorrhagic episodes associated with platelet count exceeding a million in various haematologic conditions, mostly polycythaemia vera or chronic myeloid leukaemia. As to the others, only a few cases are fit to be included in the present paper as being consistent with HT by all standards of diagnosis. In order to clarify the possible mechanism of the haemorrhagic manifestations in patients with haemorrhagic thrombocythaemia, a close study has to be made of the whole haemostasis. This paper gives a full account of two cases and presents the essential data of two others,

with a review of the diagnostic criteria of HT. On the basis of our limited material and of the data in the literature, we shall then outline our view as to the nature of the bleeding tendency, ascribing them to an abnormality of primary haemostatic platelet function, in opposition to the current concept of "coagulopathy".

Case records

Case No. 1. A 80-year-old male patient had been first hospitalized four years previously because of repeated gastrointestinal haemorrhage. The diagnosis was diverticulosis of the sigmoid and posthaemorrhagic anaemia. Leucocyte and platelet counts had been normal at that time. The patient's earlier history included protracted haemorrhage after tooth extractions. During the last four years he had repeatedly experienced intestinal haemorrhage and had moreover been treated for osteoarthrosis, pulmonary emphysema and atherosclerosis. In January, 1964, persistent melaena associated with severe anaemia called for urgent hospitalization. It was then that a thrombocyte count of over one million was first noted. The leucocyte count was 13,000 with a slight shift to the left. The most striking feature of the hypercellular bone marrow was a hyperplasia of the megakaryocyte system.

The patient was admitted for the last time in July, 1964, after repeated melaena. The spleen was not palpable. The haematocrit value was 25 per cent, the platelet count 2,190,000 with marked anisocytosis, the leucocyte count 58,000, with 2 myelocytes and one normoblast per 100 cells. The bone marrow was hypercellular with reduced fat tissue. There was hyperplasia of all three systems with a striking preponderance of megakaryocytes, mostly mature, functioning elements. The data received by the study of haemostasis are shown in Tables I—III (Pat. No. 1). As a treatment, Myleran was prescribed, but the condition deteriorated before the drug could have acted. Heart failure, azotaemia ensued, the patient became eventually confused, this gave rise to the suspicion of cerebral thrombosis or haemorrhage. Death was inevitable.

Clinical diagnosis: haemorrhagic thrombocythaemia. Diverticulosis in the colon. Osteoarthrosis. Atherosclerosis.

Autopsy revealed generalized atherosclerosis, pulmonary and peripheral congestion, diverticulosis of the large intestine. The bone marrow was hypercellular with hyperplasia of all three systems and a striking preponderance of thrombocytopoiesis. The megakaryocytes were partly immature, mostly with evidence of platelet formation. There was a minor degree of myelosclerosis in the femoral bone with no apparent fibrosis. Extramedullar haemopoiesis involving all three systems was found in the spleen, liver and lymph nodes. The spleen was reduced in size but had preserved its structure.

Epicrisis: On the evidence of clinical and autopsy findings the primary disease was HT. The concomitant condition resulting in heart failure was generalized atherosclerosis. An additional remarkable finding was the diverticulosis of the large intestine.

Case No. 2. A 66-year-old male was admitted for melaena. The history included hospitalization 11 years earlier for melaena and haematemesis attributed to duodenal ulcer which had made operation necessary. Apart from repeated epistaxis the patient had been symptom-free for the last years. On admission in July, 1964, his liver and spleen were erlarged and there were some palpable axillary lymph nodes. Haematocrit was 21 per cent, the leucocyte count ranged from 12,000 to 20,000 with a shift to the left. Repeated platelet counts were found to exceed one million with striking anisocytosis and giant platelets nearly of lymphocyte-size. The sternal marrow showed hyperplasia of all three systems with predominant megakaryocytosis. The megakaryocytes were mostly mature functioning cells. The fat tissue of the bone marrow was reduced. The axillary lymph node showed histologically simple lymphadenopathy. At the site of the excision an extensive haematoma developed, responding very sluggishly to local treatment. Gastrointestinal examinations failed to disclose any possible source of the melaena. (Data on the haemostasis are shown in Tables I—III, Pat. No. 2.) Leukaemia could be ruled out. The findings were consistent with HT. The patient was started on Myleran but did not attend for follow-up.

Case No. 3. A 58-year-old female had a ten years' history of crural vein thrombosis. Splenectomy for splenic vein thrombosis had been performed eight years earlier. Ever since this operation the leucocyte count had ranged between 12,000 and 18,000, the patient had repeatedly intestinal, nasal and gingival haemorrhages. Repeated X-ray findings were sugges-

tive of varicosities in the upper intestinal tract. Therefore a shunt operation had been considered a few years ago and laparotomy had been undertaken but massive haemorrhage made the operation impossible. In November, 1964, when admitted to the Clinic, the platelet count exceeded one million, the sternal marrow was hyperplastic with preponderant megakaryocyte increase. (Some data are presented in Table I, Pat. No. 3.) The present picture is consistent with the diagnosis of HT.

Case No. 4. A 32-year-old female patient had been subjected to splenectomy for splenic vein thrombosis in 1961. She had then nasal and gingival haemorrhages, menorrhagia and repeated intestinal bleeding. On radiological evidence the latter have been ascribed to duodenal ulcer. She had a severe anaemia consistent with the blood loss. A platelet count exceeding one million was noted in 1964. (Some data are presented in Table I, Pat. No. 4.) The splenic vein thrombosis a few years earlier may well have been the consequence of HT.

Materials and methods

Materials

Oxalated and citrated blood plasma;
blood serum obtained from clotted whole blood without anticoagulant;
adsorb-plasma, i.e. plasma prepared with $BaSO_4$;
platelet-rich plasma, resulting from fresh citrated plasma centrifuged at 800 r.p.m.;
platelet suspension obtained by differential centrifugation and washing with physiological saline, using silicone-treated instruments and containers;
silicone-serum resulting from clotting of whole blood in silicone-treated glass tubes;
tissue thromboplastin (complete) prepared with acetone from human brain in our laboratory;
partial thromboplastin (PTP) extracted from human brain with chloroform by the method of BELL and ALTON [11].

Platelet count was performed by phase contrast microscopy according to FISCHER and GERMER [12].

Bleeding time was estimated according to IVY [13]. The inflated manometer cuff was maintained at 40 mm Hg during the procedure. A wound 1 mm deep and 6 to 8 mm long was made in a non-vascularized area of the volar surface of the forearm, over the extensors. The blood was blotted up every 30 seconds. Under normal conditions the bleeding time measured in this manner was less than 7 minutes, ranging mostly between 3 and 4 minutes. This test may be combined with the determination of the adhesivity of platelets *in vivo* described by BORCHGREVINK [14]. The difference of the platelet count of the blood sample from the wound and that from "venous" blood gives the number of platelets retained on the wound surface, i.e. of the "*in vivo* adhesive" platelets. This may be also expressed in percentages of the platelets in peripheral (venous) blood. For "venous" platelet count silicone-treated glass tubes were used. For "wound" platelet count the blood was collected from the wound 3 to 5 minutes after the wound has been made.

Aggregation *in vitro* and viscous metamorphosis were studied by phase contrast microscopy using platelet-rich plasma, to which highly diluted thrombin (a few units per ml. saline) and a 0.025 molar solution of $CaCl_2$ had been added.

For the TGT we used partly PTP, partly a platelet suspension adjusted to the concentrations shown in the Table III after repeated washing with physiological saline. The time of appearance of the clot has also been noted, this being, in our experience a reliable indicator of the rate of thromboplastin generation.

The degree of retraction has been established on the grounds of the behaviour of the whole blood clot and of that of recalcified oxalated plasma.

As to the other methods figuring in the Tables, we refer to the corresponding literature.

Results

Results for the four patients relating to the vascular and thrombocytic system are shown in Table I. Bleeding time was prolonged in three cases, and at the upper limit of normal in one case. Tourniquet test and blood clot retraction were normal. When diluted thrombin was added to the plasma,

platelet aggregation ensued in 60 seconds; the platelets were enmeshed in the fibrin network appearing later. The balloon-like appearance of the clumping platelets was clearly discernible. Recalcification caused similar changes. By these standards, aggregation capacity *in vitro* of the platelets as well as their viscous metamorphosis was considered normal. The platelet count exceeded one million in all the four cases, and two million in one of them. The platelets retained on the wound surface, i.e. the "*in vivo* adhesive" platelet count was 200,000 or more, thus outside the normal range indicated by BORCHGREVINK [14] and by two subsequent investigators [64, 65]. With the exception of one case, the percentual values were lower than normal. Considering, however, the excessive "venous" platelet count, the absolute number of platelets retained on the wound surface seemed to be far more informative than the percentage index.

Table I

Study of vascular and platelet systems

Patient No.	Bleeding time (Ivy), minutes	Tourniquet test	Retraction	Platelet aggregation Viscous metamorphosis (in vitro)	Platelets per ml. (direct) × 1000	"In vivo adhesive" platelet count	
						per ml × 1000	%
1	15 20	negative	normal	normal	2190	750	34
2	6.5 8	negative	normal	normal	1550	250	16
3	12 19	negative	normal	normal	1300	200	15
4	15 16	negative	normal		1150	280	24
Normal value < 7					150—300	45—185	25—58
						average: 96	average: 37
						(Borchgrevink)	

Tables II and III show the coagulation data in Cases Nos. 1 and 2. Results of clotting time, fibrinogen titration and exploratory fibrinolysis were within the normal range. Partial thromboplastin time as well as Quick's time were prolonged in a variable degree, indicative of a moderate reduction of prothrombin activity. Correction was not possible either with stored serum (factors VII and X) or with adsorb-plasma (factor V). The reduction prothrombin activity was therefore due to a moderate reduction of the prothrombin level. Prothrombin consumption values pointed to a normal, if not increased prothrombin utilization. The silicone sera were found to contain fairly equal amounts of prothrombin in all the cases. (A decreased surface activity may have compensated the individual differences in consumption values due

Table II

Study of clotting system

Test	Method	Patient no. 1	Patient no. 2	Control
Clotting time (min.)	LEE-WHITE [15]	4	5.5	5—10
Partial thromboplastin time (PTT) (sec.)	modif. LANGDELL et al. [16]	72	83	66±3.2
Prothrombin activity (sec.)	QUICK [17]	18	17	15±1
The same with 1/10 vol. stored serum	QUICK [17]	14.5	14	12
The same with 1/10 vol. adsorb-plasma	QUICK [17]	18.5	17	15.5
Prothrombin consumption (4 hours, sec.)	modif. QUICK [18]	95	71	>40(44)
The same with silicone-serum	modif. QUICK [18]	45	35	36
Thrombin time (sec.)	HORN et al. [19]	23	22	20
Fibrinogen titration (plasma-dilation)	SHARP et al. [20]	1 : 128	1 : 128	1 : 128
Fibrinolysis (degree of lysis within 24 hours)	BIGGS—MACFARLANE [21]	Ø	Ø	Ø
Thromboplastin generation test (TGT)	modif. BIGGS—DOUGLAS [22]	See Table III		

Table III

Results of thromboplastin generation test (TGT)

Platelet	Plasma	Serum	3	6	9	12	Time of appearance of clot in incubation fluid (min.)
components			seconds				
PTP	K	K	16	*9*	10	12	4—5
PTP	1	1	25	*9*	10	11.5	5—6
PTP	2	2	49	20	*16*	17	8—9
PTP	2	K	30	*10.5*	11	12.5	5—6
PTP	K	2	31	11	10.5	12	6—7
K	K	K	17	*12*	14	17.5	4—5
1/a	K	K	13	*12*	13	14.5	3—4
1/b	K	K	45	18	*17*	18.5	5—6
2/a	K	K	15	*12*	13	17	4—5
2/b	K	K	47	19	*18*	20	5—6

Platelet concentrations:

K 0.45 M per c. mm — 2/a 0.4 M per c. mm

1/a 0.5 M per c. mm — 2/b 4.5 M per c. mm

1/b 5.0 M per c. mm

to different platelet counts.) Certain difficulties in evaluation arose from the reduced plasma-prothrombin levels. Thrombin times were slightly prolonged. TGT-values by the use of PTP were normal in Case No. 1, while in Case No. 2, a thromboplastin of deficient activity was generated in the presence of PTP and Ca; substitution with normal plasma or serum components (K) resulted, however, in full correction. This seems to indicate a moderate factor XI (PTA) deficiency. At the conventional concentrations of 0.5 M/c.mm the platelet suspensions of the patients showed a satisfactory thromboplastin production in the presence of normal serum and plasma factors. At tenfold concentrations, thromboplastin generation was somewhat delayed and their activity reduced. (It did not succeed to obtain a similarly concentrated platelet suspension from a control subject.)

Discussion

It has been found preferable to classify the various conditions associated with an increased platelet count on the basis of their origin rather than labeling them as primary ("thrombocythaemia") or secondary ("thrombocytosis"). (For instance, increased platelet count encountered in certain primary diseases, as in polycythaemia, has been regarded by many as secondary though being, actually, the very instance of primary thrombocytosis of unknown origin, with high platelet counts, frequently over 1,000,000; thrombocytosis is therefore not necessarily secondary.)

MALLARMÉ and AUZÉPY [23] discerned the following groups: 1. polycythaemia vera; 2. thrombocytosis due to splenectomy or atrophy of the spleen; 3. chronic granulocytic leukaemia; 4. myelofibrosis with myeloid metaplasia; 5. "essential" thrombocythaemia, i.e. the actual HT syndrome; 6. thrombocytosis associated with infections, malignant disease, sarcoidosis, blood loss, etc.

Groups 1, 3, 4, and 5 merge today into the so-called myeloproliferative syndrome [24]. This also includes pathogenetically the HT which has been figuring until recently or even today as essential, primary or idiopathic. On the whole, HT is regarded today as a distinct clinical syndrome rather than as a clear-cut pathogenetic entity [3, 4, 5, 6, 25, 26, 27].

OZER et al. [4] follow the strictest lines in the *diagnosis* of HT. In their view, distinction of HT within the myeloproliferative syndrome rests upon the following criteria: *a)* haemorrhagic episodes coupled in many instances with a tendency to thrombosis; *b)* the spleen must be preserved and be not smaller than normal; *c)* an erythrocyte count less than 6,000,000, haemoglobin less than 18.0 g per 100 ml, a leucocyte count of less than 50,000, a platelet count persistently over 800,000; *d)* medullar panmyelosis with predominant megakaryocytosis and aggregated platelets; *e)* absence of leukaemic infiltra-

tion. The authors stress the therapeutic significance of strict differentiation. As to criterion *b)*, the spleen in EPSTEIN's [28] second case was atrophic and led the author to assume a "splenic subfunction". There are several cases consistent with HT and associated with an enlarged spleen and even cases where the spleen had been removed prior to the onset of the symptoms. Thrombosis of the splenic vein (BANTI-like syndrome) which has been the indication for splenectomy in many instances, may have been the earliest manifestation of HT [17, 29, 30]. According to the present view, however, the part played by hyposplenia or asplenia in the myeloproliferative syndrome lies in enhancing its progress rather than in initiating it. It may probably favour the process of transformation as suggested by the relative frequency of HT following the splenectomy in cases of myeloid metaplasia [27, 31], or, on the faith of certain observations [25], it may be responsible for the conversion of non-haemorrhagic HT into its haemorrhagic form. It is therefore felt that the second criterion laid down by OZER should be considered in the light of these observations.

Obviously, it is essential to differentiate HT from megakaryocytic or megakaryoblastic leukaemia which is an entirely different process, closely related to acute myeloid leukaemia and generally unattended by thrombocytosis [5].

The aspects outlined above show that our Cases Nos. 1 and 2 meet the diagnostic criteria, though in the first the spleen was somewhat smaller than normal and the leucocyte count exceeded 50,000. The absence of leukaemia was confirmed at autopsy. In our Cases Nos. 3 and 4 the spleen had been removed earlier and it remains a matter for speculation whether we are justified in considering the splenic vein thrombosis for which the operation has been undertaken as the earliest sign of HT.

In all four cases the tendency to haemorrhage was the most conspicuous feature. The occurrence of gastrointestinal or gingival haemorrhage, epistaxis, menorrhagia as well as surgical haemorrhage corresponds to the findings of other observers. Purpura or intraarticular haemorrhage and extensive haematomas characteristic of haemophilia, were likewise absent.

The current views regarding the *pathogenesis* of the *bleeding tendency* associated with HT may be resumed as follows.

1. The platelets are assumed to be defective in function as well as abnormal in number, first of all as regards their *thromboplastic* function essential to the process of clotting (intrinsic platelet defect).

2. Platelets in HT display an abnormal behaviour *in vitro* at high concentrations only. At physiological levels, for instance in the thromboplastin generation test after dilution of the platelet component, their function becomes normal. It is therefore believed that at high platelet levels the effect of an anticoagulant factor becomes manifest.

3. The tendency to haemorrhage or the minor haemorrhagic episodes are ascribed to some *plasmatic* coagulation defect instead of the platelets.

4. Thrombosis of the small *vessels* leading to infarction, vessel-wall injury, anoxaemic haemorrhages in consequence of disturbed local blood supply, have also been incriminated, in the same way as a generalized vascular fragility.

Ad 1 and 2

Between the two extreme views, i.e. the platelets regarded as being completely normal in function and vice versa, as being completely devoid of any clotting capacity (SIEDE [32]), there are various opinions. SIEDE found a reduced tendency to agglutinate of the platelets associated with marked anisocytosis. While the morphological abnormalities of the platelets have been mentioned by numerous authors, investigations into platelet functions have been relatively scarce. MACPHERSON [33] had noted only a reduced platelet adhesiveness which, however, has not been confirmed by others [34].* BIGELOW found the serotonin content of the platelets abnormally low [35] and thought that this was to account for a supposed vasoconstrictive deficiency. This assumption is not very convincing; it is for instance at variance with the fact that a reduction of the serotonin level entails no disturbance of haemostasis. The clotting function of the platelets seemed to be a more promising topic of research. The TGT-values first studied in HT by HARDISTY and WOLFF [3], point to a deficiency in platelet function. The findings of SPAET [1] confine this abnormality to concentrated platelet suspensions, adequate dilution of which results in normal TGT-values. He even showed that normal platelets behave abnormally in concentrated suspensions; this was later confirmed by KOLLER and BOUNAMEAUX [36]. These findings suggest that the platelets can inhibit coagulation when they are in excess. In 27 cases of HT reviewed by SOULIER et al. [37] normal TGT-figures, as well as values pointing to an altered platelet function may equally be found. There were not only substantial individual differences, but the same patients yielded different results on various occasions. Suggestive evidence in favour of the surmised anticoagulant effect was brought by BAUMGARTNER and VUILLE [38]; HIEPLER [39] even went so far as to connect the inhibitory effect with the isolated alpha-granulomer fraction containing the 3rd platelet factor. PERLICK [40] could demonstrate an inhibitory effect only in the patients' platelets. An inhibitory effect was demonstrable in both our cases Nos. 1 and 2. We must not forget that what we examined were damaged platelets; intact ones would be most unlikely to display an inhibitory effect (HIEPLER [39]).

* Recently, CRONBERG et al. (Scand. J. Haemat. 1965. **2.**, 208) have found defective platelet adhesiveness in HT by an *in vitro* test. However, the *absolute* numbers of adhesive platelets were in every case in or above the normal range.

It must be stressed that, in agreement with other observations, the coagulation abnormality ceases parallel with the normalization of the peripheral platelet count. This would be a case in support of the inhibitor theory. On the other hand, in agreement with SPAET [1] we find it hard to believe that the excessive number of platelets should fail to make up for a partial platelet defect.

Ad 3

Reduction in the level of practically all of the plasma coagulation factors has been recorded by one or several authors, the abnormality having been ascribed by certain investigators to a disturbed production, by others to an increased utilization. The notion of "consumption coagulopathy" as conceived by LEVIN [41] has been built upon the assumption that minor thrombi, though clinically unnoticed, give off clotting components released from massive platelet agglomerates into the streaming blood, this results in a consumption of fibrinogen, prothrombin, and possibly of other factors. This mechanism would seem to account for the haemorrhagic episodes.

Extensive study with the TGT has shed light on the disorders of the early phase of coagulation. Reduction in factors VIII (AHG) and IX (PTC) was demonstrable. Abnormal TGT-values indicative of a reduction in factor XI (PTA) have been found in our second case. This is the first such case to our knowledge. The minor reduction in the activity of the coagulation factors must be sought in a failure of the optimal platelet concentration which is supposed to be necessary for their activation, whereas the production of these factors seems to be undisturbed [43, 44]. The presence of antithromboplastin has been demonstrated repeatedly by PERLICK [40].

Evidence of a plasma abnormality is not uncommon, but the defect is slight and by no means consistent. As soon as the platelet count falls below 500,000, the normal conditions are restored.

Ad 4

Of all conjectures as to the possible causes of haemorrhage in HT, that of vascular origin is the least convincing. To begin with, the clinical type of haemorrhage widely differs from that associated with increased vascular fragility. The laboratory findings and, above all, the tourniquet test, show, apart from exceptional cases, no abnormality. Local disturbances of blood supply cannot be ruled out with absolute certainty, nevertheless it is most unlikely that the common prolongation of bleeding time should be of vascular origin, the less so as, just like the other defects, it is restored to normal as soon as the platelet count has fallen below a certain level. (In fact, the number of conditions in which prolongation of bleeding time finds no other explanation than vascular abnormality, has shrunk to a low figure, if not to zero, as more

and more of these anomalies are being found to be corrigible by one or the other of the plasma factors or by platelets. It is a question whether there is any basis for the concept of "vascular pseudohaemophilia" [46].)

On the evidence of the literature, bleeding time is prolonged in 60 to 70 per cent of the cases with HT, particularly when IVY's method is being used. The other current method, that of DUKE, is less sensitive [47]. Bleeding time was prolonged in three out of our four cases, in the fourth it was at the upper limit of normal. The theories outlined above fail, however, to explain the cause of this abnormality and here we have arrived at the crucial point of our discussion.

The most consistent haemostatic feature of HT is the increased platelet count far in excess of normal values, associated with quantitative and perhaps qualitative changes of the thromboplastic (coagulant) platelet factor. Bleeding time is usually prolonged. On the evidence of the TGT, the excessive mass of platelets displays an anticoagulant effect, but the global clotting tests (coagulation time, prothrombin consumption) are within the normal range and the vascular fragility tests are also negative. The type of haemorrhages are not purpura-like, neither do they show any sign of the coagulopathies. They rather remind of bleedings associated with thrombasthenia, thrombocytopathies and VON WILLEBRAND's syndrome (also considered recently a thrombocytopathy), as regards the prevalence of haemorrhages of the mucous membranes, particularly of the gastrointestinal tract, nose, gums, menorrhagia, as well as of bleedings associated with surgical or other injuries. The actual bleeding tendency is closely correlated with the peripheral platelet count and less strongly, but confirmed by various observers, with the bleeding time. In the face of this evidence there is as little justification to ascribe the haemorrhages in thrombocythaemia to a coagulopathy, as there would be to connect the prolonged bleeding time with a coagulation abnormality. There is more reason to seek the cause of prolonged bleeding time, and of the consequent haemorrhages in some defect of primary haemostasis which concerns the platelets alone leaving the plasmatic clotting system out of consideration.

Since it was shown by ZUCKER [48] in 1947 that the first step of the haemostatic reaction taking place in the small vessels was the appearance of a platelet plug at the site of the endothelial damage, the participation and decisive role in haemostasis of the platelet system has been confirmed and clarified in many of its aspects by extensive studies (LÜSCHER [49], ZUCKER and BORRELLI [50], ROSKAM and his school [51, 52, 53], OWREN and his school [54, 55, 56, 57], SHARP [58], SPAET [59], etc.)

The graph constructed according to HELLEM and OWREN's scheme illustrates the dynamic concept of platelet functions.

After injury to the endothelial cells, the platelets adhere to the raw surface of collagen fibres. Little is known about the mechanism of this process.

It is, however, certain that it invariably occurs as the first step of haemostasis even in pathologic conditions, with the exception of thrombocytopenia. Damaged cells, consequently also damaged platelets, release adenosine diphosphate which, together with other factors, causes a *reversible platelet aggregation* (Fig. 1). This process fails to appear in thromboasthenia, in WILLE-

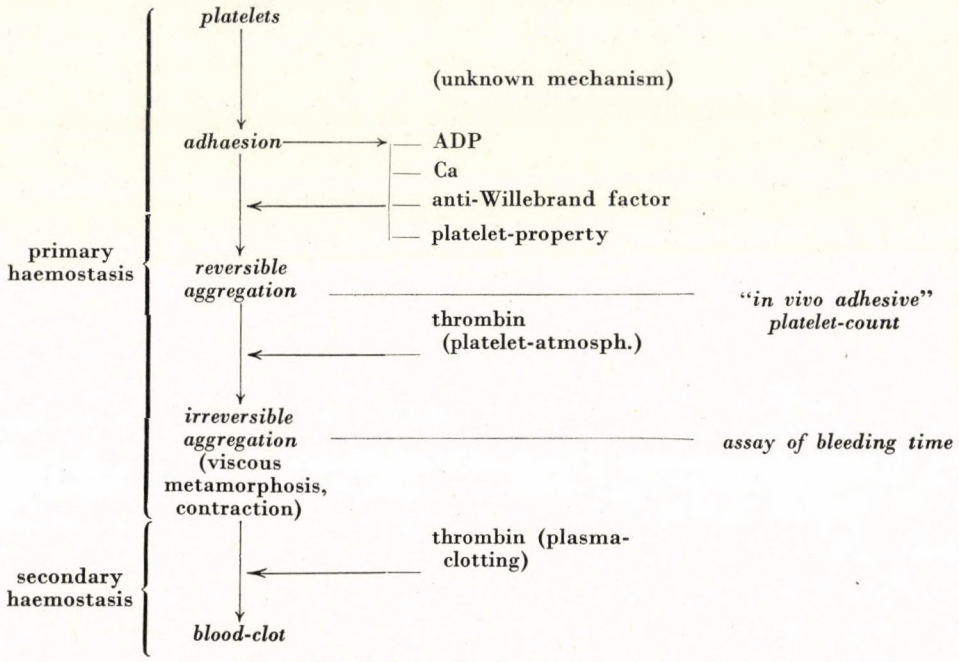

Fig. 1. The role of platelets in primary haemostasis

BRAND's disease, and in certain, mostly acquired, thrombocytopathies, particularly in cases caused by macroglobulinaemia. Athrombia was the original term applied to this anomaly by FRANK [61], in 1925. In the process representing the first step of *primary haemostasis* no clotting mechanism is involved. Its disturbance results in a prolongation of the bleeding time. Examination of the *in vivo* platelet adhesiveness or perhaps more exactly of the platelet aggregation or platelet consumption *in vivo*, described recently by BORCHGREVINK [14] is best suited for the study of this phase. In fact it has definite practical advantages over the various *in vitro* aggregation and adhesion tests.

The next step of haemostasis is that of *irreversible aggregation*, viscous metamorphosis and contraction of platelets. In the course of this process the platelet plug, which at first has been permeable, acquires a firmness and becomes impermeable to blood. This is the second step of primary haemostasis, which, according to the general belief, requires the presence of thrombin in minimal amounts. But the source of this thrombin is not and cannot even be the plasma

at this stage, since it is only at the terminal phase of viscous metamorphoses, upon the very effect of thrombin, when the clotting factor of intrinsic thromboplastin is liberated from the platelets together with other substances. It is difficult to account for the presence (though in traces) of a substance at a stage when it cannot yet be possibly formed in the regular way. OWREN et al. have provided an answer to this question by demonstrating that the required amount of thrombin is formed on the surface of the platelets, in the platelet atmosphere, under the effect of either intrinsic or extrinsic factors. Massive amounts of anticoagulants are necessary to block this mechanism which proceeds normally for instance in haemophilia and in the majority of coagulopathies. This is the reason why in the latter types the bleeding time is normal this being a reliable indicator of the whole process of primary haemostasis.

When reversible aggregation is undisturbed, as confirmed by *in vivo* tests, but bleeding time is prolonged, then the second phase must be defective. This is probably the case in certain congenital thrombopathies, as for instance in BERNARD—SOULIER's disease where, at least on the evidence of *in vitro* tests, adhesivity and aggregation are normal. If this can be confirmed *in vivo* then the cause of the prolongation of bleeding time must be sought in some abnormality of thrombin production. This also applies to exceptionally severe cases of haemophilia [62] and to certain induced coagulopathies, e.g. by massive doses of heparin or dextran sulphate. Only scanty observations exist concerning the congenital haemorrhagic abnormalities associated with a prolonged bleeding time and normal platelet aggregation *in vitro* and *in vivo*, where platelet transfusion — applied *ex juvantibus* — resulted in full correction [63].

Secondary haemostasis, the role of which is to protect and to secure primary haemostasis, involves the plasmatic clotting system.

Returning to the prolongation of bleeding time in HT, it may now be attempted to localize the defect. The number of platelets is amply sufficient for adhesion and aggregation. It could be ascertained in our cases that aggregation *in vivo* was duly achieved and that the number of platelets forming the plug was excessive. (To our knowledge, this aspect of HT has not been investigated.) On the grounds of assays *in vitro*, the factors required by viscous metamorphosis show no abnormality, likewise, there is no suggestion of any circumstance interfering *ab ovo* with the production of the required amounts of thrombin. Nevertheless, we have every reason to believe that the abnormality involves the *second phase* of *primary haemostasis*, presuming that the aphysiologic, massif platelets entail an undesirable shift in the optimal relation between platelet plug and thrombin-supply. This question is by no means solved, all we know is that for some reason, production of an impermeable platelet plug capable of sealing up the small vessels fails to be formed in most cases of HT.

In HT the secondary haemostasis, in other words, the coagulation process may also be affected. At high concentrations, the platelet-phospholipids may have a local inhibitory effect on the production of thromboplastin, for which reason formation of the definitive clot may be delayed. This probably plays a part in surgical or traumatic haemorrhages including rebleedings. Coagulation studies *in vitro* usually fail to detect this abnormality, in all probability because the medium- and concentration relationships as well as the time factor are quite different *in vivo*.

The part played by platelets in the process of haemostasis is now generally recognized, but not yet clear in all its aspects. Inquiry into the haemorrhages associated with HT may provide further informative evidence.

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CHANGES IN CEREBRAL ELECTRICAL ACTIVITY IN EXPERIMENTAL HYPEROXIA

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Electroencephalograms made with the use of implanted cortical electrodes of rats exposed to O_2 at 3.7 atm. pressure revealed gross, non-specific changes in frequency and amplitude, as well as spikes. These changes presented themselves before any change in respiration or cardiac action had become apparent, and before the first phase of pulmonary oedema had set in; at that time arterial O_2 saturation was still normal. Absorption of exhaled CO_2, i.e. prevention of rebreathing it, delayed the EEG changes and, to some extent, warded off spike potentials. — Impaired cerebral activity is the first symptom of O_2 poisoning.

The pathomechanism of lesions elicited by high pressure O_2 still awaits clarification. Some authors have stressed the causative role of local pulmonary damage [18, 21, 24, 26, 27, 28, 29], impaired pulmonary circulation or right ventricular heart failure [6]. Others implicate the neuroendocrine system [3, 4], and yet others incriminate the central nervous system [17] on the grounds that exposure to high pressure oxygen is known to give rise to spasms, opisthotonus, ataxia, and paralysis of the extremities in laboratory animals. Histological changes occur in the cerebral cortex and the reticular formation of such animals. Since these changes appear before the pulmonary oedema characteristic of oxygen poisoning, they cannot result from the associated respiratory failure [15, 16]. Evidence has also been produced that under high O_2 pressure, inhalation of 1.5 to 4 per cent CO_2 aggravates symptoms and shortens survival [13, 34].

The present paper reports on investigations into cerebral electrical activity in oxygen poisoning, the first appearance of changes in wave pattern, and the influence exerted on them by the presence or absence of CO_2. These are questions to which the few electro-encephalographic studies to be found in the literature [10, 14, 33] have failed to supply elucidative answers.

Materials and methods

Two frontal and two occipital electrodes were built in each of 29 albino rats of 110 to 250 g body weight. As soon as the wounds had healed, which took 5 to 7 days, an electroencephalogram was made of the conscious animal using a four-channel direct-writing apparatus. The experiments proper began when the tracings were found to have become normal. Singly or in pairs, the rats were then placed in a chamber of 60-litre capacity at 3.7 atm. O_2 pressure.

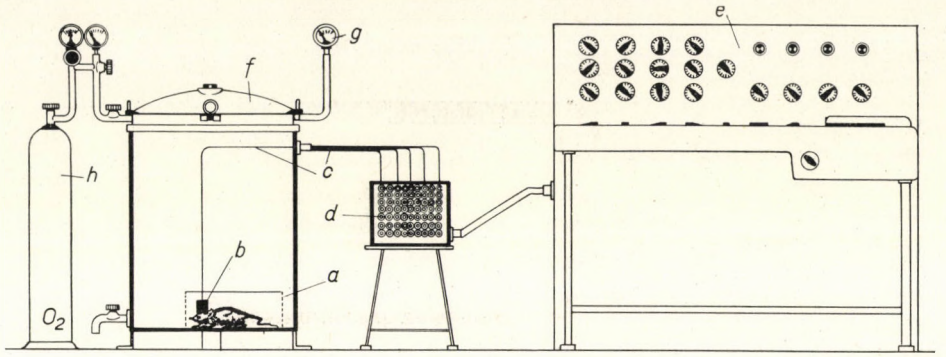

Fig. 1. Experimental arrangement *a*: Cage inside oxygen chamber. *b:* Electrode holder fixed on the rat's skull. *c:* Cables conducting cerebral electrical activity. *d:* Cable terminal box. *e:* Electroencephalograph. *f:* Hyperbaric oxygen chamber. *g:* Manometer indicating pressure inside chamber. *h:* Oxygen cylinder with pressure reductor

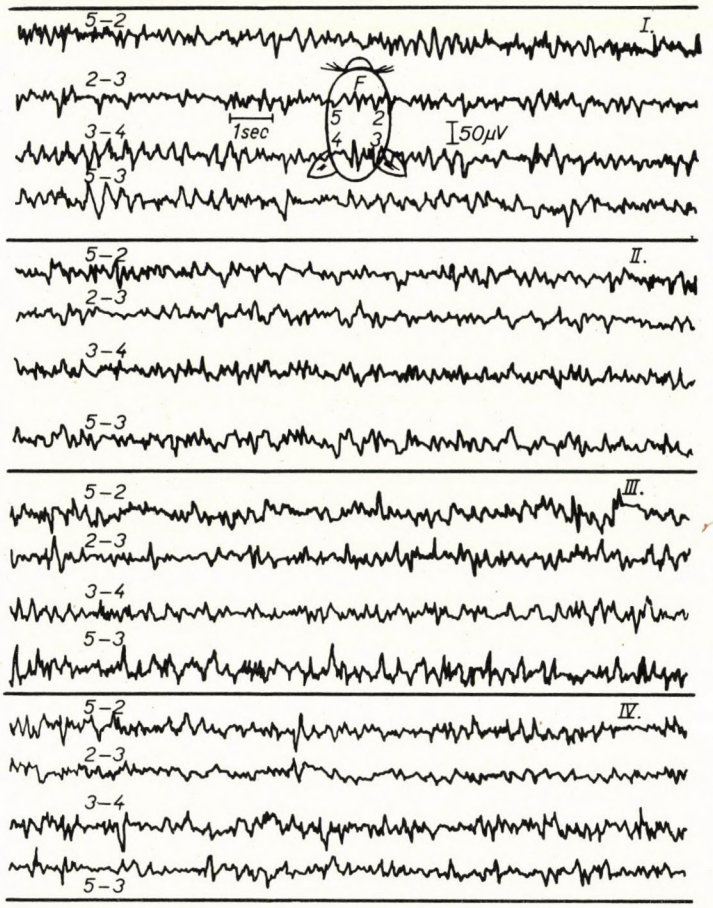

Fig. 2. EEG of rat AK, observed for 120 minutes in chamber containing air of room temperature at atmospheric pressure. I: Starting record. II, III and IV*:* Records at 30, 60 and 120 minutes, respectively. Frequency and amplitude changes within the ± 15 per cent range; spike discharges absent

To prevent intertanglement of the cables or disconnection of the plug inside the chamber, the animals were immobilized in comfortable plaster boxes lined with a soft material adjusted to body size. In some experiments the CO_2 contents of the chamber were augmented artificially. In others, in order to study the effect of CO_2 deficiency, the exhaled gas was freed from CO_2 by means of 2000 g of NaOH and $CaOH_2$ spread over wide areas in the chamber. At the end of each experiment, the CO_2 contents of the chamber were determined by the Scholander method. Fig. 1 presents the diagrammatic view of the experimental arrangement.

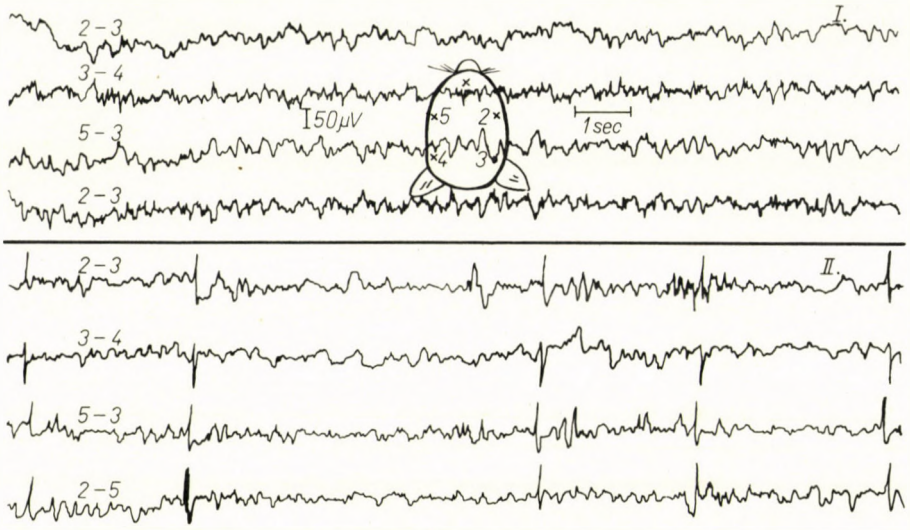

Fig. 3. EEG of rat H3, exposed to oxygen containing 1.85 per cent CO_2 at 3.7 atm. pressure. I: Starting record. II: Record made after exposure for 90 minutes showing spike discharges in all leads and significantly lowered frequency

On the EEG made before beginning the experiments, several 6-sec curve sections were analyzed, curve frequencies determined by counting, and the amplitudes measured. Values found for normal animals were considered 100 per cent. Each animal was subjected to continuous electro-encephalography throughout the experiment.*

The severity of pulmonary oedema was graded on histological evidence as follows.

+++ means oedema extending to more than one lobe, filling alveoli, bronchi and perivascular spaces;

++ oedema restricted to one lobe;

+ focal lung oedema.

In addition, severity was determined by the relative lung weight

$$\left(\frac{100 \times \text{lung weight}}{\text{body weight}}\right)$$

which was less than 1 for the intact and more than 1 for the oedematous lung.

* Although the changes in wave pattern were marked, attempts were made to qualify them mathematically. Frequency analysis by simple counting and by Fourier's method with the use of an Ural II electronic computer, yielded essentially the same data. — The authors wish to thank the Central Statistical Office, Budapest, for permission to use their computer, and Mr. B. Hajtmann, Mathematician of the Institute of Experimental Medicine, Budapest, for his co-operation in the studies.

Results

Six animals each were fixed firmly in a given posture and kept for 100 minutes on the average in a dark chamber, breathing air of room temperature and normal pressure, to be studied for the effect of these conditions on

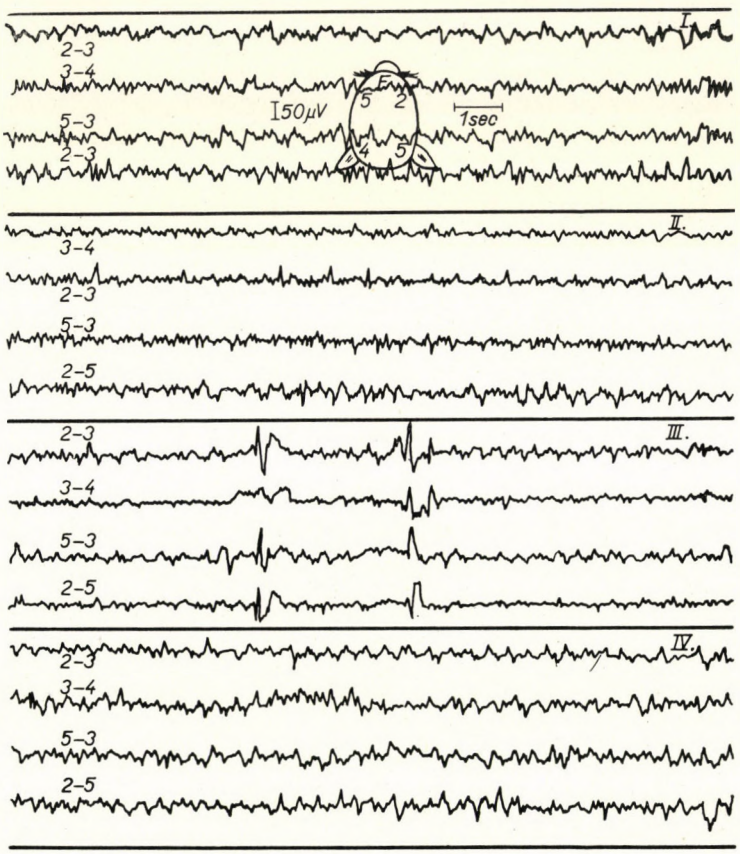

Fig. 4. EEG of rat R. 1.: Starting record. II. Record made after exposure for 70 minutes to CO_2-free O_2 at 3.7 atm. pressure showing significant rise in frequency and decrease of amplitude. III. After exposure for 150 minutes spike discharges appear. IV: 24 hours after termination of exposure to oxygen, cerebral activity is again normal

cerebral electrical activity. Spikes were absent and changes in frequency and amplitude remained within ± 15 per cent of the initial values (Fig. 2). In subsequent experiments, changes exceeding ± 15 per cent were only considered pathological.

Nine animals were studied in pressurized O_2 chambers of high (1.57 to 3.05 per cent) CO_2 content. EEG records were made in 6 of them; all showed spikes and significant changes in frequency and amplitude, though varying

in direction (Fig. 3). Spike potentials made their appearance in one animal after 50, in three after 90, and in one each after 105 and 160 minutes of exposure (Fig. 3 II). As seen in Table I, in the animals sacrificed after 90 to 110-minute exposures, cerebral electrical activity was damaged first and pulmonary oedema followed it. The surviving animals developed grave pulmonary oedema.

In a third series of experiments an answer was sought to the question whether removal by absorption of the CO_2 from the chamber would prevent or at least delay the disturbance in cerebral activity. All the rats survived the exposure to high pressure O_2 for an average duration of 170 minutes. At the end of the experiments not more than 0.03 to 0.034 per cent of CO_2 was found in the gas samples taken from the chambers (Table I). The EEG of the survivors revealed changes in frequency and amplitude exceeding ± 15 per cent

Table I

Relative lung weights, histology, and time of first appearance of EEG changes

Experiment	Animal	Exposure time (min)	First appearance of		Relative lung weight	Pulmonary histology (oedema)	Note
			EEG changes	spikes			
			(min)	(min)			
3.7 atm O_2 + 1.57—3.05% CO_2	H 1	160	100	160	1.32	++	sacrificed
	R 1	90	22	50	1.24	+	,,
	R 2	110	100	105	0.52	neg.	,,
	R 3	100	90	90	0.59	,,	,,
	H 3	90	90	90	0.63	,,	,,
	R 5	105	75	90	0.87	,,	,,
3.7 atm O_2	101	180	90	absent	survived		
	CKH	180	90	absent	,,		
	AKR	180	90	140	,,		
CO_2 absorption	R8	150	70	150	,,		
	SH	180	180	180	,,		
	H8	150	150	absent			
Control	H 4	90	absent	absent	survived		
	R 4	90	,,	,,	,,		
	CK	90	,,	,,	,,		
	AK	120	,,	,,	,,		
	H 7	75	,,	,,	,,		
	R 6	125	,,	,,	,,		

of the initial values (Fig. 4 II and III). Spike potentials appeared in half the animals, and after an average exposure of 158 minutes. Twenty-four hours later, frequency and amplitude returned to normal, and spike disappeared (Fig. 4 IV).

Arterial O_2 saturation was determined at the 150th minute of the experiment, and found to be normal in each of the 8 animals examined (Table II).

Table II

Oxygen saturation of arterial blood at 3.7 atm. oxygen pressure

Animal No.	Blood sample taken at min.	Relative lung weight	Pulmonary histology (oedema)	Arterial O_2 saturation, %
1	90'	0.87	neg.	96
2	90'	0.87	neg.	96
3	120'	0.81	neg.	96.5
4	120'	1.20	+	96
5	120'	0.94	neg.	97
6	150'	0.94	+	98
7	150'	1.54	+	95
8	150'	1.09	neg.	94

Discussion

Studies of the effects of high pressure O_2 have progressively gained in importance since hyperbaric oxygen has been used for resuscitation of the newborn [19], in the treatment of certain types of poisoning, principally CO poisoning [32], and in heart surgery [21].

In the present experiments, inhalation of 3.7 atm. O_2 was found to exert an appreciable effect on the rat's cerebral activity in the early phases of exposure. When the rats breathed in O_2 containing 1.57 to 3.05 per cent CO_2, the EEG showed significant changes in frequency and amplitude after exposure times varying between 22 and 100 minutes, and in all animals there appeared spike potentials signifying excitation of the central nervous system. When the gas exhaled by the animals was freed from CO_2, the aforementioned significant EEG changes presented themselves after exposure times ranging from 70 to 180 minutes, and spike potentials appeared in only half the number of rats. The EEG showed the appearance of changes when respiration rate and heart action were still perfectly normal and convulsions absent. Absorption of the exhaled CO_2, i.e. prevention of rebreathing it, delayed but did not ward off changes in cerebral activity, which are of course non-specific. Such changes constitute the first sign of O_2 poisoning.

The EEG changes preceded the onset of pulmonary oedema characteristic of O_2 poisoning. This supports our earlier view, based on data bearing on

other problems [15, 16, 34, 35], that the central nervous system plays an important role in the pathogenesis of hyperoxic pulmonary oedema; severe neurohistological lesions [15] and tissue hypercapnia [34, 35] had been observed to develop in rats exposed to O_2 poisoning. The EEG informs early of damage to the central nervous system occurring during the development of experimental pulmonary oedema.

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BLOOD PRESSURE AND EXTROCEPTIVE PRESSOR REFLEX IN LYMPHOGENOUS ENCEPHALOPATHY

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1. Cervical lymphatic obstruction is followed by statistically significant oscillations of blood pressure.

2. In lymphogenous encephalopathy, as opposed to normal conditions, the painful stimulus due to conjunctival instillation of capsaicin elicits an increased pressor response and restricts the duration of blepharospasm.

3. The phenomena are attributed to disturbances of the central vegetative equilibrium and to morphologic damage suffered by certain central nervous structures.

Arterial hypertension is a common occurrence in conditions associated with increased intracranial pressure. Numerous papers concerned with the pathogenesis of hypertensive disease have quoted the experiments of HELLER [1a, b] who found increased blood pressure values after raising intracranial pressure by the intracisternal injection of kaolin. GRIFFITH [2] not only was able to reproduce these results but also to throw light on the mechanism of increased intracranial pressure. While, on the evidence of radiography, thorium dioxide injected intrathecally normally appears in the submandibular lymph glands, this does not occur after the intracisternal application of kaolin since this substance blocks the lymphatics draining the subarachnoid space. This observation has, however, remained unnoticed, and we could find no reference attributing any consequence to the obstruction of the lymphatics draining the subarachnoid space.

The present authors have produced a new experimental entity — "lymphogenous encephalopathy" — by ligation of the cervical lymphatics and lymph nodes. Among the features of the new syndrome, increased intracranial pressure, cerebral oedema and papilloedema are relevant with respect to the present studies concerned with the behaviour of blood pressure in lymphogenous encephalopathy.

Methods

22 mongrel dogs of both sexes were used. The common carotid of one side was prepared through a median cervical incision under hexobarbital anaesthesia, and a polyethylene catheter was passed into the aorta, to allow the electromanometric recording of blood pressure by a Hellige-type multiscriptor apparatus in the alert, freely moving animal, as described by THURÁNSZKY [19].

In 13 animals insertion of the catheter was followed by ligation of the cervical lymphatics and lymph nodes, as described earlier [13]. In 9 animals the lymphatics and lymph nodes were exposed but not ligated. Daily measurements were carried out

1. of the resting blood pressure;
2. of the intensity and duration of the pressor response elicited by the conjunctival instillation of a 0.5 per cent solution of capsaicin. The duration of blepharospasm was also recorded. In normal animals intraconjunctival instillation of capsaicin represents a strong chemical pain stimulus which consistently elicits a distinct elevation of blood pressure and blepharospasm without any damage to the tissues [20].

The data obtained were evaluated statistically.* Since the technique used made non-surgical blood pressure readings impossible, the animals with lymphogenous encephalopathy were compared with controls subjected to a sham operation.

Results

Blood pressure was found to oscillate during the postoperative days. The extreme values for the animals with lymphogenous encephalopathy were significantly higher than those for the control group (Tables I to III). It was furthermore analyzed whether, in relation to the first postoperative day, the subsequent values tended to rise or to decline. Evaluation on the basis of maximal and minimal deviations showed that in the lymphogenous encephalo-

Table I

Blood pressure in dogs with lymphogenous encephalopathy

No. of animal	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
	day after operation											
40	105	125	105	120	82.5	—	—	—	—	—	—	—
44	82.5	95	125	132.5	115	60	95	105	—	—	—	—
12	95	95	105	117.5	125	145	115	—	—	—	—	—
11	85	85	85	122.5	122.5	122	95	92.5	—	—	—	—
7	105	137.5	115	92.5	72.5	95	82.5	105	70	62.5	—	—
8	95	125	125	122.5	105	105	105	70	80	80	115	105
6	115	115	125	—	—	—	—	—	—	—	—	—
5	115	125	125	85	40	105	—	—	—	—	—	—
4	115	135	80	115	105	135	80	105	105	75	85	105
76	105	137.5	135	87.5	115	135	105	80	115	70	105	107.5
14	95	115	125	105	150	85	130	100	105	85	125	100
35	115	142.5	125	87.5	127.5	105	80	100	72.5	105	130	100
18	105	125	132.5	125	60	110	135	105	82.5	—	—	—

The figures in the upper horizontal line represent the days after operation.

pathy group both increase and reduction were significant while in the control group the increase of blood pressure proved only significant, not its decrease. The extent of increase and decrease was likewise significantly greater in the lymphogenous encephalopathy group than in the control group (Table IV).

* We are indebted to the Section of Biometry (Head: Dr. I. Juvancz), Institute of Adapted Mathematics, Hungarian Academy of Sciences, for the statistical computations.

Table II

Blood pressure after cervical sham-operation

No. of animal	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
	day after operation											
46	105	95	105	107.5	107.5	105	100	105	105	105	—	—
45	107.5	105	110	107.5	105	105	105	100	105	112.5	—	—
24	105	105	105	112.5	105	107.5	105	—	—	—	—	—
16	95	105	105	105	105	105	110	105	105	117.5	105	105
17	105	100	100	105	107.5	105	—	—	—	—	—	—
34	110	105	102.5	105	112.5	105	105	110	110	105	105	105
78	105	105	117.5	105	105	110	110	110	105	105	105	110
69	117.5	105	110	100	105	110	100	105	105	—	—	—
37	95	105	105	105	105	105	100	100	105	105	110	—

The figures in the upper horizontal line represent the days after operation.

Table III

Statistical evaluation of blood pressure oscillations

	Lymphogenous encephalopathy	Sham-operation
n	13	9
$\bar{x}$	59	12
s	20	4
t	8.4	
P%	<0.1	

Table IV

Statistical evaluation of blood pressure changes

	Increase		Decrease	
	lymphogenous encephalopathy	sham-operation	lymphogenous encephalopathy	sham-operation
n	13	9	13	9
$\bar{x}$	31	7	27	4
s	14	9	22	9
t	7.8	2.4	4.4	1.4
P%	<0.1	<5	<0.1	>10
t	4.5		3.4	
P%	<0.1		<1	

The peaks of blood pressure occurred on the average 2.2 days after operation in the animals with lymphogenous encephalopathy, and 5.1 days after operation in the control group. In other words, the blood pressure elevation after cervical lymphatic blockade was almost immediate, while after sham operation it was relatively delayed. The intensity of pressor response to capsaicin did not significantly differ in the two groups, but from the fifth day onwards blood pressure rose significantly higher in the lymphogenous encephalopathy group than in the control group where the maximal blood pressure values were unchanged during the eleven-day period (Table V).

Table V

Pressor response to capsaicin after sham-operation (s.op.) and lymphatic obstruction (l.ob.)

Days after operation	1		5		7		9		11	
	s.op.	l.ob.	s.op.	l.ob.	s.op.	l.ob.	s.op.	l.ob.	s.op.	l.ob.
n	6	4	6	4	6	4	5	4	3	3
x	26.7	32.5	28.3	71.2	25.0	85.0	32.0	69.8	26.7	51.7
s	16.6		17.1		16.4		20.0		14.0	
t	0.5		3.9		5.7		2.8		2.2	
p%	>60		<1		<0.1		<5		<5	

Table VI

Duration of pressor response after sham-operation (s.op.) and lymphatic obstruction (l.ob.)

Days after operation	1		5		7		9		11	
	s.op.	l.ob.	s.op.	l.ob.	s.op.	l.ob.	s.op.	l.ob.	s.op.	l.ob.
n	6	4	6	4	6	4	5	4	3	3
$\bar{x}$	3.2	5.8	3.2	8.1	2.7	11.2	3.0	8.5	2.3	5.5
s	1.9		1.2		1.4		2.8		1.2	
t	2.0		6.1		9.6		2.9		3.3	
p%	>5		<0.1		<0.1		<5		<5	

It was likewise from the fifth day onwards that the duration of the pressor response to capsaicin began to diverge significantly in the two groups. In the animals with lymphogenous encephalopathy normalization of blood pressure took longer than in the controls where the time required for the blood pressure to regain its original level was unchanged during the eleven-day period (Table VI).

In the controls, the duration of blepharospasm elicited by capsaicin became progressively shorter until the eleventh day as compared to the initial

Table VII

Duration of blepharospasm elicited by capsaicin

a) Changes correlated to preoperative findings

Day after operation	Sham-operation		Lymphatic obstruction	
	5	7	1	11
n	7	7	8	8
diff.	6.6	20.9	51.0	20.6
t	0.9	5.8	9.5	2.7
p%	>30	<0.1	<0.1	<5

b) Correlation of changes

Day after operation	1		9		11	
	s.op.	l.ob.	s.op.	l ob.	s.op.	l.ob.
n	7	8	7	8	7	8
diff.	10.4	51.0	20.3	58.9	25.4	20.6
t	5.6		2.5		0.6	
p%	<0.1		<5		>50	

values. In the lymphogenous encephalopathy group the duration of blepharospasm successively diminished until the fifth day, to rise after this time without, however, attaining the initial values during the whole course of the experiment. Until the 9th day, the reduction was significantly more marked in the lymphogenous encephalopathy group than in the controls (Table VII).

The duration of blepharospasm correlated with that of the pressor response was significantly shorter in the lymphogenous encephalopathy group from the fifth day onwards (Table VIII).

Table VIII

Correlations between pressor response and duration of blepharospasm

Days after operation	1		5		7		9		11	
	s.op.	l.ob.	s.op.	l.ob.	s.op.	l.ob.	s.op.	l.ob.	s.op.	l..ob
n	6	4	6	4	6	4	5	4	3	3
$\bar{x}$	99.2	48.7	101.0	4.3	114.0	10.5	99.8	32.0	146.0	53.1
s	34.2		31.6		40.1		34.0		34.8	
t	2.3		4.7		4.0		3.0		3.3	
p%	=5		<1		<1		<5		<1	

Discussion

GRIFFITH et al. induced hypertension in 39 out of 85 rats by the intracisternal injection of kaolin. According to their graphs, the rise of blood pressure reached its peak between the fourth and eighth days after the injection of kaolin. In our studies obstruction of the lymphatic drainage of the intracranial space, whether carried out from inside by injection of kaolin, or from outside by surgical means, entailed a transitory rise of blood pressure. Return of blood pressure to its initial level or still farther below, as witnessed in our experiments, might have been due to the following factors:

a) The pressor effect of lymphatic ligation might have released a parasympathetic counterregulation.

b) Surgical obstruction of the cervical lymph channels is hardly ever complete. The lymphatics which have remained patent dilate. Further lymphatic channels may open up making thus the consequences of lymphatic obstruction reversible. FÖLDI et al. have pointed out in an earlier paper [13] that the animals with lymphogenous encephalopathy are indolent, drowsy, sluggish in response to painful stimuli and their EEG findings are consistent with those recorded under the effect of chlorpromazine. This peculiar observation is in line with the finding of THURÁNSZKY in that capsaicin blepharospasm is restricted in duration by chlorpromazine, and in a lesser degree, by reserpine. In these experiments the pressor response to capsaicin was also reduced by the two drugs. On the other hand, no agent could be found which would have enhanced the pressor response but shortened the duration of blepharospasm. In other words, in normal animals the two reactions are of the same direction. The pressor response in lymphogenous encephalopathy thus differs from that elicited after the application of tranquillizers. In lymphogenous encephalopathy the pressor response to capsaicin is enhanced in all probability because higher inhibitory structures have been damaged. Our earlier studies [7] have revealed severe neuropathologic changes in lymphogenous encephalopathy.

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QUANTITATIVE CHANGES IN THE SERUM HEPARIN LEVEL AFTER THYMECTOMY, HYPOPHYSECTOMY, ADRENALECTOMY OR CORTICOID TREATMENT IN THE RAT

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Treatment with formalin or glycocorticoid has been found significantly to lower the serum heparin level. Removal of the adrenals raised it, but thymectomy prevented this rise in the adrenalectomized animal. Hypophysectomy raised the level significantly.

DOCA treatment increased and hydrocortisone treatment decreased the serum heparin level to a lesser extent in the thymectomized animal.

Heparin inhibits adrenocortical activity in the rat [1, 2] and reduces 24-hour urinary 17-ketosteroid excretion in man [3]. Glycocorticoid reduces the number of mast cells [4, 5] and the amount of mucopolysaccharides in tissues [6, 7]. The number of mast cells in the thymus is high [8, 9, 10, 11]; the gland contains PAS-positive substance [12, 13] and heparin [14]. Adrenocortical hyperactivity or glycocorticoid treatment causes thymo-lymphatic involution [15, 16].

Recent experiments have shown serum and heart lipoprotein lipase activity to decline in the thymectomized animal [17]. As this decline might be one of the causes of the fall of the heparin level, this being the co-ferment of the enzyme, the present work was undertaken to study in the rat the quantitative changes of the serum heparin level under the effect of thymectomy and glycocorticoid treatment, known to result in thymo-lymphatic involution.

Material and methods

A total of 223 male Wistar-strain rats was selected for uniformity in body weight (120 ± 20 g) and groups of them varying in number (cf. Fig. 1 and Table I) were subjected to one of the following procedures:

(a) Thymectomy following sternotomy under ether anaesthesia.

(b) Bilateral adrenalectomy from the lumbar approach under ether anaesthesia. Postoperatively, these animals were given 0.9 per cent physiological saline to drink.

(c) Exposure of the cerebral base from the parapharyngeal approach and removal of the pituitary by aspiration. Data for animals with successful hypophysectomy at autopsy and histological examination, have only been included in this series.

Formalin and corticoid treatments were as follows, all per 100 g of body weight:

(a) A single dose of 0.3 per cent formalin was injected subcutaneously, and the animals were decapitated one hour later.

(b) Thymectomized and sham-thymectomized animals were treated either with 0.5 mg of intramuscular hydrocortisone (Richter, Budapest), or 1 mg of intramuscular DOCA (Docaquosum, Organon, Oss) daily for eight days beginning 48 hours after surgery, and were decapitated one hour after the last injection. The control (sham-operated) group was treated with 0.9 per cent physiological saline. The animals were decapitated on the tenth day after thymectomy, adrenalectomy, or the corresponding sham operation. The blood obtained at decapitation was centrifuged without the addition of an anticoagulant. Serum heparin levels were estimated by the thrombin inactivation method described by GERENDÁS [18, 19, 20]. Statistical analyses were performed with Student's t test.

Results

The serum heparin level fell insignificantly after thymectomy ($p > 0.10$) and rose significantly after adrenalectomy ($p < 0.001$). Thymectomy following removal of the adrenals prevented this rise. Treatment with formalin reduced

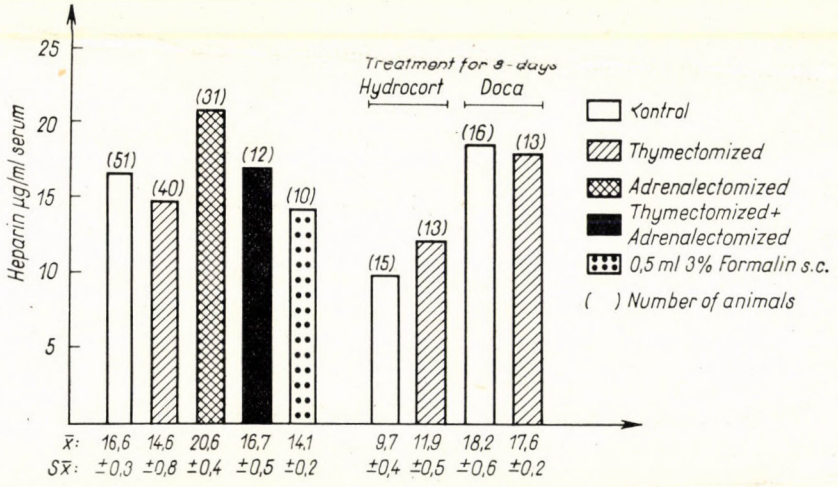

Fig. 1. Effect of thymectomy, adrenalectomy and corticoid treatment on serum heparin level in the rat

the serum heparin level ($p < 0.001$). Hydrocortisone also reduced it in thymectomized rats but much less markedly than in the sham-operated controls ($p < 0.01$ and $p < 0.001$, respectively). DOCA raised the serum heparin level insignificantly in the thymectomized animals ($p < 0.10$) but significantly in their sham-operated controls ($p < 0.01$) (Fig. 1).

As expected, hypophysectomy reduced body weight, adrenal weight ($p < 0.001$), and lienal weight ($p < 0.01$), and raised the serum heparin level significantly ($p < 0.001$) (Table I).

Discussion

In previous works [1, 2, 3] heparin had been studied for its inhibitory effect on adrenocortical activity. The present experiments were carried out to study the effect on the serum heparin level of the adrenals, treatment with

Table I

Effect of hypophysectomy on serum heparin level, adrenal weight, thymus weight and lienal weight

Experimental groups	Number of animals	Body weight g	Weight of adrenal mg/100 g body weight	Weight of thymus, mg	Weight of spleen, mg	Serum heparin, ug/ml
Control	9	175.6±7.1	19.78±1.25	210.8±14.3	187.1±9.4	17.20±0.11
Hypophys-ectomyzed	13	134.6±4.9	11.15±0.67	201.5±10.0	157.1±7.2	20.26±0.22
Significance			$p<0.001$	$p>0.50$	$p<0.05$	$p<0.001$

The values are means ($\bar{x}$) with standard deviations ($S_{\bar{x}}$)

corticoid, and thymectomy. In these experiments it was found that glycocorticoid lowered and DOCA raised this level to a lesser extent in thymectomized than in sham-operated rats. Its hould be noted that in earlier experiments [21] these corticoids had been demonstrated to have a similar effect on the serum properdin level of thymectomized animals.

In the present experiments thymectomy reduced the serum heparin level insignificantly, but prevented the marked rise of it which follows adrenalectomy. Thus they failed to supply information about the role of the thymus in the regulation of the serum heparin level, though in our view the gland may have a part in heparin synthesis and the heparin formed in the thymus may have a share in increasing lipoprotein lipase activity. In favour of this view argue the high number of mast cells in the thymus [8, 9, 10, 11] and the decline of serum and heart lipoprotein lipase activity observed in the rat following thymectomy [17].

CSABA et al. [22] observed that under the effect of cortisone a rise occurs in the serum acid mucopolysaccharide content which they determined by nephelometry of the turbidity caused by protamine sulphate [23]. The discrepancy between their data and ours may be explained by the difference in the methods used, among others.

CANNON [24] reported as early as 1914 that fright or fear (stress) shortens blood clotting time. MEYER et al. [25] confirmed this but demonstrated that clotting time is not shortened in the adrenalectomized animal. In the present experiments formalin, as a stressor agent, lowered the serum heparin level, and hypophysectomy raised it. We incline to the view that the formalin-induced stress lowered it by mobilizing endogenous ACTH, and hypophysectomy raised it by reducing ACTH secretion and adrenocortical activity.

The present results appear to show that adrenocortical activity is one of the factors controlling the serum heparin level. This agrees well with the data referred to above [4, 5, 6, 7] and those published by COSGRIFF [26] and

other authors [27, 28, 29] that treatment with ACTH or glycocorticoid increases the incidence of thrombo-embolic episodes.

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SERUM LIPIDS IN EXPERIMENTAL JAUNDICE

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The appearance in serum of abnormal beta-lipoproteins in obstructive jaundice has been demonstrated in animal experiments. An attempt has been made to clarify the causes of this abnormality by the chemical analysis of lipoproteins isolated from the serum of the animals under study.

Extra- or intrahepatic biliary obstruction belongs to the major predisposing factors of abnormal lipid metabolism. Absence of bile is known to interfere with the digestion of fats in the intestines [17, 13]. Parallel with the development of jaundice, the level of total lipids in blood serum increases [3, 4, 20]. The fractions largely responsible for this increase are beta-lipoproteins, and also constituents of an abnormal structure [7, 12, 15].

On the basis of an empirical test (JIRGL, 1957) the present authors (KREMMER, FERENCZY, 1964) have demonstrated a hitherto unknown abnormality of fat metabolism in obstructive jaundice [11]. The pattern is made up of abnormal beta-lipoproteins, labile to aromatic sulphonic acids, with easily split off lipid components. A study of an extensive clinical material has shown that changes of lipoprotein stability ensuing upon specific influences of this kind are characteristic of conditions associated with jaundice, but are extremely rare in other conditions. The phenomenon seemed to deserve further study.

Material and methods

According to an earlier communication of JIRGL [9] ligation of the common bile duct can conveniently be carried out in rats. We used inbred rats of both sexes weighing between 100 and 150 g. Semi-synthetic food and water was offered ad libitum. High ligation of the common bile duct was performed in groups of 5 animals, then 48 hours after the operation the animals were killed under ether anaesthesia by bleeding through the femoral vein. The control animals which had been subjected to laparotomy only, were bled in the same manner. From the clear centrifuged blood serum of the jaundiced and control animals the following tests were performed: serum bilirubin by the method of JENDRASSIK-GRÓF [1]; total lipids, by the gravimetric procedure of SPERRY and BRAND [16]; then, after redissolving the lipids, total cholesterol according to LIEBERMAN [1] and phospholipids by the method of ZIELVERSMIT [21]. Beta-lipoproteins were isolated with dextran sulphate according to BURNSTEIN and SAMAILLE [2]. After repeated extractions with $CHCl_3 : CH_3OH$ (2 : 1), the eluted precipitate was analysed for total lipid, cholesterol, and phosphatide contents. The residual proteins left after extraction were determined by the KJELDAHL micro-method.

JIRGL's test was carried out in an aliquot of serum. Extraction and analysis of the mucoprotein and lipid containing precipitate was performed by the above methods.

The studies were repeated in eight series (40 jaundiced and 40 control animals.) Lognormal distribution of the results of lipid determination was checked by the GAUSS-probit graphical method. Since the zero-hypothesis could be rejected, the identical number of icteric and control animals permitted to use STUDENT's two-sample *t*-test for the comparison of the icteric and control group.

Results

Results are shown in Tables I and II.

Ligation of the common bile duct was invariably followed by jaundice with serum bilirubin levels reaching 10.4 mg per 100 ml. JIRGL's test was strongly positive in each group, apart from a case where the material was insufficient for serum lipid determination (Group V). A positive test of elevated serum bilirubin levels was observed in none of the laparotomized control animals; the amount of lipids in the mucoprotein-containing precipitate of these animals remained within the limits of error inherent to gravimetry.

Table I

Control groups

No. groups	Serum lipids mg per 100 ml			beta-Lipoproteins mg per 100 ml			
	Total lipid	Chol.	P-lip.	Total lipids	Chol.	P-lip.	Protein
I.	288	73	168	100	21	44	96
II.	388	60	125	230	30	66	75
III.	490	69	146	300	33	64	122
IV.	363	104	207	150	33	42	115
V.	375	63	125	210	31	47	70
VI.	390	78	139	240	40	46	109
VII.	525	80	134	360	64	89	209
VIII	740	91	111	310	64	94	174
Average	440	77	144	237	39	62	121

Chol. = total cholesterol (free and esterified)
P-lip. = phosphatides expressed in lecithin, calculated from organic phosphorus content.

The rise in serum lipids due to ligation of the common bile duct was strongly significant ($p < 0.1$ per cent) and involved an extreme elevation of phosphatides at the expense of neutral fats. While the total lipids reached 2.5 times and the beta-lipoproteins 3.5 times their original level, the value for lipids bound to alpha-lipoglobulins was practically unchanged. These changes,

Table II

Icteric groups

No. groups	Serum lipids mg per 100 ml			beta-Lipoproteins mg per 100 ml				Abnormal lipids mg per 100 ml			Serum
	Total lipids	Chol.	P-lip.	Total lipids	Chol.	P-lip.	Proteins	Total lipids	Chol.	P-lip.	Mucoproteins
I.	1190	335	680	800	218	422	158	425	105	223	99
II.	1550	453	1050	1370	342	800	256	900	190	145	126
III.	1110	198	398	750	170	323	167	160	25	37	79
IV.	1210	310	590	1010	224	482	240	300	98	65	68
V.	1290	255	640	950	204	464	215	—	—	—	—
VI.	925	273	490	540	174	310	171	270	48	87	49
VII.	1055	221	453	710	142	283	233	170	26	68	28
VIII.	1110	222	404	740	156	281	114	120	9	15	32
Average	1180	283	588	859	204	480	194	335	71	91	69

Abbreviations as in Table I.

as well as the pattern of labile lipids appearing in the filtered serum tested for mucoproteins are illustrated in Fig. 1.

The described changes involved the beta-lipoproteins in the first place and the increase in phosphatides and the fall in neutral fats were the most conspicuous features. The changes in the protein content of the beta-lipo-

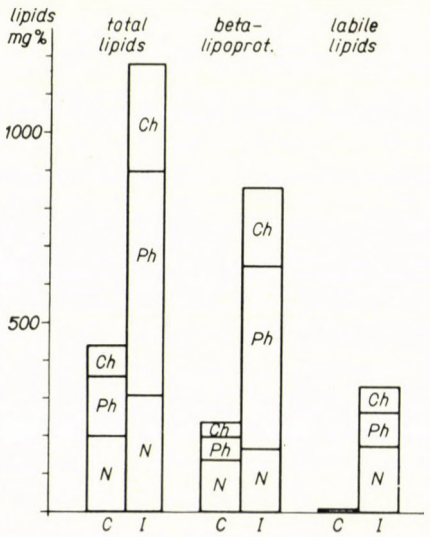

Fig. 1

proteins did not go parallel with those of the lipid fraction. Table III shows the changes in the composition of beta-lipoproteins ensuing upon ligation of the common bile duct.

In the jaundice of animals, the binding of nearly one third of the lipids forming the structure of beta-lipoproteins was abnormally labile (Table II). They were split off by aromatic sulphonic acids such as sulphosalicylic acid,

Table III

Beta-lipoprotein pattern

Content	Control group		Icteric group	
	mg per 100 ml	rel.%	mg per 100 ml	rel.%
Total cholesterol	39	10.9	204	19.4
Phosphatides	62	17.3	480	45.6
Other (neutral) fats	136	38.0	175	16.5
Total lipids	237	66.2	859	81.5
Protein	121	33.8	194	18.5

p-toluolsulphonic acid, etc., during the precipitation of proteins, and passed into the filtrate in a colloidal state. Thin-layer chromatography showed them to contain all the lipid components of lipoproteins. Their origin from the beta-lipoproteins has been established on the following grounds:

1. Paper electrophoresis of serum stained for lipids revealed an isolated increase in beta-lipoproteins.

2. The carriers of abnormal changes were the beta-lipoproteins.

3. Dextran sulphate added to a positive serum abolished the positivity of the test by blocking beta-lipoproteins.

4. The beta-lipoproteins isolated from positive sera (but not from controls) also gave a positive test.

Discussion

Ligation of the common duct is followed by a complexity of profound changes in lipid metabolism. When the fats are inadequately absorbed from the intestine, their accumulation in the blood cannot be explained otherwise than by an increased mobilization from the stores. The various normal and abnormal types of fat mobilization are generally associated with an increase in beta- and $alpha_2$-lipoglobulins. In the present case, the excess of lipids in blood was integrated into the structure of lipoproteins, thus changing their physico-chemical nature.

In this connection the following questions arise:

1. Which are the factors determining the production of abnormal lipoproteins?

2. What causes the lability of the lipoprotein complexes in question?

According to present knowledge, the main site of beta-lipoprotein production is the liver [14]. Biliary obstruction is associated with an increased production of lecithin by the liver and with its integration into the lipoprotein structure. Lipoproteins of a structure different from that occurring in serum are excreted in the bile also under normal conditions [10, 18, 19]. The bile gives generally a positive JIRGL-test. It is a further point of interest that in biliary obstruction the difference between the patterns of serum and biliary lipids tends to disappear. This would suggest a mechanic regurgitation of bile, possibly associated with a hyperfunction of liver cells and accumulation of lipoproteins.

FRIEDMAN et al. [6] connect the production of abnormal lipoproteins with the accumulation of bile acids. Though it is actually possible to induce hyperlipaemia by the administration of bile acids, earlier observations in jaundiced subjects did not suggest any correlation between the levels of labile lipoproteins and those of bile acids in the serum [5]. This, however, does not exclude the possibility of an intracellular mechanism affecting the liver cell. At any rate, the phenomenon under study must be closely associated with intra- or extrahepatic biliary obstruction and must involve the liver in the first place.

HANAHAN investigated the relative lability of lipoprotein complexes [8]. On the evidence of radio-isotope studies the most stable fraction of the molecule is the protein and next to it the phosphatide, while the other lipids are easily interchanged as a result of intermolecular collision.

In the present case the increase in the relative lipid content of the lipoprotein complex was associated with a reduction of its more stable component, the protein fraction. A further feature of this condition, in opposition to others, is the integration of components of greater polarity, particularly of phosphatides, into the molecular structure. The absence of any correlation between the lipid and protein fractions in the filtrate of the sulphosalicylic acid precipitate suggests that only a loosely attached portion of the abnormal lipoproteins is split off by aromatic sulphonic acids. However, the question still awaits a closer study.

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CARE OF THE PATIENT WITH RENAL DISEASE

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A total of 400 patients with renal disease has been followed up over the last five years.

1. Long-term care is required in addition to chronic renal disease, by patient with acute nephritis taking a protracted course. Cure of this condition may take two years. In the presence of two or more renal abnormalities, i.e. of combined renal disease, long-term care offers little prospect.

2. The terms "residual proteinuria", "residual haematuria", "asymptomatic bacteriuria", "defective healing", though giving confidence to the patient, may blunt the alertness of the physician unless he keeps in mind that there is no complete cure without a normal filtration fraction.

3. Slow progression of chronic glomerulonephritis is due in all probability to auto-immunization while the short latency of acute exacerbations finds its best explanation in the classical theory of streptococcal sensitization (sessile antibodies). The predisposing factors of chronic pyelonephritis are: 1. calculi; 2. congenital malformations of the urogenital tract; 3. pregnancy.

4. Signs of activity in chronic nephritis are microhaematuria by the standards of the Addis count and/or a low complement titre, while the dynamics of chronic pyelonephritis is reflected by quantitative urine cultures and leucocyte Addis counts.

5. Appropriate measures to brake the progress of chronic condition are as follows. 1. Abstinence from harmful influences. 2. Suppression of activity by long-term steroid therapy in glomerular affections, and protracted chemotherapy and antibiotics in pyelonephritis. 3. If necessary, eradication of foci and surgical correction of uropoietic abnormalities. Steroid treatment is contraindicated in renal amyloidosis.

6. Patients with chronic renal disease showing signs of activity are unfit for work. Rehabilitation in the stage of latency is an attempt calling for the closest possible supervision.

7. Long-term care and follow-up of patients with chronic renal disease should be entrusted to nephrologic units with suitable laboratory facilities. This should be complemented by occasional overall studies of the renal condition. The medical practitioner is in the best position to notice any unexpected change. Care of the renal patient along these lines is a lifelong necessity.

Owing to the recent medical and surgical advances, the life of chronic renal patients could considerably be lengthened and the number of patients requiring long-term medical care has greatly increased. This leaves the physician with new responsibilities, particularly as concerns preserving the patient in the best possible condition and of enabling him to pursue an activity compatible with his state of health.

The problems involved by the care of the renal patient have not received the attention they deserve. We have followed up closely 400 renal cases over the last five years. Since the majority of our material consisted of cases of

glomerulonephritis and pyelonephritis, these two types will be discussed in the present paper. Neither the period of observation nor the number of the cases provide sufficient evidence to decide the pertaining issues, nevertheless provisory lines of conduct can be given.

Which patient requires long-term care?

First of all the chronically ill with the following diagnoses:

1. Chronic glomerulonephritis
2. Nephrotic syndrome;
3. Chronic pyelonephritis;
4. Renal ptosis;
5. Congenital anomalies of the urogenital tract, particularly polycystic kidney;
6. Renal calculi;
7. Renal tuberculosis;
8. Renal tumours.

If two or more types combine, proper care is a particularly difficult task, though for obvious reasons there can be no question of a long-term care fatal outcome being unavoidable in practically every case. The possibilities of combinations are unlimited, as illustrated by some cases from our material:

Interstitial nephritis with nephrosis;
Hypersensitive angiitis (Zeek) + salt-losing kidney;
Systemic lupus erythematosus + scleroderma involving the kidney;
Polycystic disease of kidney + pyelonephritis;
Pyelonephritis + renal amyloidosis;
Renal amyloidosis + renal vein thrombosis;
Kimmelstiel—Wilson's syndrome + pyelonephritis;
Pyelonephritis + essential (central) hypertension + Goldblatt mechanism in consequence of atheromatous plaques obliterating the right renal artery + generalized atherosclerosis with reduced elasticity of the aortic wall.

It must be pointed out that acute nephritis when taking a protracted course, may require two years for its cure. Such patients, too, are in need of long-term care. Very often, the 14-year-old belong to no man's land, since they have grown out of a paediatric unit before being actually cured, and fail to report at a medical department.

Criteria of healing

The cured patient needs no further care. However, it is not easy to decide when he is to be regarded as cured. The ideal requirement would be a full restitution on the following evidence:

1. Freedom of clinical symptoms;
2. Immunological inactivity;
3. Normal ultrastructure as revealed by electron microscopy of renal biopsy material;
4. Normal renal functions on the evidence of functional tests including clearance.

The terms "residual proteinuria", "residual haematuria", "defective healing", "asymptomatic bacteriuria", though giving confidence to the patient, may blunt the physician's vigilance and are therefore best discarded from our terminology or made very limited use of. By our standards, one is not justified in speaking of a "residual proteinuria" unless the renal function tests and the filtration fraction give consistently normal results. We limit the term "residual haematuria" to cases with an Addis count below 8 to 10 million. We keep a careful watch on the behaviour of the filtration fraction, this being one of the most sensitive functional indicators. When improvement of a glomerular lesion has advanced so far that the filtrate is practically normal in volume, the significant reduction of the filtration fraction will still indicate the glomerular disturbance until recovery is complete. "Three minor signs make up a major one" is the rule of French clinicians. If we apply it to the present issue, then residual proteinuria, residual haematuria and residual reduction of the filtration fraction add up to chronic nephritis in the latent stage. The patient, as concerns future care is a candidate for chronic nephritis.

When does chronic nephritis begin?

The limits are indistinct. According to HETÉNYI [27] chronic nephritis starts three weeks after the onset of acute nephritis if the accompanying hypertension has not completely ceased by this time. We cannot, however, subscribe to this view as in our experience acute nephritis may affect many hyperreactive individuals, moreover central (essential) hypertension may be misinterpreted as chronic nephritis [24]. Impairment of the concentration capacity leaves, however, little doubt about the stage either of glomerulonephritis or of pyelonephritis.

Bacteriuria whether symptom-free or associated with mild bouts of pyelonephritis, calls for special attention since it may have a severe chronic

pyelonephritis in its background, as illustrated by the carefully observed cases of THRUPP et al. [48]. The question arises when bacteriuria should be considered "true". As recently stressed by WHALLEY et al. [52], bacteriuria has no significance unless the same microorganism grows from the urine in large numbers on two consecutive occasions. Usually, 10^5/ml is said to be the critical count, in our experience 10^3/ml is critical in the case of clear voided urine of males and catheter urine of females [21, 22]. A growth of different microorganisms from different cultures repeated at close intervals is no evidence against "true" bacteriuria. In a person susceptible to infections the urogenital tract may harbour different microorganisms and it may sometimes occur that even more than one kind may be present simultaneously.

Natural history of the chronic process

Certain renal diseases assume a chronic form at the onset, as for instance the chronic type of renal tuberculosis. Certain renal abnormalities such as congenital malformations, obviously last through a lifetime. But it is hard to understand how acute glomerulonephritis or pyelonephritis become chronic.

Glomerulonephritis is doubtlessly an allergic disease, but the underlying immunological mechanism is not fully understood. The following explanations have been put forward, among others.

1. The classical school claims streptococcal allergy to be responsible for the disease (SCHICK [45], PIRQUET [42]). The streptococcal proteins are supposed to sensitize the human organism, the first phase of the process being an attachment of antibodies to the tissues and the first manifestation of the disease is ascribed to the reaction with the sessile antibodies of the antigen repeatedly invading the blood stream.

2. Inverse active anaphylaxis. The theory presumes, as the first phase of the process, the binding of the antigen to the tissues (KELLETT [31], SARRE [44]). The disease becomes manifest when the antibodies produced during the latent stage react with the antigen bound to the kidney.

3. Auto-immunization (SCHWENTKER and COMPLOIER [46], CAVELTI [11], VANČURA [49]).

4. The pathogenic role of soluble antigen-antibody complexes (DIXON et al. [13], MCCLUSKEF and BENACERRAF [37]). Recent investigations clearly point to the possibility that in the latent stage of serum sickness an antigen-antibody reaction occurs in the blood stream where toxic complexes are formed and these are supposed to be the responsible factors not only while circulating in the blood but also at a later stage when they are deposited in the vascular wall and give rise to proliferative glomerulonephritis.

A review of the literature makes one conclude that all of the alleged mechanisms have their part in the development of the process, at least in some

of its features, such as the slow progression or conversely the acute exacerbation of chronic nephritis. We ignore, however, whether the said factors operate together or in succession. The latter conjecture is more likely to be true. It is for instance in line with the theory which ascribes two phases to the origin of Masugi's nephritis and finds conclusive evidence in the rabbit kidney—duck serum experiments of KAY [29, 30]. The model experiments of HÁMORI, TOMPA and KÁDAS [26] also point to two different allergic mechanisms coupled

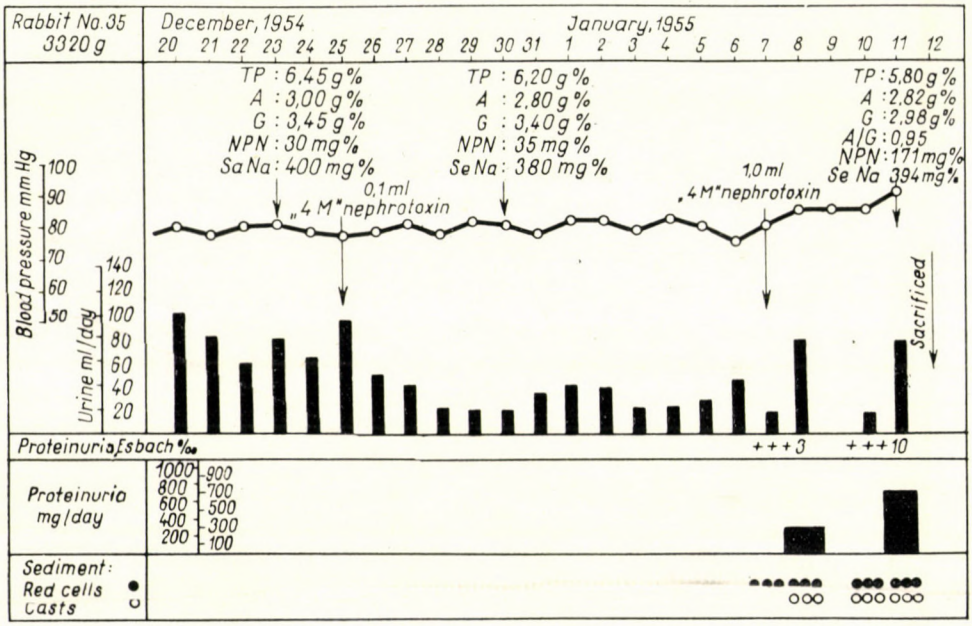

Fig. 1. Effect of anaphylactic shock on silent Masugi's nephritis. Sensitization: ineffective dose of nephrotoxic duck serum. Reinjection: 1.0 ml nephrotoxic duck serum. Abbreviations: TP = total protein; A = serum albumin; G = serum globulin; NPN = non-protein nitrogen; SeNa = serum sodium; +++ = intense precipitation by sulphosalicylic acid

in succession. These authors induced non-lethal anaphylactic shock in rabbits sensitized with minimal amounts of nephrotoxic duck serum. Under the effect of the shock the latent process became manifest whether normal or nephrotoxic duck serum had been used for reinjection (Figs. 1 and 2). As exposed in detail in an earlier paper [19], the auto-immunization provides the closing link of the process and is also responsible for the slow progression of chronic nephritis. PFEIFFER and BRUCH [41] expressed a similar view ascribing chronic nephritis to infective allergy which generally abates but may occasionally start off an auto-immune mechanism leading to chronic nephritis and resulting in secondary renal atrophy.

Acute exacerbations of chronic nephritis associated with repeated streptococcal infections and liable to cause sudden relapses have a different

origin. The latency period of acute exacerbations is shortened as found by EARLE and SEEGAL [14] and confirmed by ourselves. In our view the classical streptococcal hypothesis offers the best explanation for the short latency period.

PETRÁNYI [40] has called attention to the possibility of acute glomerulonephritis becoming chronic under the effect of hypertension, through a kind of vitious circle leading to a hypertensive transformation of the kidney.

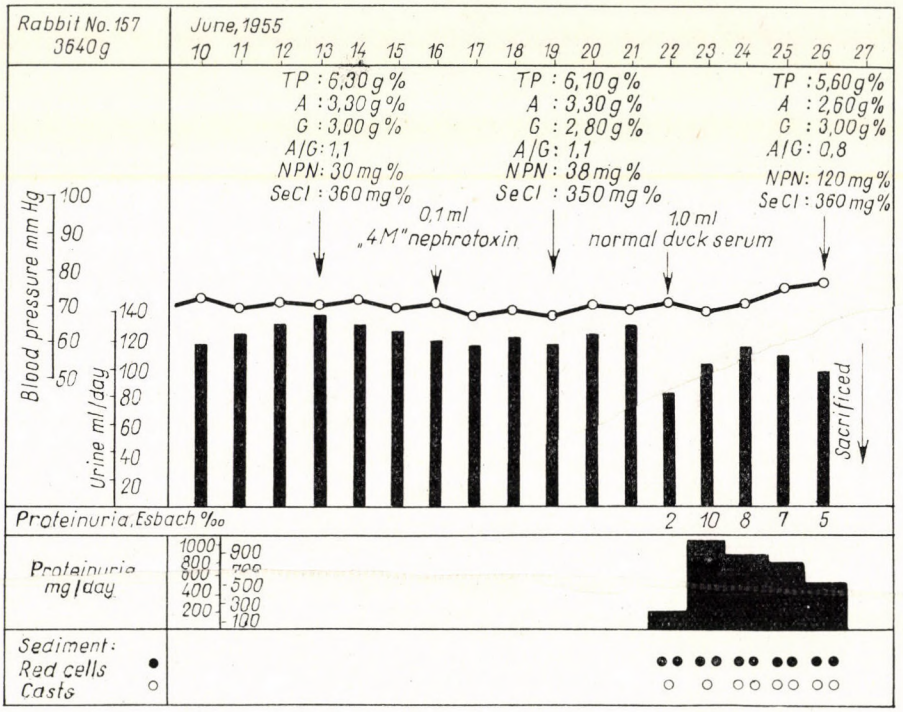

Fig. 2. Effect of anaphylactic shock on silent Masugi's nephritis. Sensitization: ineffective dose of nephrotoxic duck serum. Reinjection: 1.0 ml normal duck serum. Abbreviations: TP = total protein; A = serum albumin; G = serum globulin; NPN = non-protein nitrogen SeCl = serum chlorides

In 50 per cent of chronic nephritis cases hypertensive changes may be found without oedema or inflammation. A transformation of this kind obviously precipitates the advent of renal failure.

The origin of the type of glomerulonephritis chronic from its very outset is still enigmatic and suggests a relationship with collagen disease [40].

What is the origin of chronic pyelonephritis? As concerns local immunity, we are still in the dark, therefore the question why bacterial infections persist in the kidney, leaves us with general conjectures. Either the infective agent is too virulent or the defence of the organ too weak, the major predisposing

factors being 1. calculi, 2. congenital anomalies of the urogenital tract, 3. pregnancy. Obstructive forms of renal disease require surgery and non-obstructive forms, eradication of the responsible foci, for their cure.

The experiments of BABICS and RÉNYI-VÁMOS [3, 4, 5] have lent convincing support to the assumption that in obstructive renal disease urine is absorbed first into the adipose tissue adjacent to the renal pelvis and later into the renal interstitium. This induces a massive release of histamine and scarring of the adipose tissue of the renal sinus. Obliteration of the lymphatics cause a mechanical impairment of renal lymph circulation and this interferes with intrarenal protein transport which is considered by the authors as the decisive factor in renal destruction.

Criteria of activity in chronic renal disease

It is a general rule that every chronic process is to be considered active if any of the presenting features predominate and still more if any of them becomes prevalent, but the various signs have not the same significance. Glomerulonephritis is the very example of this. Increase of proteinuria is an unfavourable sign and massive proteinuria is indicative of the nephrotic syndrome. On the other hand, as decrease of the proteinuria need not be a sign of improvement, the protein being of glomerular origin, and therefore the destruction of the glomerular architecture may result in a reduction of proteinuria. Evaluation of hypertension is rendered difficult by clinical types involving a dual mechanism, in other words, renal hypertension originating in the classical renin-angiotensin mechanism combines with neurogenic factors [24]. In our experience the Addis count of red cells is the most reliable sign [20, 22, 23, 25]. Fig. 3 shows that a considerable sudden increase ensues in erythrocyte excretion, as a rebound phenomenon, if steroid treatment is interrupted in acute glomerulonephritis. BROD [8] set down the following criteria of activity: proteinuria, haematuria, leukocyte excretion, cylindruria, sedimentation rate.

Reduction in serum complement titre is an evidence of immunologic activity [20, 22, 23, 25, 34, 35]. There is still little information about the correlation between auto-antibody titres and the clinical course of the disease. The fact is, however, that significant titres are consistently found in the case of chronic nephritis [12, 33 41, 50]. Persistent presence of anti-renal auto-antibodies on the basis of serial tests is claimed by KRAMER et al. [32] to be an unfavourable sign in chronic nephritis.

In our experience, significant microhaematuria and complement reduction need not be associated with each other in cases of acute glomerulonephritis taking a protracted course, or in cases of chronic glomerulonephritis. Quantitative analysis of the sediment is generally a more sensitive

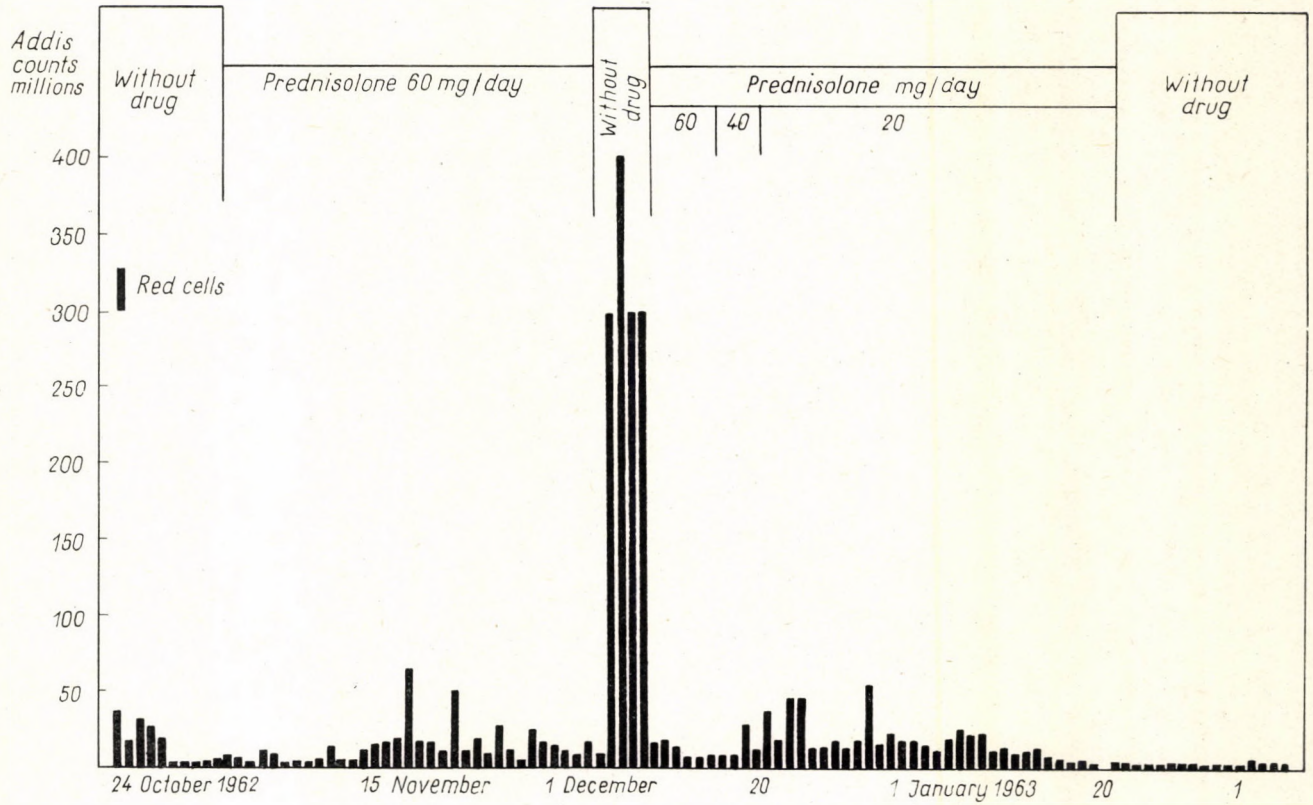

Fig. 3. Male, 40 years. Consequence of abrupt interruption of prednisolone therapy and effect of prednisolone on reactive haematuria. Diagnosis, acute glomerulonephritis of a protracted course

indicator than the serum complement titre. Occasionally, the latter remains low despite the clinical cure of acute nephritis, but nothing decisive can be said as yet about the further course of such cases. Long-term follow-up is certainly required.

The serum complement titre is a valuable indicator of activity in lipoid nephrosis. According to LANGE et al. [35, 36] and WEDGWOOD and JANEWAY [51], changes of the titre closely reflect the clinical course of lipod nephrosis. Though in our experience this correlation is not quite as close, still we consider a reduction in complement titre a fairly reliable sign of progression. Two facts restrict, however, the diagnostic significance of the complement titre: 1. The serum complement titre may remain normal even in cases with fatal outcome as evidenced by serial tests in one of our cases. 2. As pointed out by FISCHEL and GAJDUSEK [15] low complement values rapidly return to normal levels if acute glomerulonephritis is joined by some inflammatory process or thyrotoxicosis. We were able to confirm the possibility of such correlations in a case of nephrotic nephritis and in one of systemic lupus erythematosus, the responsible inflammatory process having been in both cases an intragluteal abscess caused by an injection.

Activity in pyelonephritis is indicated by the presence of "true" bacteriuria combined with pyuria, whether or not the patient is febrile. In our experience the behaviour of the bacterial and leukocyte counts gives a true reflection of the dynamics of the process. An Addis count of leukocytes requires a catheter specimen in women, but not every day of course.

Increase in non-protein nitrogen levels associated with chronic renal disease indicates the terminal stage of the process rather than its activity.

How to prolong the progress of chronic renal disease?

Chronic glomerulonephritis or pyelonephritis predict the terminal stage of the process. The ideal way of averting it would be to control the process during its acute stage. But when we are faced with an established chronic process, all we can do is to attempt to keep away every possible influence that may precipitate the fatal outcome. The essential harmful factors are 1. reinfection, 2. physical strain, 3. mental stress, 4. incorrect regimen. Our other endeavour is to gain control over the activity of the process by means of long-term steroid treatment in chronic glomerulonephritis [8, 9, 20, 22] and the nephrotic syndrome [1, 17, 20, 22, 43], and sulpha drugs and antibiotic treatment in chronic pyelonephritis [6, 7, 10, 21, 22, 28, 39, 47].

Prolonged steroid treatment has its hazards. We find it unwarranted in cases of nephrosis due to amyloidosis, as in one case we had the definite impression that it had precipitated the fatal event. MAXWELL et al. [38] expressed a similar view.

Our procedure is as follows. Patients with X-ray evidence of peptic ulcer are not given steroids. Those who have been found negative are kept on daily oral doses of 50 to 60 mg prednisolone until the activity of the process subsides or side effects have developed. This may take 4 to 8 weeks or more. Long-term administration of medium doses in this range is limited by irritability, heartburn, abdominal colics, insomnia, palpitations or reluctance to take the drug. This calls for an adjustment of the doses to the individual tolerance. Daily doses in the range of 10 to 15 mg are generally free of any adverse effect even when taken over long periods. Medium doses are liable to cause hypopotassaemia and therefore 3 g potassium chloride daily should be prescribed and sodium bicarbonate according to need. The patient should be made aware of the dangers of dietary errors. He must particularly avoid coarse, heavy food which may bring on ulcer perforation or steroid pancreatitis.

During intensive steroid treatment, close supervision of the patient is imperative. Barium-meals should be repeated at three-week intervals in the interest of early recognition of a peptic ulcer. The appearance of ulcer forbids any further administration of prednisolone, but 20 mg of cortisone daily have to be given i. m. in order to ameliorate the rebound phenomenon and iatrogenic adrenocortical failure, otherwise the long-term prednisolone therapy will be gradually reduced over a period of two weeks. No ACTH is required. Small doses of prednisolone may also be taken outside hospital.

According to the prevalent view, the decisive point in the outcome of pyelonephritis is the control of bacteriuria. This is generally attempted by specific antibiotic treatment. BROD [6, 7] emphasized that sterility of the urine found on one occasion does not justify bringing treatment to an end, and even when several cultures have remained negative, it is still long before the antibiotic can be discontinued. For long-term, antibacterial treatment he recommends small doses of sulphonamides soluble in urine. Though subscribing to BROD's view as concerns the necessity of long-term treatment, we prefer the use of large-spectrum antibiotics in small doses, the maintenance dose being 120 to 250 mg of oxytetracycline daily. Prolonged intermittent antibiotic and sulphonamide treatment has also been attempted, but in order to be rewarding this too must be started at an early stage as long as creatinine clearance is still normal or nearly so [10].

What are the possibilities if the causative agent is polyresistant? An attempt may be made with some sulphonamide of high solubility. According to clinical observations, resistance *in vitro* by no means excludes responsiveness *in vivo*. It is, however, defensible to renounce chemotherapy or antibiotics. Personally, we prescribe some dye preparation, obviously for psychic reasons rather than in the hope of therapeutic effect.

Congenital abnormalities of the urogenital tract should be looked upon as potential sources of pyelonephritis.

After surgery for renal malignancy a careful watch must be kept on the patient for early signs of relapses.

The importance of adequate relationships between the physician and the patient need not be emphasized. Encouragement and sympathy offered by the physician is an invaluable factor in efficient long-term care.

Renal disease and pregnancy

Two circumstances have prompted us to reconsider the question, how far the maintenance of pregnancy is warranted in renal disease. First, with antibiotics at our command, we can do more in the way of stabilizing the renal condition during pregnancy than we could in the past. Second, owing to the progress achieved in the field of premature care it is no longer vital to carry a pregnancy to term. Induced delivery between the 7th and 8th months takes a substantial load off the kidneys. The problem, obviously, does not present itself unless the parents absolutely insist on the maintenance of pregnancy.

It stands beyond doubt that chronic nephritis, especially if it verges to uraemia, represents an absolute indication for the interruption of pregnancy. But what if a woman with nephrotic nephritis and in the 5th to 7th month of pregnancy reports for the first time? It is difficult to decide what to do in that case, since six more weeks would be enough for a viable foetus to be borne.

Yet, though there is little evidence in this respect, we must face the necessity of giving up the foetus as we did in the past if close clinical observation showed further deterioration in the mother's condition.

Renal disease and military service

The presence of chronic nephritis settles the question, the patient being in that case obviously unfit for military service. But what should be the attitude in the case of an apparently cured acute nephritis? For instance, if the patient had acute nephritis of a few weeks' duration a year ago and he has been free of any symptom since that time? In our opinion, military service must be postponed for two years after the subsidence of acute nephritis, since we have no absolute proof of its full restitution. As GÖMÖRI [18] puts the time during which improvement is still possible at 18 months and the present authors at two years, the patient needs those two years to be on the safe side.

Rehabilitation

Provision for suitable activity adapted to the patient's condition belongs to the major practical problems of chronic renal disease. Rehabilitation committees work with standardized schemes based on the character and the stage of the disease. Such standards have certainly their use. FÖLDI [16] does justice

to these claims in his study about rehabilitation in renal disease. Schemes as such, have obviously a limited value. BROD [8] greatly relies on the endogenous creatinine clearance in assessing the working capacity of a renal patient. As long as the average GFR is over 50 ml/min, he sees no objection to a sedentary full-time job. If, however, GFR falls below that limit or if hypertension appears, he limits the patient's activity to a half-time job which may be continued until signs of uraemia appear. Personally, we would not undertake to set up hard-and-fast-rules of this kind, as two cases of the same disease are never similar. We have only to remember the wide variety in nephritis ranging from abortive cases to the fulminant type leading to death within 24 hours. In our view, rehabilitation is not more than an attempt with entirely unforeseen results. We had for instance a patient with polycystic kidney on the verge of uraemia. On his request we let him stay on his administrative job. The attempt was successful. Another patient with ureteral obstruction associated with microhaematuria works as a joiner without any adverse consequence. But there are more than enough examples to the contrary. A young collier for instance just on the way to recovery from an acute glomerular nephritis of protracted course with minor signs of activity, took up his work, definitely against our advice. Eighteen months later he was brought back to us in the terminal stage of uraemia and died soon after admission. A young man in the latency stage of chronic nephritis entered the High School of Forestry. The strain involved by the studies and, possibly, an acute exacerbation resulted in uraemia and death in a few months.

Return to work must be regarded in every case as a tolerance test, which makes close supervision of the patient imperative, at least in the first time. Here lies the role of the factory medical consultant. In cooperation with the attending physician of the hospital unit, he must carefully assess the safety range involving the minimum risk and it is up to him to find an activity for the patient which he may safely continue without endangering his condition. It must, however, be admitted that the problem of rehabilitation in renal disease is as difficult as in any other disabling condition.

When should the renal patient be allowed to resume his work? The obvious answer would be, as soon as he is cured clinically. There are, however, cases which will take a chronic course in spite of adequate measures, so we shall have to come to terms with the disease, allowing the patient back to work if his condition is stationary or showing very slow progress.

When should the renal patient be forbidden to resume his work?

1. If the process is definitely active.

2. In case of renal disablement, for instance, on the verge of uraemia, or with excessive renal hypertension.

What is the strain which presumably leaves the renal conditions unaffected? In all likelihood intellectual occupation or not too hard physical work.

The patient should stand as little as possible. He should not lift heavy objects. High temperature workrooms should be avoided, particularly where renal poisons, e.g. mercurial compounds are used.

Residual proteinuria constitutes a special problem. In agreement with GÖMÖRI [18] in these cases we apply the same criteria as in the early stage of chronic nephritis. Such cases should be excluded from hard physical work.

As concerns school attendance, it should be borne in mind that with acute nephritis taking a protracted course, it is better to lose a school-year than to jeopardize the chance of recovery.

Problems of organized care

Care in renal disease has the same objectives as in any other condition. The network of care has similar tasks as the family doctor had in the old days, only at a higher level. The patient's condition must be followed closely so as to take the necessary measures in case of impairment. Organized care is of substantial help in rehabilitation as it allows to check the consequences of the resumed work. The ultimate objective of organized care is efficient prevention, in other words the protection from any harm and provision for an activity compatible with the patient's condition. The main issues of this task are:

1. Overall check-up at regular intervals;
2. stabilization of the satisfactory condition;
3. early recognition of relapses;
4. rehabilitation.

Who is to be entrusted with the care of renal patients? In our opinion, out-patient clinics with a suitable laboratory background are best suited for this task. There is no need for a special network, but laboratory facilities are indispensable as they alone provide the necessary basic information. We have to rely on the general practitioner, too, who is generally familiar with the patients of his district and is in the best position to note the slightest change though not always to interpret it in the light of objective evidence. Shortage in beds makes regular check-up in a clinical ward illusory.

Care of patients along the above lines requires no special expenditure. The out-patient clinics of hospitals can undoubtedly cope with the task. The patients would have to report for follow-up at regular dates, and it would be in their interest to be seen by the same physician. In this manner, invaluable material could be assembled in respect to the dynamics of renal disease. A special institute for renal diseases would be desirable from the scientific aspect, but the existing hospitals or clinics are well suited for the practical tasks, particularly if specially equipped nephrologic units could be set up within their framework.

The most expedient means of organized long-term care would be to have the patient referred by the general practitioner or the medical consultant of the factory, to a nephrologic consultation at intervals of two months or so. In addition, annual check-up at a suitably equipped ward would be necessary. The tasks include postgraduate training of the medical practitioner in the field of renal diseases.

How long is the renal patient in need of care? In our opinion, to the end of his days.

Future prospects

Until recently, the terminal stage of renal disease has not been accessible for medical care. Two new possibilities, have, however, brought a change in these gloomy prospects. The first is renal transplantation, and the second, the artificial kidney. Widespread use of the first is limited by immunologic incompatibility which, it is to be hoped, will soon be overcome.

Today there are many persons who live without kidneys and report twice weekly for being dialyzed. This possibility must be developed on a larger scale in the future.

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DAS HISTOLOGISCHE BILD EINIGER OLIGO-ANURISCHEN ZUSTÄNDE

Von

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UROLOGISCHE KLINIK (DIREKTOR: PROF. DR. A. BABICS) UND II. PATHOLOGISCHES INSTITUT (DIREKTOR: PROF. DR. L. HARANGHY) DER MEDIZINISCHEN UNIVERSITÄT BUDAPEST

(Eingegangen am 22. Dezember 1965)

In den dargestellten Krankheitsbildern konnte die Pathogenese der Oligo-Anurie in vivo nicht mit Sicherheit festgestellt werden, der histologische Befund trug jedoch zur vollkommenen bzw. partiellen Klärung des Prozesses bei. Diesem Umstand wird hinsichtlich der Feststellung der entsprechenden Therapie und der Prognose eine wesentliche Bedeutung beigemessen.

Das Krankenmaterial der Kunstnierenstationen besteht in erster Reihe aus anurischen bzw. oligurischen Kranken. Im Hintergrund dieser klinischen Diagnose stehen zumeist Krankheitsbilder verschiedener Pathogenese, die im allgemeinen leicht diagnostiziert werden können (Quecksilbervergiftung, inkompatible Bluttransfusion, septischer Abort usw.), manchmal jedoch lediglich anhand des bioptischen- bzw. Sektionsbefundes zu klären sind. In einigen Fällen — z. B. wenn der Patient ohne sichere Diagnose und Biopsie heilt, besteht die Möglichkeit der genauen Klärung überhaupt nicht.

Außer den diagnostischen Schwierigkeiten ist manchmal auch die Klärung zahlreicher, mit der Pathogenese der verschiedenen Anuriearten verbundenen Fragen problematisch.

Die Zielsetzung vorliegender Arbeit ist nicht die statistische Bewertung unseres Krankenmaterials, sondern die Darstellung einiger Fälle, in denen die Pathogenese *in vivo* nicht mit Sicherheit festgestellt werden konnte, die histologische Untersuchung der Niere dagegen die Klärung des Krankheitsbildes — teilweise oder vollkommen — ermöglichte.

Krankenmaterial

An unserer Kunstnierenabteilung wurden in 5 Jahren 367 Kranken behandelt: 211 blieben am Leben, 156 starben. Das Sektions- bzw. bioptische Material wurde in 4 Gruppen eingeteilt und zwar erfolgte die Gruppierung dementsprechend, welches Nierengebiet derartig schwere histologische Veränderungen aufwies, mit denen die Entstehung der Oligo-Anurie *in erster Reihe* erklärt werden konnte.

1. *Glomerulum-Veränderungen* lagen bei 93 Kranken vor; die klinische Diagnose war in diesen Fällen eine sich infolge einer „internistischen" Nierenerkrankung entwickelte Oligo-Anurie. Die histologisch nachweisbaren Ursachen der Oligo-Anurie waren folgende: subakute Glomerulonephritis (Abb. 1), Endstadium chronischer Glomerulo- bzw. Pyelonephritis, Amyloidose, akute Aufflackerung chronischer Glomerulo- bzw. Pyelonephritis (Abb. 2), Schwangerschaftstoxämie (Abb. 3), WEGENERsches Syndrom (Abb. 4), GASSERsches hämolytisches urämisches Syndrom (Abb. 5).

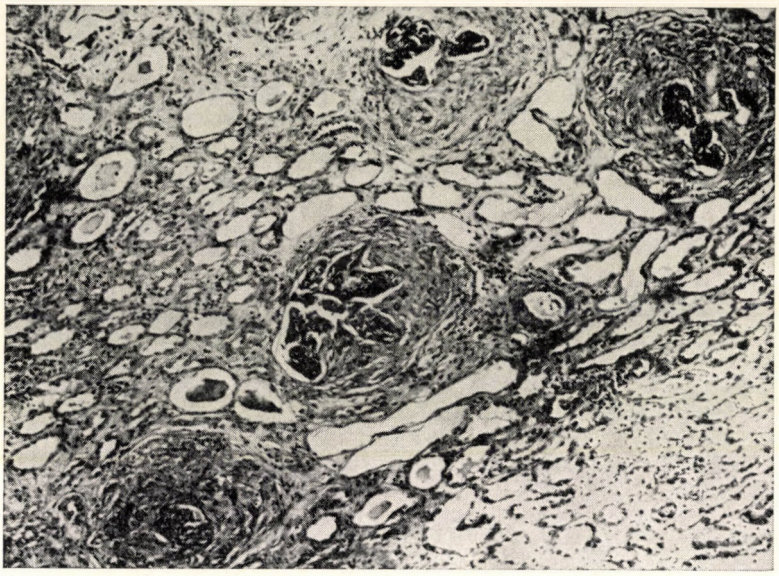

Abb. 1. Intra- bzw. extrakapilläre Form der subakuten diffusen Glomerulonephritis. Periglomeruläre und interstitielle rundzellige Infiltration. Parenchymatöse Dystrophie und Atrophie (Hämatoxylin-Eosin)

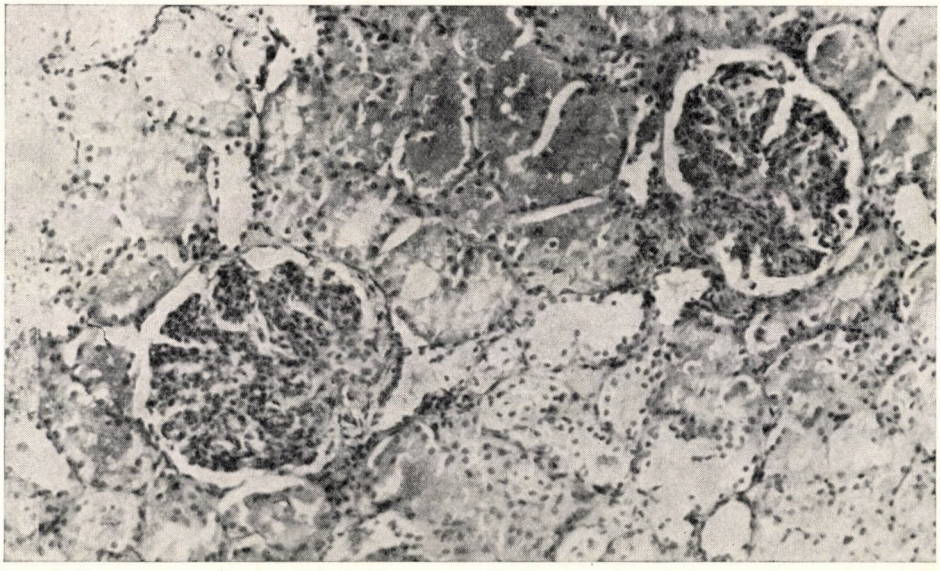

Abb. 2. Akute Exazerbation einer chronischen Pyelonephritis. Leukozytengruppen zwischen den angeschwollenen und zellreichen Glomerulumschlingen. Leukozytäre Infiltration im Interstitium (Hämatoxylin-Eosin)

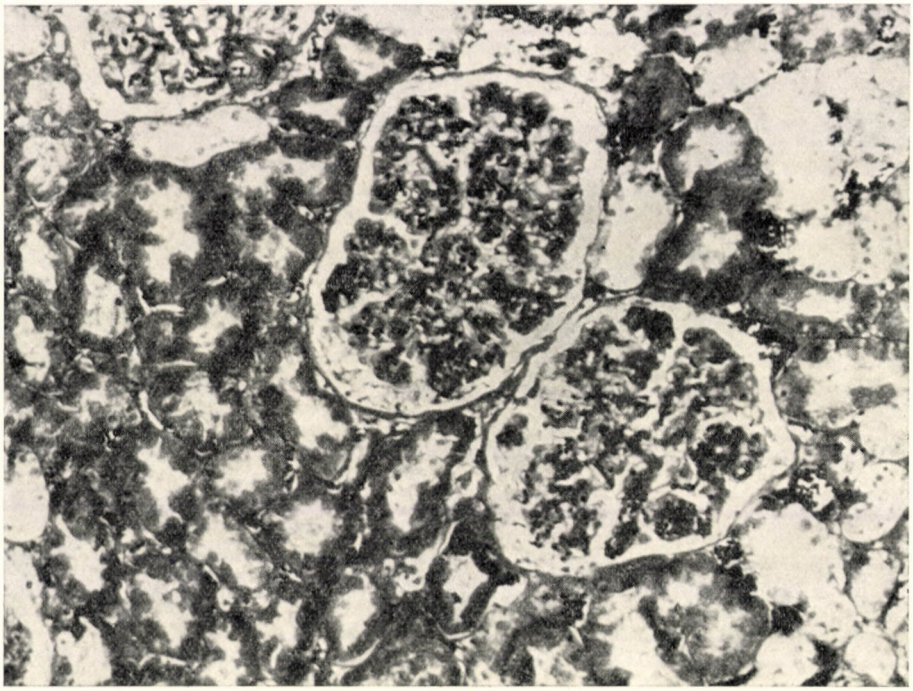

Abb. 3. Schwangerschaftstoxämie. Verdickung des Mesangiums. In den Kanälchen vakuoläre und hydropische Dystrophie (Azan)

2. *Tubulusveränderungen.* Mehr oder minder ausgeprägte Tubulusveränderungen sind bei sämtlichen Anuriearten vorzufinden, die histologische Veränderung erklärt jedoch die Pathogenese nur selten. In 8 bilateralen Nierenrindennekrosefällen (1 fleckige und 7 Totalnekrosen) sowie in 1 Fall, in dem es sich wahrscheinlich um eine Äthylenglykolvergiftung handelte (Abb. 6) ließen sich dagegen die Fragen — weshalb die Diurese trotz mehrmals wiederholter Hämodialyse nicht in Gang gesetzt werden konnte und weshalb die Menge des Tagesharns, falls die Harnausscheidung begann, nicht in dem Maße anstieg, daß anstelle der Oligurie eine Polyurie trat — anhand des histologischen Bildes beantworten.

3. *Interstitielle Veränderungen.* Da im Interstitium zumeist Ödem und zellige Infiltration zu beobachten sind, vertraten HENSCHEN [2] und ZOLLINGER [3] die Ansicht, daß der intrarenale Druckanstieg (Nierenglaukom) auch ohne Schock Anurie herbeizuführen vermag. Bei akuter, hämatogener Aufflackerung einer chronischen Pyelonephritis standen im Mittelpunkt des bioptischen- bzw. Sektionsbildes nicht selten schwere interstitielle Veränderungen (Abb. 7).

4. *Gefäßveränderungen.* Der Totalverschluß der A. oder V. renalis kann ebenfalls die Ursache des tödlichen Ausgangs sein.

Es sei betont, daß außer den hervorgehobenen schweren Veränderungen auch die übrigen Komponenten der Nierensubstanz in kleinerem-größerem Maße lädiert waren.

Besprechung

Falls der auslösende Faktor der Oligo-Anurie eine schwere Glomerulum- oder interstitielle Veränderung bzw. ausgedehnte Kortikalnekrose ist, so kann die Ursache des Krankheitsbildes bei der histologischen Untersuchung geklärt

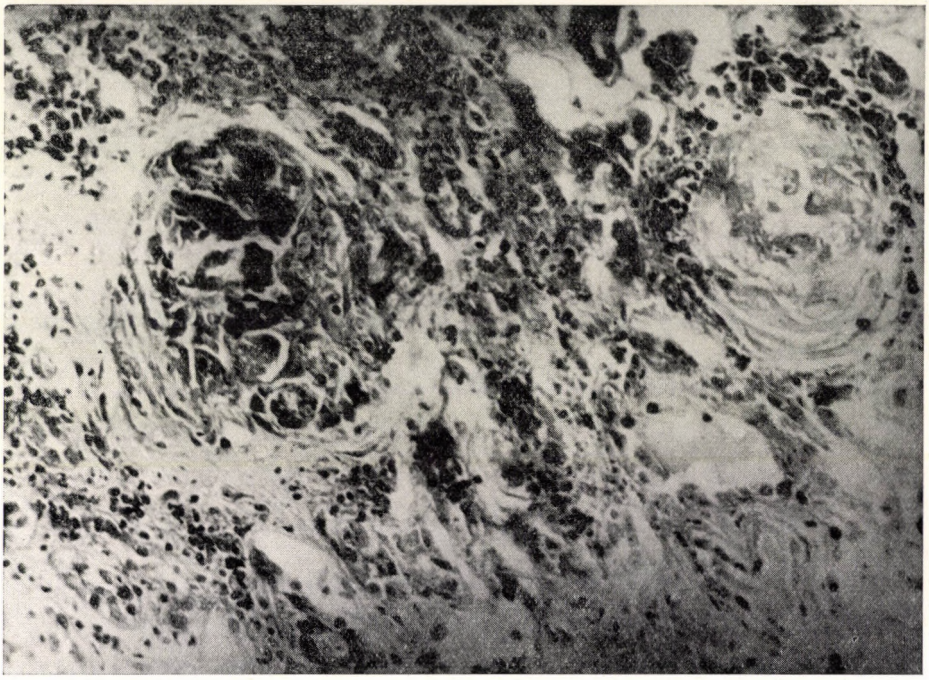

Abb. 4. WEGENERsche Granulomatose. Partielle Nekrose der Glomerulumschlingen, Granulombildung. In der Gefäßwand Fibrinoidnekrose und Entzündung (Phosphorwolframsäure-MALLORY)

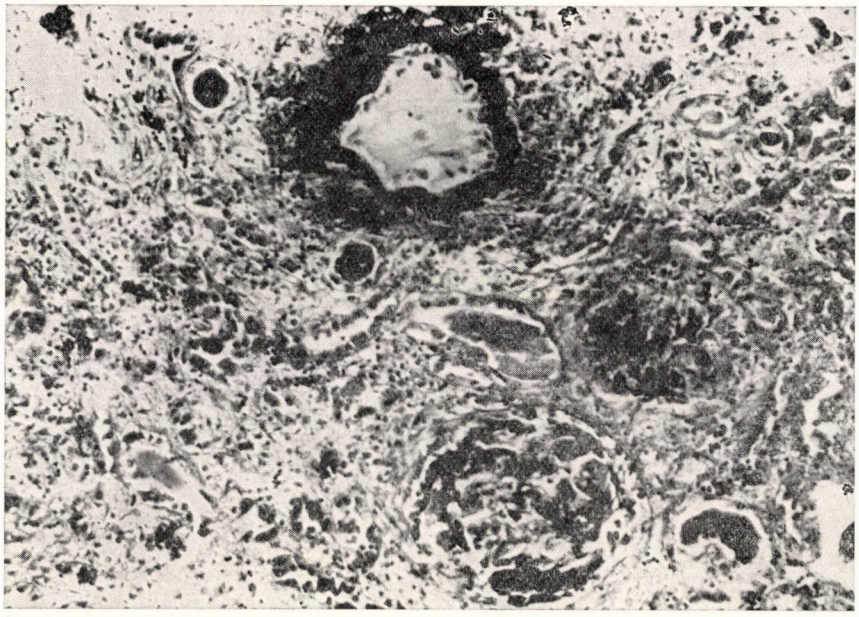

Abb. 5. GASSER I.-Syndrom. In den Glomerulumschlingen und in den Gefäßen Mikrothromben (Hämatoxylin-Eosin)

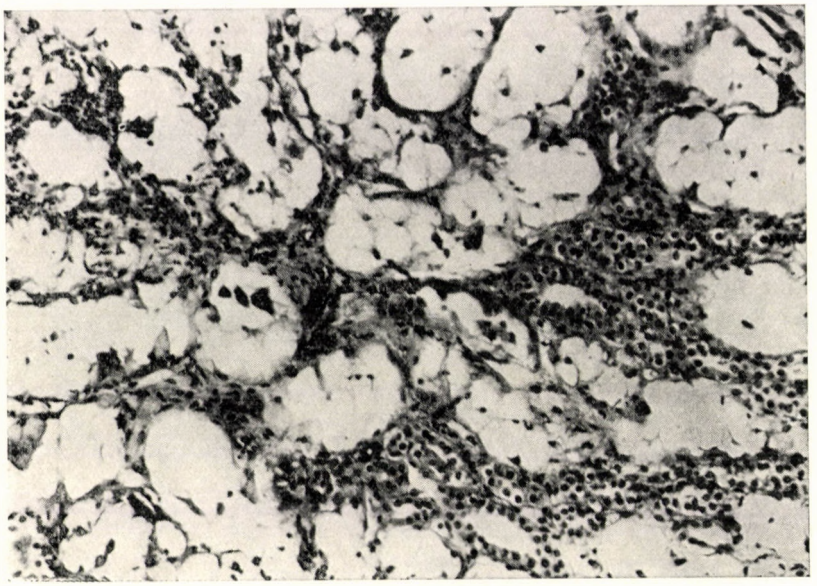

Abb. 6. In den Epithelzellen der Tubuli ausgeprägte Vakuolisation. Plasma ist lediglich an den Randteilen zu finden (Hämatoxylin-Eosin)

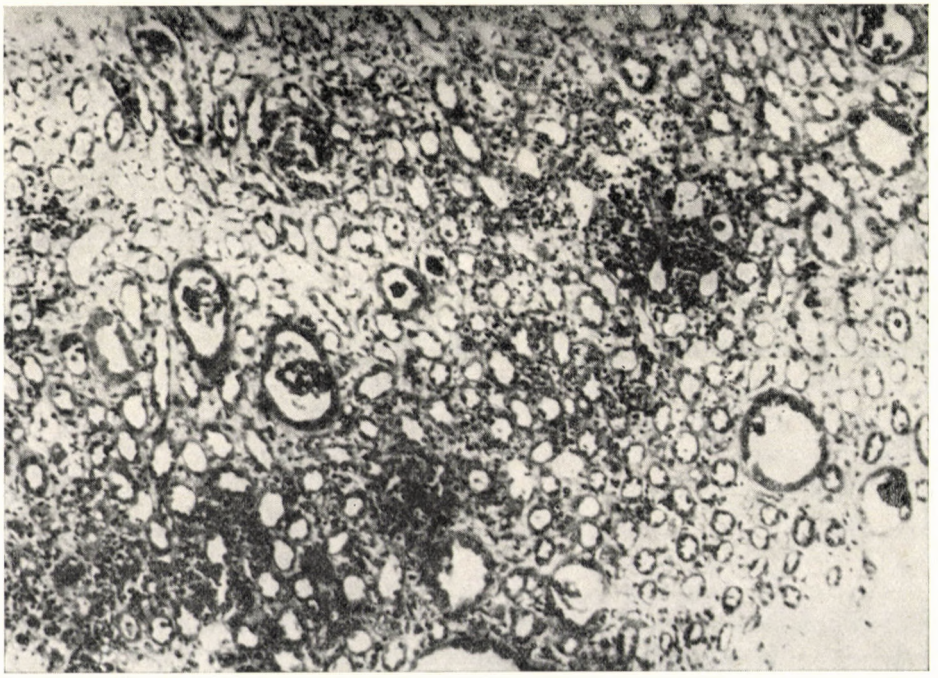

Abb. 7. Ausgeprägte interstitielle Entzündung, in den distalen Harnkanälchen Epithelzylinder (Hämatoxylin-Eosin)

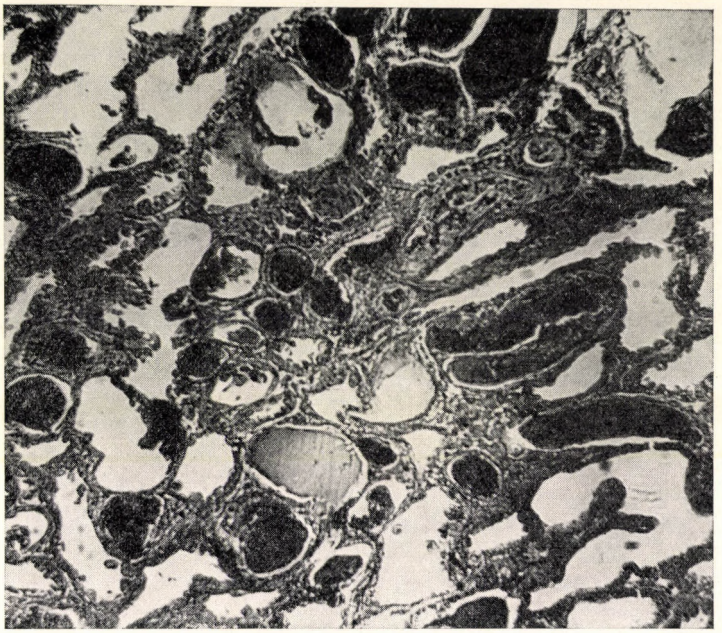

Abb. 8. Histologisches Bild der Niere bei septischem Abort. In den Tubuli zahlreiche Hämoglobinzylinder (Hämatoxylin-Eosin)

werden. Die Anurie entwickelte sich in diesen Fällen infolge des vollkommenen oder fast vollkommenen Aufhörens der Filtration. Nebst verhältnismäßig intakten Glomerula sind für die Oligo-Anurie im Falle ausgedehnter Tubuluszerstörung vornehmlich die Tubuli verantwortlich. Bei der akuten Exazerbation chronischer Nierenprozesse hat die akute Entzündung die Verminderung der noch verhältnismäßig intakten Oberfläche der Glomerula zur Folge, während die interstitielle Entzündung unter Umständen die Kapillaren komprimiert. Außer den histologisch nachweisbaren Veränderungen muß selbstverständlich auch mit einer, histologisch nicht nachweisbaren Funktionsstörung gerechnet werden.

Das Syndrom der akuten Niereninsuffizienz (Trauma, inkompatible Bluttransfusion, septischer Abort, Blutung, Operation usw.) gehört in eine andere Gruppe der Nierenerkrankungen; während der klinische Verlauf dieser Prozesse in bezug auf die Oligo-Anurie häufig ebenso schwer ist als die der übrigen Anuriearten, weist das histologische Bild wesentlich mildere pathologische Veränderungen auf. Das Aufhören der Harnproduktion ist in diesen Fällen wahrscheinlich hauptsächlich den funktionellen Störungen (durch präglomerulären Gefäßspasmus bedingte Filtrationsverminderung) zuzuschreiben. Zu den funktionellen Störungen gesellen sich selbstverständlich auch histologisch nachweisbare Veränderungen, sowie Tubulusläsion, Ödem und Infiltra-

tion des Interstitiums, ferner die in den Tubuli erscheinenden Zylinder, deren Anzahl in Anurien hämolytischen Ursprungs (inkompatible Bluttransfusion, septischer Abort) bedeutend sein kann (Abb. 8).

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OLIGO-ANURIE BEI CHRONISCHEN NIEREN-PROZESSEN

Von

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UROLOGISCHE KLINIK (DIREKTOR: PROF. DR. A. BABICS) DER MEDIZINISCHEN UNIVERSITÄT BUDAPEST

(Eingegangen am 22. Dezember 1965)

Bei 96 von 413 an der Kunstnierenstation der Klinik behandelten Kranken entstand Oligo-Anurie infolge der akuten Aufflackerung eines chronischen Nierenprozesses. 44 der Patienten konnten gerettet werden, 52 starben (54,1%). Die Grundkrankheit war in 61 Fällen chronische Pyelonephritis, in 20 chronische Glomerulonephritis, in 9 subakute Glomerulonephritis, in 3 Amyloidose, in 2 Nephrosklerose und in 1 Fall Periarteriitis. Nach Erörterung der histologischen Nierenveränderungen und der Ursachen der akuten Exazerbation, wird ausdrücklich betont, daß bessere Ergebnisse nur dann zu erwarten sind, wenn die Patienten früher und in besserem Allgemeinzustand an die Kunstnierenabteilung gebracht werden.

Eine verhältnismäßig selten auftretende Komplikation der chronischen Nierenprozesse ist die akute Oligo-Anurie, deren klinisches Bild in erster Reihe an den Kunstnierenabteilungen bekannt ist.

Krankenmaterial

An unserer Kunstnierenabteilung wurden in 5 ½ Jahren 413 oligo-anurische Kranken behandelt; in 96 dieser Fälle war der schwere Zustand auf die akute Aufflackerung eines chronischen Nierenprozesses zurückzuführen (23,2%).

Die wesentlichsten Angaben des Krankenmaterials sind in Tabelle I dargestellt. Wie ersichtlich, entwickelte sich die Oligo-Anurie als Komplikation verschiedener Grundkrankheiten: chronische Pyelonephritis (61 Fälle = 63,5%), chronische Glomerulonephritis (20 Fälle = 20,8%), subakute Glomerulonephritis (9 Fälle = 9,3%), Amyloidniere (3 Fälle), Nephrosklerose (2 Fälle), Periarteriitis (1 Fall).

Die den verschiedenen Grundkrankheiten entsprechende Altersverteilung war folgende: chronische Pyelonephritis 46,5 Jahre (17—73 Jahre), chronische Glomerulonephritis 37,4 Jahre (3—69 Jahre), subakute Glomerulonephritis 21,4 Jahre (3—36 Jahre).

Mortalität: Pyelonephritis 23,2% (26 Fälle), chronische Glomerulonephritis 70% (14 Fälle), subakute Glomerulonephritis 77,7% (7 Fälle). In den letzterwähnten 2 Gruppen ist die prozentuale Aufschlüsselung wegen der geringen Zahl der Fälle nur mit Vorbehalt zu bewerten. 2 der 3 Patienten, bei denen die Grundkrankheit eine Amyloidniere war, starben; die beiden Nephrosklerotiker konnten ebenfalls nicht gerettet werden, und auch der in 1 Fall beobachtete periarteriitische Prozeß war tödlichen Ausgangs. Die Gesamtmortalität beträgt somit 54,1% (52 Fälle). Vollständigkeitshalber sei hinzugefügt, daß die Gesamtmortalität der Kunstnierenabteilung 42,6% ausmacht (176 Todesfälle von 413 Kranken).

In den sezierten Fällen verursachte die Feststellung der Grundkrankheit, d.h. des chronischen Nierenprozesses keine Schwierigkeiten (54 Fälle); bei jenen überlebenden Kranken, bei denen eine Biopsie durchgeführt wurde, konnte die Grundkrankheit ebenfalls mit Sicherheit festgestellt werden (12 Fälle); bei den übrigen geheilten Kranken wurde die Diagnose der seit langem bestehenden Nierenkrankheit anhand der Anamnese, der nach Abklingen der oligo-anurischen Periode durchgeführten Röntgenuntersuchung sowie der Kontrollunter-

suchungen gestellt. Außer diesen Daten wurde selbstverständlich auch die Tatsache geprüft, ob für die Anurie nicht etwa irgendeine andere Ursache (Operation, Schock, Ureterverschluß, Nephrotoxine usw.) verantwortlich sei. Die Ergebnisse der mikroskopischen Harnuntersuchung bieten keine wesentliche Hilfe, da die Patienten vor der Aufnahme auf die Kunstnierenabtei-

Tabelle I

Krankenmaterial

Grundkrankheit	Zahl der Fälle (%)	Durchschnittsalter (Jahre)	Mortalität (%)
Chronische Pyelonephritis	61 (63,5)	46,5	26 (23,2)
Chronische Glomerulonephritis	20 (20,8)	37,4	14 (70)
Subakute Glomerulonephritis	9 (9,3)	21,4	7 (77,7)
Amyloidniere	3		2
Nephrosklerose	2		2
Periarteriitis	1		1

lung bekanntlich mehrmals katheterisiert (Blasen- und Ureterenkatheterisierung) werden, woraus folgt, daß die eventuell bestehenden Pyurie, Hämaturie bzw. Bakteriurie der durch die Katheterisierung herbeigeführten Ureteritis, Zystitis bzw. Prostatitis zugeschrieben werden können.

Besprechung

Im oligo-anurischen Krankenmaterial der Kunstnierenabteilung war für die Entstehung der Oligo-Anurie in fast 25% der Fälle die akute Aufflackerung einer seit langem bestehenden chronischen Nierenerkrankung verantwortlich. Die Grundkrankheit war in der Mehrzahl der Fälle chronische Pyelonephritis, ferner chronische und subakute Glomerulonephritis, in einigen Fällen waren aber auch Nephrosklerose, Amyloidose und Periarteriitis zu verzeichnen.

Anhand dieser Feststellung erhoben sich 2 Fragen:

1. Welche Art der Nierenveränderung erklärt die plötzlich auftretende Oligo-Anurie? Diese Frage läßt sich in zahlreichen Fällen mit Hilfe der histologischen Untersuchung beantworten: Die sich zu einem chronischen Nierenprozeß gesellenden akute Glomerulonephritis oder schwere akute interstitielle Veränderungen (Ödem, Infiltration) können teilweise für die Entwicklung dieses schweren Zustandes verantwortlich sein. Anschließend sei betont, daß wir das Zustandekommen der Oligo-Anurie nicht allein anhand der histologischen Veränderungen feststellten und diese lediglich deshalb hervorheben, weil ihre Anwesenheit einen offenkundigen Beweis darstellt. Die funktionellen Nierenveränderungen (Gefäßspasmus, Kreislaufsstörungen usw.) können in der klinischen Praxis wegen des schweren Zustandes der Patienten nicht untersucht bzw. nicht genau geklärt werden. Diejenigen Fälle, in denen die Oligo-Anurie

sich langsam, stufenweise, als das Endstadium der Grundkrankheit entwikkelte, wurden in diese Gruppe selbstverständlich nicht eingereiht.

2. Welche vorangehende Krankheit löste die Oligo-Anurie aus? Der Oligo-Anurie ging in sämtlichen Fällen eine mit Fieber verbundene Krankheit voraus. In der pyelonephritischen Gruppe waren die auslösenden Faktoren in 7 Fällen Cholezystitis, Cholangitis und Cholangiolitis, in 7 Fällen ein Nierensteinanfall (ohne Verschluß), in je 4 Fällen Sepsis bzw. Enterokolitis, in 3 prostatahypertrophischer Reflux, in 2 Fällen Pankreatitis (ohne Schock) und in 1 Fall Enzephalitis. In den übrigen Fällen kamen in der Anamnese mit Fieber verlaufende Grippe oder Pharyngitis vor. Bei 17 der 20 an chronischer Glomerulonephritis leidenden Kranken war die auslösende Ursache der Oligo-Anurie mit Fieber einhergehende Grippe, während in je 1 Fall Hepatitis bzw. Cholezystitis vorlagen. In der Gruppe der subakuten Glomerulonephritiker wurde das Fieber in 8 von 9 Fällen durch Grippe und in 1 Fall durch Gastroenteritis verursacht. Aus dem Gesagten geht hervor, daß die Oligo-Anurie sich nach irgendeiner, mit Fieber verlaufenden Krankheit, auf Wirkung derselben entwickelte.

Unsere statistischen Angaben weisen darauf hin, daß die Mortalität der infolge der akuten Exazerbation einer chronischen Nierenerkrankung entstandenen Anurien höher liegt als die des oligo-anurischen Gesamtmaterials. Schlechtere Ergebnisse waren lediglich bei nach beiderseitiger Nierenrindennekrose (8 von 8 starben), nach Nierengefäßverschluß (6 von 7 Kranken starben) und bei nach Operationsschock aufgetretener Oligo-Anurie (35 von 47 starben) zu verzeichnen. Vollständigkeitshalber sei erwähnt, daß 4 Patienten, die nach der Aufnahme binnen 12—24 Stunden starben, in derartig schwerem urämischen und Kreislaufzustand eingeliefert wurden, daß bei ihnen die Hämodialyse nicht durchgeführt werden konnte. In den übrigen Fällen, in denen 1 oder mehr Hämodialysen vorgenommen worden waren bzw. in einigen Fällen, in denen es nicht zur Kunstnierenbehandlung kam, war die Todesursache zumeist Kreislaufinsuffizienz. Mit Ausnahme der an subakuter Glomerulonephritis leidenden Kranken konnte die Harnausscheidung in sämtlichen Fällen in Gang gesetzt werden, bei einigen Patienten stieg die tägliche Harnmenge sogar über 2—3000 ml an, was darauf hinweist, daß sich die Nierenfunktion besserte, der Kreislauf infolge des schweren Allgemeinzustandes jedoch insuffizient wurde.

Die nach der akuten Exazerbation eines chronischen Nierenprozesses auftretende Anurie beansprucht keine besondere »antianurische« Behandlung. Sowohl die übliche Regelung des Wasser- und Salzhaushaltes, wie auch die Indizierung der Kunstnierenbehandlung und die Verordnung der antibiotischen Therapie usw. werden ebenso durchgeführt wie bei den übrigen anurischen Kranken; besondere Erfahrungen bzw. Umsicht erfordert lediglich die Behandlung der bejahrten pyelonephritischen Kranken.

Wir wollen es nicht unterlassen, auch an dieser Stelle ausdrücklich darauf hinzuweisen, daß falls die oligo-anurischen Kranken — und selbstverständlich auch die übrigen anurischen Patienten — früher und in besserem Allgemeinzustand eingewiesen wären, wir aller Wahrscheinlichkeit nach bessere Resultate erzielen könnten. In den meisten Fällen werden wir nur dann zum Konsilium gebeten, wenn der Patient sich wegen der fortgeschrittenen Urämie bereits in einem schweren Zustand befindet. In einem Teil dieser Fälle war die zwecks Regelung des Salz- und Wasserhaushaltes angewandte Behandlung nicht richtig, was Ödem und Kreislaufinsuffizienz zur Folge hatte.

Das Ziel vorliegender Arbeit war ein doppeltes: Teils beabsichtigten wir eine seltenere Komplikation der chronischen Nierenprozesse anhand unseres Krankenmaterials bekanntzugeben, teils wollten wir es abermals betonen, daß in Fällen, in denen eine Oligo-Anurie diagnostiziert wird, unmittelbar eine Kunstnierenstation konsultiert werden soll, da auf diese Weise der sich in lebensgefährlichem Zustand befindliche Patient eine, besondere Erfahrung und Praxis beanspruchende, entsprechende Behandlung erhalten wird.

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ANAEMIA AFTER THERMAL INJURY

II. IRON METABOLISM

By

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Thermal injury has been found to be associated with a profound disturbance of iron metabolism, as reflected by reduced iron levels associated with an impaired iron binding capacity of blood serum.

Oral iron tolerance curves in 20 burned patients have been found to start with low values and to remain flat over 7 hours.

In 10 patients with burns intravenously administered iron disappeared from the blood stream in 3 to 5 hours, as a sign of increased iron metabolism.

Histochemical studies involving 40 albino rats have shown that the iron leaving the blood stream at an increased rate is taken up and stored by the RES.

The described changes become manifest within the first 24 to 72 hours after thermal injury and persist during the entire course of the process, to return to normal values during convalescence.

In order to gain insight into the pathomechanism of anaemia associated with burns, we have studied the behaviour of iron metabolism after thermal injury. As reported in an earlier paper, thermal injuries are followed by a fast and significant decline of the iron level and iron binding capacity of blood serum. This change becomes manifest within the first 72 hours after injury, often in a few hours. The abnormality persists during the entire process and, though resistant to oral or parenteral iron administration, subsides spontaneously in the course of convalescence.

The present paper reviews the results of our recent investigations into iron metabolism.

Material and methods

1. Oral iron tolerance tests were performed in 20 burned patients. Eight healthy subjects served as controls.

After determination of the fasting serum iron level, the test subjects were given 850 mg of $FeSO_4$, corresponding to 170 mg metallic iron. The serum iron level was then determined at 1, 3, and 7 hours, using the BOTHWELL—MALLETT-method modified by LAKOS and LEHOTAI [1960].

2. In 10 burned patients intravenous iron tolerance tests were performed in order to determine the rate at which iron is cleared from blood plasma. Five healthy persons served as controls.

3. In order to ascertain whether there is any histochemically demonstrable change in the reticuloendothelial storage of iron, 40 albino rats of 200 to 250 g body weight were subjected to thermal injury involving 20 per cent of the body surface. Two days later the surviving animals were killed by ether and the spleen, liver, lung, kidney and skin (normal and burned) were tested for iron content by the Prussian blue reaction. Twenty-five rats served as controls.

Results and discussion

1. As revealed by the oral iron tolerance tests ingestion of 170 mg metallic iron after thermal injury elicits no or a very slight rise in the serum iron level. The tolerance curve starts with low values and remains flat over 7 hours, in contrast to healthy persons where significant peak values are reached after the same dose (Table I, Fig. 1).

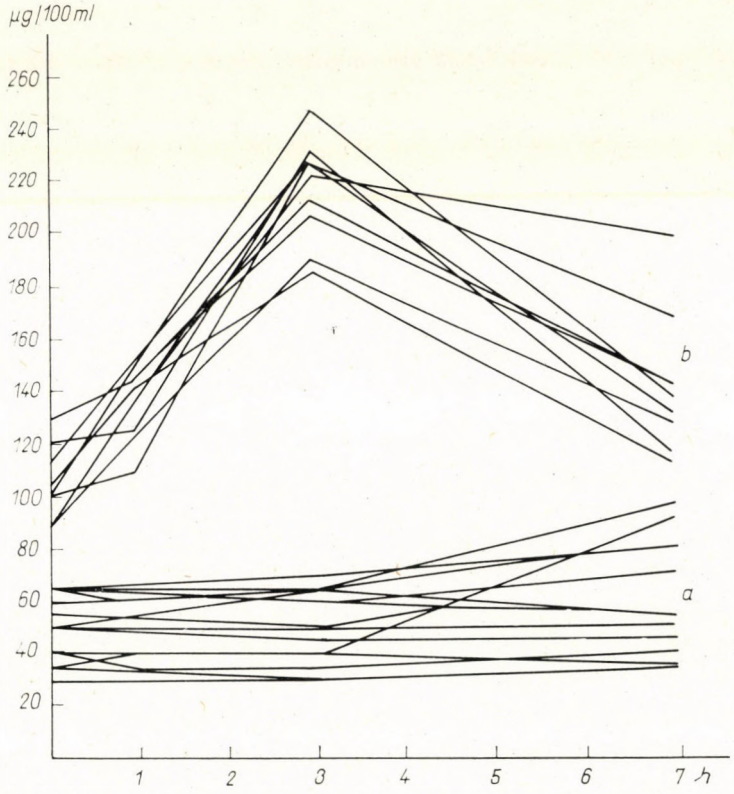

Fig. 1. Oral iron tolerance tests; *a)* patients with burns; *b)* healthy controls

Impaired absorption of iron is, however, not necessarily the sole cause of the flat tolerance curve observed after thermal injury. Disturbances of iron metabolism of infectious or malignant nature are characterized by a flat iron tolerance curve, though, on the evidence of isotope studies, absorption of iron from the intestines is generally increased. Actually, the pattern of oral iron tolerance curve results from two factors, i.e. absorption of iron from the intestines on the one hand and its clearance from the blood stream, on the other. Further studies regarding iron absorption after thermal injury are in progress.

Table I

Oral iron tolerance tests

No.	Serum iron level μg/100 ml			
	Fasting	1 hr	3 hrs	7 hrs
Patients with burns				
1	65	60	65	80
2	60		65	55
3	50	60	50	50
4	40	35	35	40
5	50		65	95
6	35	40	40	90
7	50		45	45
8	65		60	70
9	35		30	35
10	55		50	70
11	65		70	80
12	65		60	55
13	40		40	35
14	30		30	35
15	45	40	85	70
16	50		60	55
17	70	70	80	60
18	65		75	75
19	50	55	65	45
20	40		55	50
Controls				
21	105	140	185	110
22	115	150	225	130
23	90		190	125
24	130	145	210	140
25	120	125	230	115
26	100	150	245	135
27	90		220	195
28	100	110	255	165

2. Intravenous iron tolerance tests resulted in curves characterized by low initial values, lower than normal peak values, and a return to the initial level as early as in 3 to 5 hours (Table II, Fig. 2), as opposed to the control subjects whose values ranged between 225 and 280 μg at 10 hours and showed

Table II

Intravenous iron tolerance tests

No.	Serum iron level μg/100 ml					
	Fasting	5 min.	1 hr	3 hrs	5 hrs	10 hrs
Patients with burns						
1	10	190	60	45	20	10
2	30	300	120	110	90	50
3	45	290	190	140	120	70
4	75	270	195	125	95	70
5	55	230	145	105	80	50
6	35	270	130	65	35	30
7	45	265	140	80	35	25
8	45	270	145	120	70	50
9	40	125	90	90	75	40
10	45	305	155	70	65	40
Controls						
11	130	450	300	275	290	280
12	150	390	250	275	250	250
13	155	385	280	265	265	240
14	135	330	255	250	245	255
15	140	375	250	230	225	225

little tendency to decline. This means that thermal injury results in an accelerated rate of elimination of *intravenously administered iron.*

Enhanced elimination of iron from the blood stream was found two hours after a burn in one case, and six hours after a burn in another case. When the test was repeated a week later, the differences were still more pronounced (Fig. 3).

3. The part played by the individual tissues in the uptake and storage of iron eliminated from the blood stream has been studied in albino rats. Increased storage of iron in the RES under the influence of thermal injury has been demonstrated by histochemical methods. Storage of iron in the spleen of healthy animals varies within wide limits, nevertheless the amount of iron stored in the spleen of burned animals was found significantly higher. While

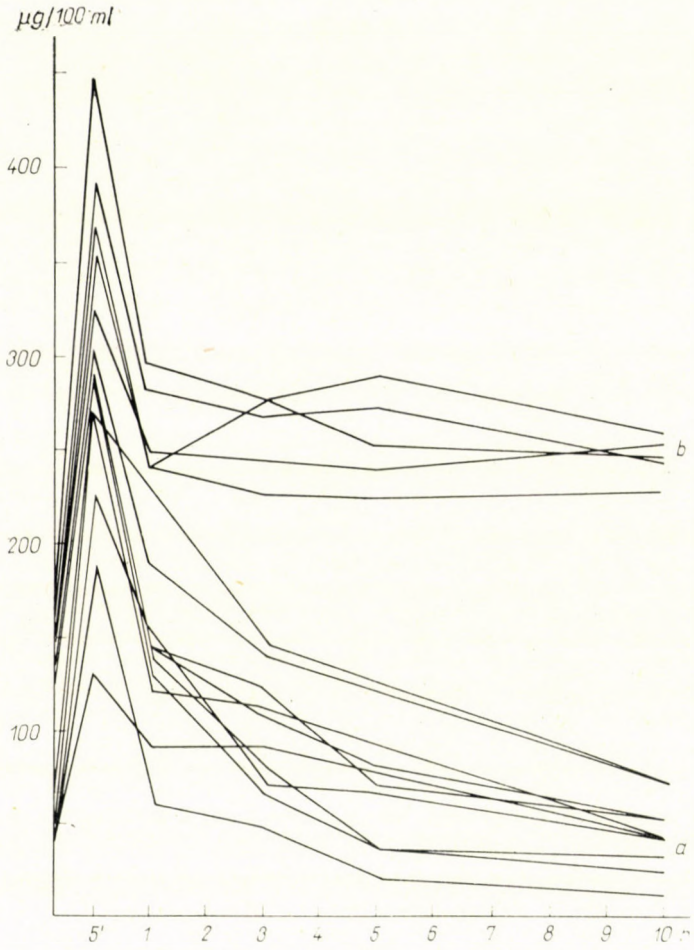

Fig. 2. Intravenous iron tolerance tests; *a)* patients with burns; *b)* healthy controls

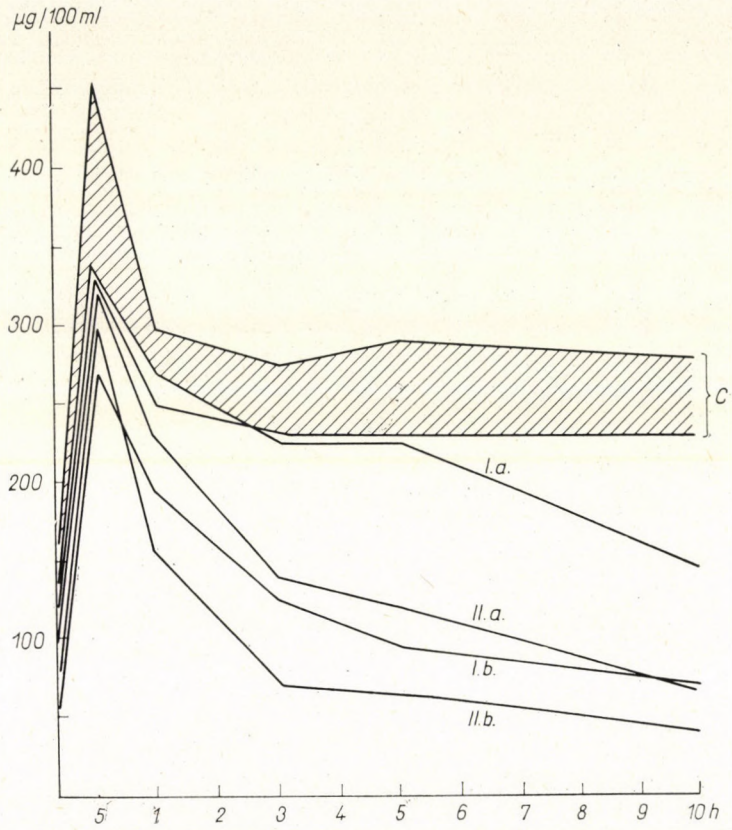

Fig. 3. Intravenous iron tolerance tests in 2 patients with burns. The tests were made in the first hours after injury (Ia and IIa) and repeated one week later (Ib and IIb) (C = limit values obtained from healthy controls)

in the other organs of healthy animals no iron was demonstrable by the Prussian blue reaction, in a number of the burned animals there was evidence of a moderate storage of iron in the liver, lung, kidney, and skin.

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EFFECT OF LATERAL HYPOTHALAMIC LESION ON PREGNANT RATS AND FOETAL MORTALITY

By

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It has been investigated how the electrolytic lesion to the lateral (dorsal tuberal and ventral infundibular) hypothalamic regions on the 16th to 18th days of gestation influenced foetal mortality, as well as the daily food and water intake, body weight and temperature of the mothers.

It has been found that lesions of this type lead to a much higher rate of stillbirth and of early foetal mortality than do sham operations or destruction of other hypothalamic regions. There was no significant difference in foetal weight at birth and in the number of offsprings per mother between the group with hypothalamic lesion and the sham-operated controls. Foetal weight increased in the control group only. Lesion to the lateral hypothalamus led to a marked temporary decrease of food and water intake, the weight of the mothers tended to decrease until delivery, whereas in the control group body weight increased by 10 to 11 per cent following the sham operation. No significant change in body temperature in any of the groups could be seen.

Previous investigations in rats have shown that hypothalamic lesions placed at the 16th to 18th days of pregnancy did not influence the duration of gestation and the course of delivery, whereas they increased the rate of stillbirths and early foetal mortality. Analysis of the effects of lesions in various parts of the hypothalamus suggested that the higher rate of foetal mortality could be correlated in the first place with damage to the ventrolateral infundibular and dorsolateral tuberal regions [1]. The present investigations deal with the effects of lesions produced in these areas and we have studied the changes in foetal mortality rate, as well as those in maternal food and water intake, body weight and body temperature.

Materials and methods

A total of 36 virgin Wistar rats, weighing 150 to 180 g each, were used in the experiments. Mating, verification of pregnancy, observation of the onset and course of delivery, were described earlier [1].

In 17 animals the regions specified above were lesioned electrolytically on the 16th or 18th day of pregnancy by means of the Kovách—Szentágothai universal stereotactic apparatus. Corresponding to the site of the regions, the electrodes were inserted at an angle of 34.5 degrees in the caudo-ventral direction. The data of the coordinates determined in 6 preliminary experiments were: frontal, 4 to 4.5 mm anterior to the bregma; sagittal, 1.4 mm right and left from the sagittal suture; vertical, 11.5 mm (ventrolateral infundibular region) and 10.5 mm (dorsolateral tuberal region). In the sham-operated control group the electrode was inserted into the external layers of the brain. For 3 days after operation 5 mg/100 g body weight of terramycin was administered. In the histological studies formalin-fixed and paraffin-embedded brain sections stained for cells and fibres were used [4].

Beginning on the 12th to 14th days of pregnancy, the food intake of the mothers (Standard high-protein mouse and rat diet), their water intake (tap water ad libitum), as well as

their body weight and rectal temperature were measured daily. Also the number of newborns, their gain in weight, as well as the foetal (newborn) mortality rate were recorded. The results were evaluated by the χ^2 and Student's t tests [3].

Results

The typical hypothalamic lesions are shown in *Fig. 1.* Histological examination revealed that 80 per cent of the lesions were in the proper regions, whereas the rest extended slightly over to the adjacent ones as well.

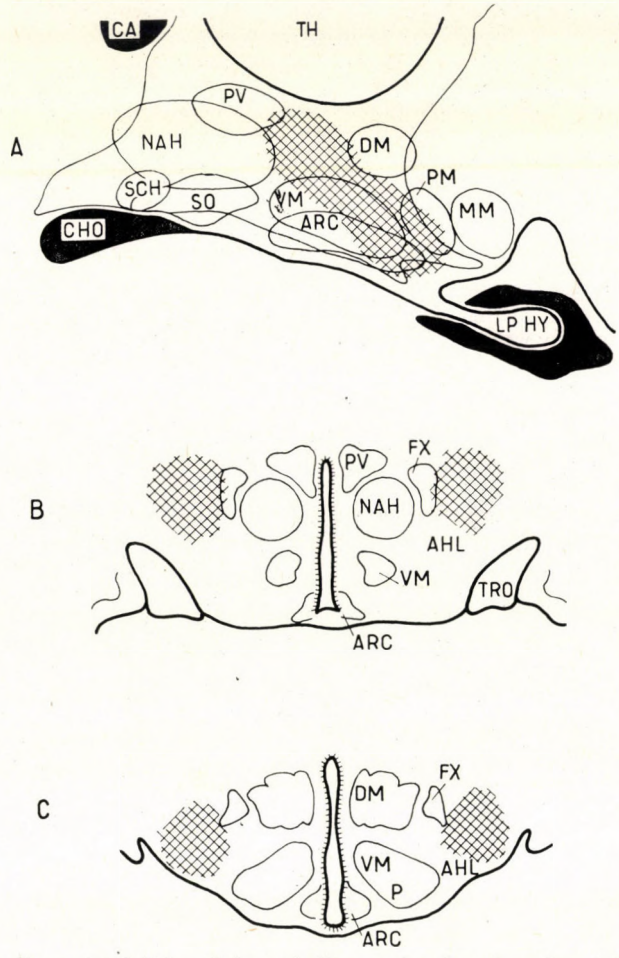

Fig. 1. Showing the sagittal (A) and frontal (B — tuberal region; C — infundibular region) sections of the hypothalamus. The checkered parts show the commonest locations of the lesions.

AHL: lateral hypothalamic area. ARC: arcuate nucleus. CA: anterior commissure. CHO: optic chiasma. DM: dorsomedial nucleus. FX: fornix. LP: posterior pituitary lobe. MM: medial mammillary nucleus. NAH: anterior hypothalamic nucleus. PM: premammillary nucleus. PV: paraventricular nucleus. SCH: suprachiasmatic nucleus. SO: supraoptic nucleus. TH: thalamus. TRO: optic tract. VM: ventromedial nucleus. VMP: posterior ventromedial nucleus

As the data in *Table I* indicate, there was no difference between the lesioned and the control groups in the duration of pregnancy, the number of offsprings per mother and in the average weight of the newborns. On the other hand, the two groups differed significantly in foetal mortality rate *(Fig. 2)*.

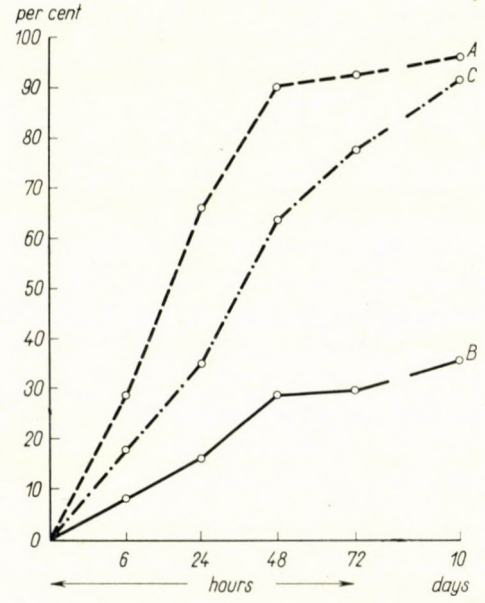

Fig. 2. Foetal death plotted against postpartum days, in percentage of all newborns. A: mothers with lateral hypothalamic lesion (locations shown in *Fig. 1)*. B: sham-operated controls. C: mothers with diffuse (mainly anteromedial) hypothalamic lesions. "p" values expressed as per cents (χ^2 test):

	6	24	48	72 hours
B—C	1.5	<0.1	<0.1	<0.1
B—A	<0.1	<0.1	<0.1	<0.1
C—A	3	<0.1	<0.1	<0.1

Fig. 2 presents the foetal mortality rate in the case of 31 rats observed under identical conditions in a previous series [1] (group C), too. In that group various areas of the hypothalamus were destroyed. When the dorsolateral tuberal and the ventrolateral infundibular regions had been lesioned (group A); mortality rate of the newborns 3 days following birth was significantly higher than in the sham-operated group (group B) or in the previous group C. The rate of stillbirth (death within six hours following delivery) in group A was more than 150 per cent of that of group C, and 300 per cent of that of group B. The differences in foetal mortality rate between groups (B—C; B—A; C—A)

Table I

	Number of rats observed	Duration of pregnancy, days	Total number of newborns	Mean body weight of newborns, g	Number of foetuses dead within	
					6 hours	72 hours
I. Lesioned group	17	23.5	121	5.23 ±0.17	35	112
II. Sham-operated group	19	23.1	142	5.50 ±0.14	12	42

were significant statistically in the 6-, 24-, 48- and 72-hour post-partum period alike. The higher mortality rate following lesion to the specified regions of the hypothalamus was due partly to the fact that there was a larger number of dead offsprings per mother and at the same time a higher percentage of the

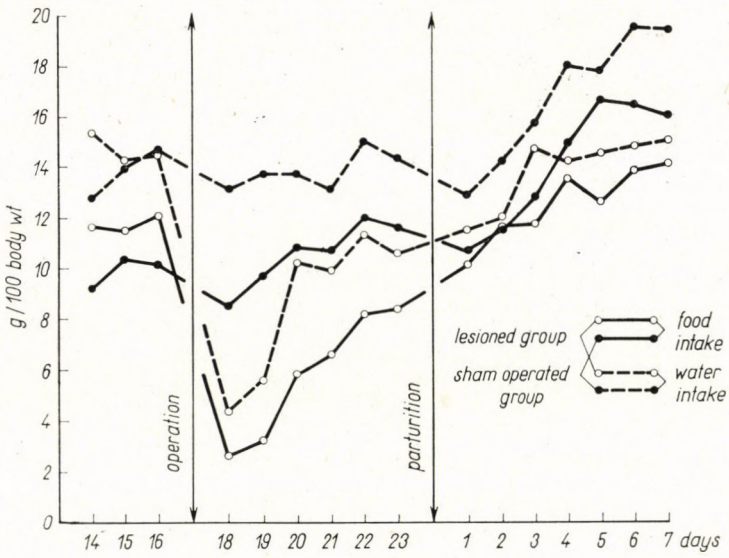

Fig. 3. Food and water intake of lateral hypothalamic lesioned and sham-operated control mothers

mothers had dead offsprings than in the other two groups. Thus, 24 hours following delivery dead offsprings were found at 88.3 per cent of the mothers of group A, 36.9 per cent in group B, and 64.5 per cent in group C; the number of dead newborns per mother averaged 5.3, 3.3 and 3.5, respectively. Average body weight of the newborns increased in the days following birth, except in group A.

In response to lateral hypothalamic lesions, food and water intake changed markedly *(Fig. 3)*. As opposed to the sham-operated group, food and

water intake decreased significantly following the lesion. The decrease was particularly marked in the pre-delivery period ($p < 0.1$ per cent) in both cases.

The hypothalamic lesions resulted in a loss of 4 to 5 per cent of the body weight of the pregnant animals till delivery, whereas in the sham-operated group body weight increased by 10 to 11 per cent ($p < 0.5$ per cent).

There was no substantial change in body temperature in any of the groups.

Discussion

Electrolytic lesions to the ventrolateral infundibular and dorsolateral tuberal regions lead to a significantly higher rate of early foetal mortality (a higher percentage of the mothers has dead offsprings and also the number of dead offsprings per mother is higher), than did a sham operation affecting the cerebral cortex, or a lesion to other areas of the hypothalamus. There was no significant difference between the lateral hypothalamic-lesioned and the sham-operated control groups in the weight of the newborns at birth and in the average number of offsprings per mother. The average weight of the newborns increased in the control group only. Lesion to the lateral hypothalamus led to a marked, temporary decrease of food and water intake; correspondingly, body weight of the mothers tended to decrease till delivery, whereas in the control group body weight increased by 10 to 11 per cent even after the sham operation.

A high rate of stillbirth and of early newborn death has been found also by KURCZ [5] following lesion to the ventromedial hypothalamic nucleus about 3 months before delivery, or on the 9th to 16th days of pregnancy. In the former case somatic factors connected with hypothalamic obesity, in the latter a disturbance of lactation were blamed for the high rate of stillbirth and for early foetal death, respectively. Maternal behaviour was considered normal in both cases. GALE and MCCANN [2] found serious disturbances in the process of labour in 22 per cent of their rats subjected to median eminence lesion on the 7th to 9th days of pregnancy, but even the mothers showing normal labour failed to feed their newborns because of the loss of the milk-ejection reflex. Protracted labour and significant maternal and foetal mortality rates were induced also by placing progesterone and testosterone implants in the areas of the arcuate nucleus and the region of the mammillary nuclei [6]. All these data indicate that the hypothalamus is involved in many ways in the organization of the factors determining the viability and raising of newborns. Similarly, various factors may be involved in the high rate of stillbirth and newborn mortality observed by us following lesion to the lateral hypothalamus. A disturbance of maternal behaviour may play a particularly significant role: most of the mothers build no nests, the newborns lie scattered in the cage, many

of them show signs of gnawing. According to KURCZ [5] maternal behaviour is not impaired following lesion to the medial hypothalamus. Other factors which may be taken into consideration are disturbances of milk production and ejection as well as an decreased vitality of the offsprings of the lesioned mothers. We have been unable to demonstrate histological differences between the breasts of the control and lesioned mothers.

It is justified to assume that the mothers with lateral hypothalamic lesions give birth to offsprings of decreased vitality also because during the days following operation, unlike in the sham-operated group, there is a very significant decrease of water and food intake and the increase of body weight seen in the control group is absent. Our observations agree with data in the literature which suggest that feeding centre would exist in the lateral hypothalamus [7]. It is remarkable that the two groups examined by us did not differ much in post-partum food intake, and the small difference in favour of the controls may be ascribed to their higher calorie demand, because they feed their newborns. The circumstances of breast-feeding, an eventual decreased vitality of the offsprings and the changes of maternal behaviour will be studied in subsequent experiments.

On the basis of the present results we are of the opinion that the structures in the lateral regions of the hypothalamus may play a significant role in the delivery and raising of viable offsprings, presumably by influencing maternal behaviour, on the first place.

Acknowledgement. Thanks are due to Miss Judit Kovács for skillful assistance.

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DATA TO THE PATHOMECHANISM OF THE SHOCK-KIDNEY

IV. REGENERATION OF THE KIDNEY AFTER TEMPORARY ANOXIC INJURY

By

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After unilateral ligation of the renal artery and vein for various periods, restitution of renal function has been investigated by means of the dye absorption method in rats. A correlation was found between the duration of renal anoxia and the length of time necessary for regeneration. Regeneration was practically complete by the 14th day. Restitution of renal function greatly depended on the severity of oxygen lack suffered by the tubular epithelium.

In an earlier paper [1] we dealt with the influence of anoxia on GFR and tubular function. The aim of these studies was to ascertain whether the functional and structural changes associated with the shock-kidney could be reproduced by isolated renal anoxia. The problem was approached by temporary ligation of the renal hilum [2] and subsequent study of the renal changes according to the method of SELLERS [2]. As the animals were sacrificed 24 hours after this intervention, the observations were necessarily limited to acute renal damage. It seemed therefore obvious to investigate the regenerative capacity of the kidney when the acute consequences of the injury had subsided. Interest in the possibilities of restoration of renal functions is enhanced by the clinical experience that patients with acute renal failure may survive the phase of oliguria or anuria thus having a chance to regain their full renal activity [3].

We were led by these considerations to study the changes of GFR and tubular function in model experiments by means of Evans-blue given at various intervals after an anoxic period. As pointed out earlier, the method is based on the fact that Evans-blue administered intravenously is bound to serum protein. Since the rat is a species with proteinuria, the protein impregnated with Evans-blue is filtered, the protein-dye complex is partly absorbed by the proximal tubules where it can be visualized in microscopic sections in the form of blue droplets. In this manner, the dye content of the proximal tubular cells permits to assess the degree of filtration and absorption.

Method

Albino rats weighing between 120 and 150 g were used. The left renal hilum was ligated under ether anaesthesia through the abdominal approach, then half or one and a half hour later the ligature was removed and the wound closed. An aqueous solution of 25 mg Evans-blue was injected intravenously 3, 4, 7 and 14 days after the intervention. Four hours after injection the animals were killed by decapitation. Pieces of the removed kidneys were partly fixed in formalin for unstained frozen sections, partly embedded in paraffin for haematoxylin-eosin staining.

Results

Group I. In 5 rats ligation of the left kidney was maintained for 90 minutes, and the animals were killed after 3 or 4 days. The haematoxylin-eosin stained sections clearly showed that the changes were essentially tubular. The damage varied in intensity and extent. It correlated fairly well with the condition of the epithelium, which showed swelling, degeneration or necrosis, the site of prevalence being the cortico-medullary border, occasionally also the area of the proximal tubules. In some cases dilatation of the tubular lumina with flattening of the epithelium and occasional signs of regeneration were predominant. Numerous tubules contained homogeneous casts. The glomerules remained practically unaffected.

Absorption of the Evans-blue-protein complex was studied in unstained frozen sections. In the areas of extensive necrosis a diffuse blue staining was seen instead of dye droplets. In the cases in which tubular dilatation and epithelial flattening were prevalent the tubules contained either no dye or only sparse dye droplets.

Quantitative evaluation of the stored dye was the basis for assessing the changes in renal function. Obviously, this method lacks the reliability of chemical analysis, but with a certain practice it was possible to give a fairly correct estimate of the amount of stain retained by the ligated kidney in comparison with the normal side.

In Group I, the amount of dye droplets was moderately reduced in 2 animals, greatly reduced in 9 (Fig. 1), while in 2 animals diffuse staining revealed a widespread necrosis; in 2 animals no absorption was evident.

In Group II, in 8 animals the hilum of the left kidney was ligated for 90 minutes, and the animals were killed after 7 days. Microscopic examination showed epithelial degeneration with occasional tubular necrosis. Dilatation of the lumina was conspicuous along the cortico-medullary border. Interstitial focal round cell infiltrations with occasional casts were present. Absorption of the dye was evident from the intensity of blue droplets, but its extent was moderately reduced in half (Fig. 2) and strongly reduced in the other half of the animals.

Group III. In the 10 animals of this group, ligation was likewise maintained for 90 minutes and the animals were killed after 14 days. There was no

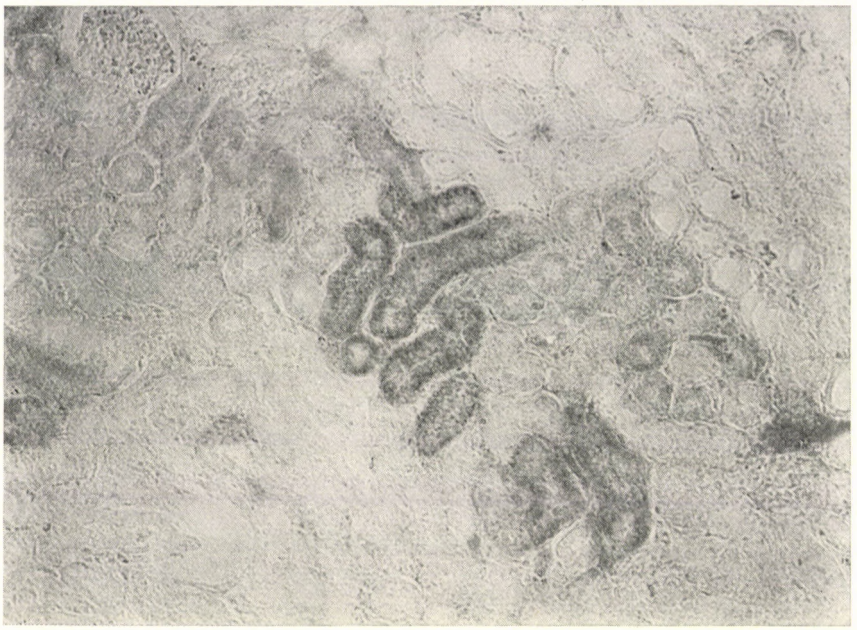

Fig. 1. Rat killed four days after renal hilum ligation for 90 minutes. Section of renal cortex. Dye content of tubules greatly reduced. Unstained frozen section. Enlargement, approx. 100 fold

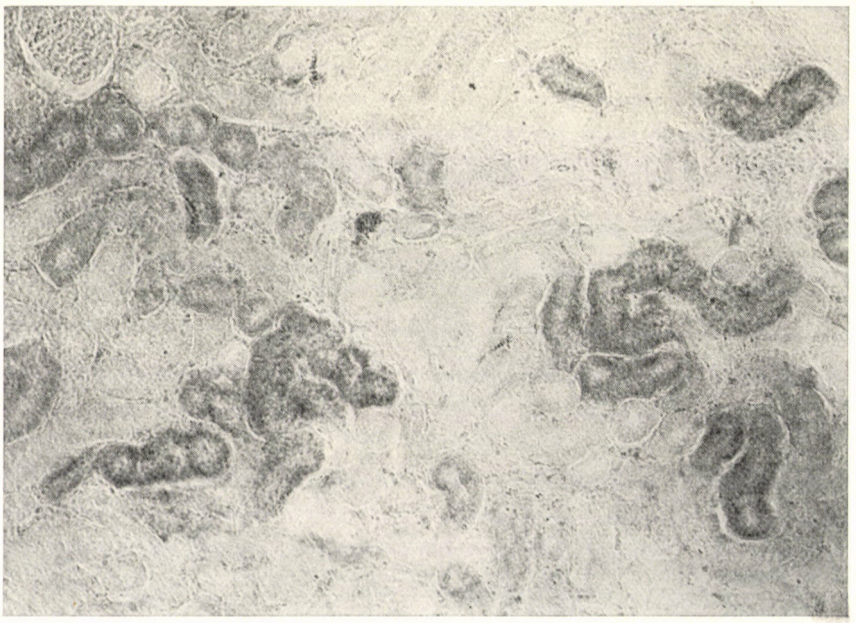

Fig. 2. Rat killed seven days after ligation of renal hilum. The renal cortex contains dye in moderately reduced amounts. Unstained frozen section. Enlargement, approx. 100 fold

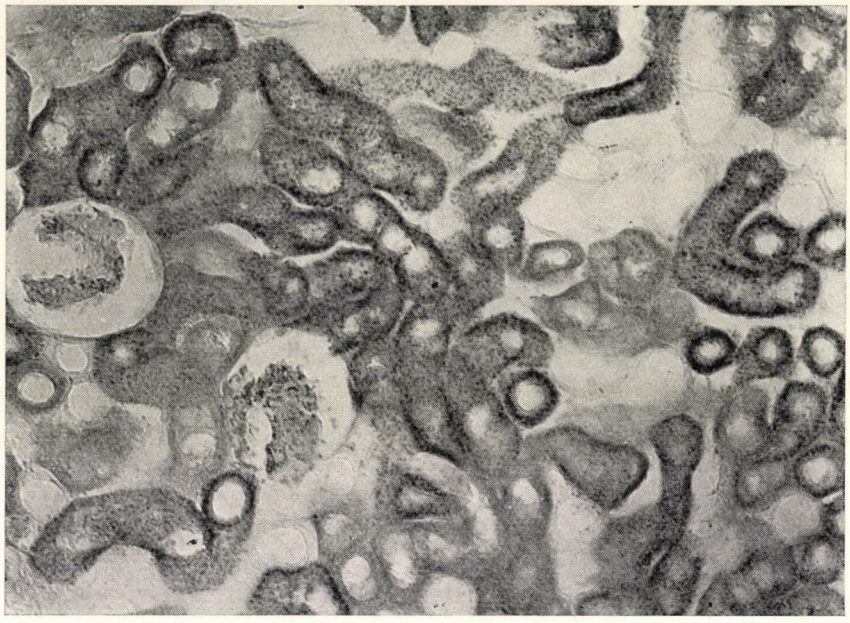

Fig. 3. 14 days after renal hilum ligation for 90 minutes. The cortex contains normal amounts of dye. Unstained frozen section. Enlargement, approx. 100 fold

evidence of tubular necrosis and epithelial degeneration was less distinct. Some of the distal tubules were dilated, their flat epithelial lining showed intraluminar protrusions in some parts. Interstitial inflammatory foci were distinct. The tubular lumina were filled at many sites with a viscous granular mass. The dye droplets in the epithel cells of the proximal tubules showed normal intensity in 3 animals (Fig. 3), moderate reduction in 2, slight reduction in 5.

Group IV. In 12 rats ligation was maintained for 30 minutes, and the animals were killed 7 days later. Epithelial necrosis was confined to a small

Table I

Group	Ligation, minutes	Time between ligature and death, days	Dye content of tubules						Number of animals Total
			Normal	Slightly reduced	Moderately reduced	Greatly reduced	Ø	Diffuse	
I	90	3—4	—	—	2	9	2	2	15
II	90	7	—	—	4	4	—	—	8
III	90	14	3	5	2	—	—	—	10
IV	30	7	3	5	2	1	—	—	12
V	30	14	7	3	—	—	—	—	10

number of tubules, degeneration was not significant. Dilated tubules were found at sites with a lining of flattened epithelium. The dye appeared in droplets in the tubular epithelium. In 4 animals the dye content was normal, in 5 slightly, in 2 moderately, in one strongly, reduced.

Group V. Ligation was maintained for 30 minutes, and the 10 animals were killed 14 days later. The microscopic changes were very slight throughout. The dye appeared in normal amounts, with the exception of 3 animals where it was slightly reduced (Table I).

Discussion

The microscopic changes subsequent to total occlusion of the vessels of the renal hilum have been closely studied [4, 5, 6, 7]. In Hungary, ZÁDOR and BALOGH [8], recently BÁLINT et al. [18] have investigated the question. CAIN and FAZEKAS [9] studied the tubular changes by histochemical methods at various intervals after clamping the renal hilum for 60 minutes; according to them the renal ischaemia was responsible for the totality of the changes. FINCKH, JEREMY and WHYTE [10] investigated the structural changes in fatal acute renal failure in man. They found the extent of necrotic tubular damage to be inversely related to the duration of the process. On the 13th day lesion of the proximal, on the 18th day of the distal tubules, was no longer demonstrable. In agreement with other investigators [11, 12] they did not connect the oliguria or anuria with rediffusion through the necrotic tubules. Though there is ample evidence to make the transitory renal ischaemia responsible for the coagulative necrosis of the proximal tubules [13, 14] it is equally true that regeneration of these changes is fairly rapid [15]. For these reasons, the authors ascribe the syndrome of acute renal failure to the sustained inhibition of renal blood supply rather than to the structural changes in question. There is no agreement in literature concerning the question whether the oliguria or anuria subsequent to acute renal damage is a consequence of the reduced GFR or rather of a passive rediffusion through the severely damaged tubular epithelium. In an earlier study [16] we investigated by SELLERS' method the changes of the tubular epithelium in experimental corrosive sublimate poisoning and found passive rediffusion to be equally severe in temporary renal anoxia or in corrosive sublimate poisoning. The question still awaits further studies for its full elucidation [17].

The aim of the present investigations was to derive information from the structural changes subsequent to temporary ligation of the renal hilum concerning the restoration of renal function. The studies were centred on the intensity of the appearance of the dye in relation to normal conditions, i.e. on the informative value of the microscopic changes as concerns the restitution of renal functions and the length of time necessary for this process. In accord-

ance with earlier results [11], tubular dye reabsorption seems to be determined by two factors, namely the structural condition of the tubules and the degree of filtration. These two factors must therefore be considered when interpreting our findings. If glomerules and blood flow remain unimpaired, normal filtration may be preserved, but the damaged or necrotized tubular epithelium is staining diffusely, being unable to absorb the dye-protein complex from the filtrate in the form of droplets. Correspondingly, in the case of widespread epithelial necrosis, dye droplets were only encountered occasionally whereas diffuse staining was invariably present. This shows that glomerular filtration was preserved also in these cases, but dye absorption was impaired. Nor are the flattened epithelial cells which make up the lining of the dilated tubules capable of retaining the dye in the form of droplets. This would suggest that the absorptive capacity, or, broadly speaking, the whole function of the tubular epithelium, particularly of the flat epithelium, is greatly impaired.

It emerges from the present experiments that the duration of renal ischaemia is correlated with the length of time required for reparation. Renal damage caused by temporary anoxia in rats is almost completely restored by the 14th day. It is likewise obvious that restoration of renal function closely depends on the duration of anoxia and on the condition of the tubular epithelium.

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HISTAMINE CONTENT OF THE CEREBROSPINAL FLUID IN MULTIPLE SCLEROSIS

A PRELIMINARY COMMUNICATION

By

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Allergic inflammation is one of the possible factors which have been made responsible for multiple sclerosis, but this claim has not been substantiated by any conclusive or at least convincing evidence. The part played by histamine in the pathomechanism of allergic phenomena has long been a controversial issue. NOAH and BRAND [1] found increased blood histamine levels in certain allergic conditions. The phenomenon of reduced histaminopexy associated with allergic diseases [2] has been studied in multiple sclerosis and the finding of a reduced or missing histamine-binding capacity has been linked with a possible allergic origin of this disease [3].

Material and methods

The present studies were concerned with the free histamine content of lumbar and cisternal CSF in multiple sclerosis and other neuropsychiatric conditions. For the extraction of histamine from 2.5 ml CSF-samples we used the method described for histamine estimation in blood plasma [4]; the histamine level was estimated by the biological superfusion method which in earlier tests was found sufficiently sensitive and specific [5, 6].

The cases of multiple sclerosis were grouped according to the stage, i.e. acute (recent cases, optic neuromyelitis) and chronic (first or repeated exacerbation, slow progression, stationary condition or remission) (see Table I). The cases showing acute and chronic exacerbation (Cases Nos. 1, 3, 5, 6, 7, 8, 11, 13, 14, 15, 19, 20, 25, 26, 27) have been dealt with separately ($N' = 15$). In the acute cases the tests were carried out in the first three months after the onset of the first symptoms. The cases under drug treatment at the date of the study have been marked separately.

The control group has been made up of unselected CSF samples (obtained from subjects with some abnormality) but normal protein levels and cell counts. The tests were invariably matched with the control samples with the exception of one case which has been, however, excluded from statistical evaluation. The matched (test and control) CSF samples were always of the same origin (lumbar or cisternal).

Results, discussion

The histamine content in the CSF of the multiple sclerosis group was higher than that of the controls; the average was 6.6 μg/1000 ml in the former and 2.2 in the latter group. There was a large standard deviation in both groups, 6.7 and 2.4 units, respectively. The range values are from 0 to 25 μg/1000 ml

Table I

Comparison of CSF histamine content in multiple sclerosis and other diseases

No. 1963—65	Multiple sclerosis Stages	CSF-histamine 1/1000 μg/ml	Other diseases Diagnosis	CSF-histamine 1/1000 μg/ml
1	acute	25	Discopathy	1
2	chronic stationary	1	Discopathy	1
3	chronic, first exacerbation	2.5	Discopathy, neuropathy	1
4	chronic stationary (Th)	1 (c)	Trigeminal neuralgia	0
5	chronic, third exacerbation	10 (c)	Epilepsy	1 (c)
6	acute	10 12 (c)	Spondylarthrosis	5
7	chronic after first exacerbation (one week Th)	1	Discopathy	0
8	acute	15	Cerebral concussion	7.5
9	chronic progressive	5 (c) 5	Epilepsy	0 (c)
10	chronic progressive for 6 months	20 (c)	Epilepsy	0 (c)
11	acute	10	Headache, neuropathy	2.5
12	chronic stationary (Th)	1	Discopathy	1
13	acute	10	Discopathy	7.5
14	acute (4 weeks Th)	5 (c)	Headache, neuropathy	2.5 (c)
15	chronic, first exacerbation (Th)	0	Headache	1
16	chronic stationary	0	Discopathy	1
17	chronic progressive	10	Discopathy	1
18	chronic remission	1 (c)	Headache, hypertension	7.5 (c)
19	chronic, second exacerbation	10	Discopathy	1
20	optic neuromyelitis	15	Discopathy	5
21	chronic stationary	1	Epilepsy	1
22	chronic stationary	0	Discopathy	0
23	chronic progressive	0	Discopathy	1
24	chronic progressive	10 (c)	Epilepsy	0 (c)
25	chronic, second exacerbation (Th)	0	Headache	1
26	acute (Th)	7.5 (c)	Parkinsonism	5 (c)
27	chronic exacerbation	5 (c) 5	Trigeminal neuralgia	5 (c)
28	acute	10	Headache	5 (c)
Average	(N = 27)	6.6±6.76		2.2±2.49
	(N′ = 15)	8.1±6.70		P < 0.01

Values 1, 3, 7 furthermore 5, 10 and 13, 14 are the results of repeated tests made in the same case. (c) = cisternal

in the multiple sclerosis group and from 0 to 7.5 μg/1000 ml in the control group. There was a significant difference between the average values for the two groups ($P < 0.01$). The average in the cases which have been evaluated separately ($N' = 15$) was 8.1 ± 6.7 μg/1000 ml, and in the remaining 12 cases, 4.2 ± 6.2 μg/1000 ml.

Confrontation of the values obtained in this separate group with those of the controls showed that, if computed from this purified value, i.e. $8.1 \pm \pm 6.7$, significance increased further ($t = 3.241$; $t_{15} = 4.031$). The elevated average histamine level was thus mainly accounted for by the cases of acute and chronic exacerbation.

No correlation was found between the protein and histamine values of the CSF. The cell counts in multiple sclerosis ranged between 0/3 and 12/3, a sole exception being 24/3. Since the low cell counts showed practically no individual variation, according to rutin tests the cellular pattern could not be correlated with the histamine content. Haemorrhagic CSF samples were not examined.

On the evidence of biologic tests, BOCCARINI et al. [7] found the histamine content of the normal CSF between 10 and 20 μg/1000 ml. Recent photofluorometric examinations failed to demonstrate any free histamine in the normal CSF. The aim of the present study was to establish whether in various pathological conditions, first of all in those of supposedly inflammatory origin, the histamine in the lumbar and cisternal CSF was increased and, if so, to what extent. In the literature we found no data concerning the histamine content of the CSF, in multiple sclerosis.

The origin of histamine in the CSF is assumed to be connected with the following possibilities.

a) It may originate from an inflammatory process in the brain;

b) it may come from the blood plasma passing the blood-CSF-barrier.

1. The blood histamine level is normal, but the barrier permeability is increased; this results in higher CSF histamine level.

2. The blood histamine may be for some reason increased to a level which causes an increase in the CSF level.

3. Reduction of histaminopexy in multiple sclerosis [3] may result in a raised free histamine level in the CSF.

4. The cellular elements passing into the CSF may be a source of histamine [7].

The present investigations have failed to give information about the possible relationship between the raised CSF histamine level and the disturbance in histamine metabolism supposed by certain authors [9] to be present in multiple sclerosis.

The recent, relatively simple but reliable and sensitive methods of histamine determination, first of all spectrophotofluorometry will make it possible

to carry out further investigations into the pathogenetic role of histamine in other allergic or inflammatory diseases. It might be rewarding to investigate the cytologic pattern and the CSF histamine content in patients at different stages of the disease with a view to providing for further data concerning pathomechanism of multiple sclerosis.

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EFFECT OF DEHYDRATION ON CARDIAC OUTPUT AND ORGAN BLOOD FLOW IN ANAESTHETIZED AND UNANAESTHETIZED RATS

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The circulatory effects of dehydration have been studied in anaesthetized, unanaesthetized-restricted and unanaesthetized-unrestricted rats. In the anaesthetized dehydrated animals the circulatory pattern was similar to that found previously in various types of stagnant hypoxia. In the alert animal even the preparatory manipulation brought about serious circulatory changes; in response to dehydration the coronary fraction of cardiac output increased, the skin fraction decreased, while the renal fraction remained unchanged.

The circulatory changes in various types of stagnant hypoxia have been investigated extensively in various species (see: GÖMÖRI and TAKÁCS, 1960).

It was concluded that stagnant hypoxia was associated with a characteristic redistribution of cardiac output. Cardiac and cerebral blood flow change slightly, renal and extremital flows decrease significantly; i.e. the coronary fraction of cardiac output increases and the renal and extremital ones undergo a decrease. These changes are initiated presumably by the hypoxia of tissues (TAKÁCS 1957 a, b; TAKÁCS and KÁLLAY 1957 a, b).

Due to methodological difficulties the effect of dehydration on organ blood flow have been studied in larger mammals (ROGER 1965). GÖMÖRI and PODHRADSZKY (1937) observed a decrease in glomerular filtration rate (GFR) with the deterioration of circulation. Then it has been shown that the decrease in renal blood flow exceeds that in cardiac output (TAKÁCS and KÁLLAY 1957 a). The circulatory changes are presumably centrally regulated (GÖMÖRI et al. 1960).

In the present experiments the circulatory effect of dehydration have been studied in rats. The animals were divided into the following groups: anaesthetized, unanaesthetized-restricted and unanaesthetized-unrestricted rats.

Methods

Male inbred rats weighing 160 to 250 g, were used. In the single groups the average weights ranged from 187 to 215 g.

Dehydration was induced by the method of SHAY et al. (1945), as modified by ROGER (1965). By this modification the otherwise lethal gastric dilatation could be avoided (since the

rat does not vomit, the fluid accumulating in the ligated stomach usually kills the animals before dehydration would have developed). The animals were fasted 24 hours before operation, water was allowed ad libitum. Under ether anaesthesia upper median laparotomy was performed, the pylorus was ligated, then the abdominal wound was closed in two layers. At 10 and 24 hours following the operation, the animals were fixed by hand without anaesthesia and the accumulated gastric juices were withdrawn by means of a soft tube. In the sham-operated animals upper median laparotomy was performed, the stomach was gently moved about and the abdominal wall was closed in two layers. In the dehydrated and sham-operated groups the circulatory studies were carried out 24 hours after operation. The control animals were fasted 24 hours before the experimental procedure, water was allowed ad libitum.

The degree of dehydration was estimated on the basis of the weight loss and azotaemia (NPN level determined by the modified Rappaport method). At 24 hours following pyloric ligation the dehydrated animals lost an average of 17 per cent of the body weight, as compared with the average losses of 6.5 per cent shown by the controls and the sham-operated animals. NPN increased to 121—127 mg per 100 ml in the dehydrated animals, as compared with the average values of 40 to 52 mg per 100 ml in the control and sham-operated groups.

Mean arterial pressure was measured in the carotid artery by means of a mercury manometer.

Cardiac output was determined on the basis of the dye dilution method (HAMILTON et al. 1932) with Evans blue, the organ fractions of cardiac output were estimated by SAPIRSTEIN's isotope indicator fractionation method (^{86}Rb) (SAPIRSTEIN 1956; 1958). The animals were anaesthetized with 40 mg/kg sodium pentobarbital intraperitoneally, as it was found that this drug is better suited for circulatory studies in the rat than urethane, chloralose or ether (KÁLLAY and TAKÁCS 1961; VIDT et al. 1959). From the values for cardiac output, organ fractions, organ weights and blood pressure, the blood flow and vascular resistance of the organs were computed for 100 g of tissue weight (KÁLLAY and TAKÁCS 1961).

The experiments were carried out on three groups: anaesthetized, unanaesthetized-restricted and unanaesthetized-unrestricted ones.

In the unanaesthetized-restricted group the procedure was as follows. The animals were fixed in supine position, then the areas of the carotid artery and femoral veins were infiltrated with 1 per cent procaine solution. Blood pressure was measured in the carotid artery, then Evans blue and ^{86}Rb were injected into the femoral veins.

In the unanaesthetized-unrestricted group a thin polyethylene cannula filled with heparin was tied into the jugular vein under pentobarbital anaesthesia; 24 hours later, without interfering with the movements of the animals, the ^{86}Rb was injected through the cannula. In this group blood pressure and cardiac output were not measured, only the organ fractions of the cardiac output were estimated.

The results were evaluated by Student's "t" test. To compile uniform and synoptical tables we presented only the within-sample standard deviations (computed from the pooled sum-of-squares), as they did not materially affect the results. We compared the sham-operated rats with the corresponding controls, and the dehydrated rats with the sham-operated ones. The controls of the alert groups were compared also with the anaesthetized controls.

The following units were used:
Cardiac output: ml/min/100 g of body weight
Blood pressure: mm Hg
Resistance (systemic): 10^3dyne.sec.cm^{-5}/100 g of body weight
Flow: ml/min/100 g of organ weight
Resistance (organ): 10^3 dyne.sec.cm^{-5}/100 g of organ weight
Fraction (organ): blood flow of the total organ expressed in percentage of total cardiac output.

Significance of the differences was denoted as follows:

No sign	not significant	$p > 0.05$
., *, °	significant	$p < 0.05$
.., **, °°	highly significant	$p < 0.01$
..., ***, °°°	very highly significant	$p < 0.001$

Results

The results are presented in *Tables I, II* and *III.*

a) In the anaesthetized group (Table I), as compared with the control, the sham operation caused no significant circulatory change, only flow in the carcass increased and coronary resistance decreased significantly.

In the dehydrated animals cardiac output and blood pressure decreased significantly. The heart fraction of cardiac output increased, this was associated with a smaller decrease of flow and increase of resistance. Renal fraction of cardiac output and renal flow decreased, renal resistance increased. Hepatic and intestinal flow decreased and resistance increased. The splanchnic fraction of cardiac output decreased but, owing to the wide scattering, the difference was not significant. Skin flow and fraction decreased, resistance increased. Flow in the carcass decreased as compared with the sham-operated animals which yielded higher values than the controls; the carcass fraction of cardiac output increased.

b) The unanaesthetized-restricted animals showed the following differences from the anaesthetized ones and from the alert controls (Table II).

Cardiac output and blood pressure increased, systemic resistance decreased, the renal fraction dropped by almost 10 per cent of the cardiac output, renal flow decreased, renal resistance increased to almost double the initial value. Pulmonary flow increased significantly, pulmonary resistance decreased. The hepatic, intestinal and splanchnic fractions decreased considerably, skin flow increased, skin resistance decreased. In the carcass the fraction of cardiac output increased very considerably, together with an increase of flow and decrease of resistance.

The sham operation caused no marked change in this group, either; blood pressure, skin fraction and hepatic blood flow were higher than the corresponding values of the unanaesthetized-restricted controls.

The changes brought about by dehydration were a decrease of blood pressure and cardiac output and an increase in systemic resistance. Coronary fraction increased, renal, hepatic and intestinal flows decreased, resistance in these organs increased. The skin fraction and flow decreased considerably, resistance increased. In the carcass, flow and resistance changed in a similar manner. In the lungs the fraction increased, flow decreased, in the splanchnic area the fraction of cardiac output increased.

c) In the unanaesthetized-unrestricted group (Table III), when the alert control was compared with the anaesthetized control, the cardiac, renal, hepatic, intestinal and splanchnic fractions decreased, but the decrement — except for that of the cardiac fraction — was considerably less than in the alert-restricted animals, particularly in the case of the renal fraction. The skin and carcass fractions increased.

Table I

Effect of dehydration on circulation of anaesthetized rats

Number of cases (n)	Control 14	Sham-operated 14	Dehydrated 14	Within-sample standard deviation
Total body	Mean values			
Cardiac output	25.2	27.5	18.6***	5.4
Blood pressure	117.0	114.0	96.0***	10.3
Resistance	396.0	377.0	460.0	131.7
Heart				
Flow	155.0	191.1	163.1	56.1
Resistance	68.8	49.9·	57.7	20.3
Fraction	2.0	2.2	3.0**	0.7
Kidney				
Flow	379.1	420.8	252.4***	77.2
Resistance	26.9	22.8	34.0**	9.3
Fraction	15.5	16.0	13.7*	2.9
Lung				
Flow	65.6	68.3	55.0	21.4
Resistance	161.2	137.7	176.2	52.1
Fraction	3.4	3.3	3.8	0.9
Liver				
Flow	53.4	59.6	31.0***	15.8
Resistance	187.8	165.1	226.6**	53.4
Fraction	9.1	10.0	9.4	1.9
Intestine				
Flow	75.3	86.2	49.6***	18.5
Resistance	132.1	109.3	171.9**	48.1
Fraction	18.3	18.9	16.8	3.5
Splanchnic area (liver + intestine)				
Fraction	28.4	28.6	26.2	3.6
Skin				
Flow	10.2	12.3	6.1***	4.1
Resistance	1056.7	875.4	1149.7**	488.0
Fraction	7.5	7.9	6.2**	1.5
Carcass				
Flow	12.1	16.9··	13.3*	4.4
Resistance	633.3	564.1	687.3	251.7
Fraction	42.3	41.4	46.6*	6.4

Signs: . = significant, as compared with control.
* = significant, as compared with sham-operated animal.

As compared with the alert-unrestricted control, the sham operation caused no significant change.

In dehydration the cardiac, pulmonary and hepatic fractions increased significantly, the intestinal, splanchnic and renal fractions, too, increased, though not significantly. Values for skin and carcass decreased.

Table II

Effect of dehydration on circulation of unanaesthetized-restricted rats

Number of cases (n)	Control pentobarbital 14	Unanaesthetized groups		Dehydrated 14	Within-sample standard deviation
		Control 14	Sham-operated 13		
Total body		Mean values			
Cardiac output	25.2	39.9··	42.4	19.9 °°°	11.6
Blood pressure	117.0	129.0·	145.0***	115.0 °°°	11.0
Resistance	396.0	300.0·	315.0	489.0 °°°	111.7
Heart					
Flow	155.0	161.0	184.3	116.0°	72.5
Resistance	68.8	82.4	88.0	89.9	38.6
Fraction	2.0	1.9	1.9	2.6°°	0.6
Kidney					
Flow	379.1	215.9···	230.4	112.1°°	88.1
Resistance	26.9	51.5·	64.0	96.0°°	28.1
Fraction	15.5	5.8···	5.9	5.9	2.3
Lung					
Flow	65.6	107.5··	108.2	65.1°°	34.8
Resistance	161.2	110.9··	123.1	152.7	46.1
Fraction	3.4	3.2	3.0	3.8°	1.0
Liver					
Flow	53.4	53.7	66.8*	37.9°°°	16.8
Resistance	187.8	213.7	184.4	275.4°°	74.2
Fraction	9.1	7.1··	8.2	9.5	1.7
Intestine					
Flow	75.3	61.8	61.3	35.0°°	23.1
Resistance	132.1	179.0	231.4	284.6	69.5
Fraction	18.3	10.4···	9.1	10.6	3.3
Splanchnic area (liver+intestine)					
Fraction	28.4	17.4···	16.1	20.1°	4.2

Number of cases (n)	Control pentobarbital 14	Unanaesthetized groups		Dehydrated 14	Within-sample standard deviation
		Control 14	Sham-operated 13		
Skin					
Flow	10.2	16.2··	18.4	7.1°°°	5.8
Resistance	1056.7	721.0·	775.6	1472.0°°°	413.0
Fraction	7.5	7.9	8.8*	6.7°°	1.6
Carcass					
Flow	12.1	40.4···	40.9	18.6°°°	14.2
Resistance	633.3	297.5···	308.2	516.3°°°	110.6
Fraction	42.3	64.5···	63.0	61.4	5.8

Signs:
· = significant as compared with the anaesthetized controls.
* = significant as compared with the unanaesthetized controls.
° = significant as compared with the sham-operated group.

Table III

Effect of dehydration on circulation of unanaesthetized-unrestricted rats

Number of cases (n)	Control (pentobarbital) 14	Unanaesthetized groups		Dehydrated 15	Within-sample standard deviation
		Controls 16	Sham-operated 16		
Fractions	Mean values				
Heart	2.0	1.6·	1.7	2.7°°°	0.5
Kidney	15.5	12.9··	12.3	13.5	2.5
Lung	3.4	2.8	2.7	4.6°°°	1.0
Liver	9.1	7.3··	7.3	8.8°°	1.5
Intestine	18.3	13.6···	12.9	14.4	2.9
Splanchnic area (liver+intestine)	28.4	21.5···	22.3	23.1	3.5
Skin	7.5	8.8·	9.5	7.0°°°	1.5
Carcass	42.3	52.3···	53.5	48.7°	5.6

Signs:
. = significant as compared with the anaesthetized control.
* = significant as compared with the alert, unrestricted controls.
° = significant as compared with the sham-operated group.

Discussion

In dehydration circulation seriously deteriorates, cardiac output decreases in both anaesthetized and unanaesthetized rats. In anaesthetized animals vasodilatation occurs in coronaries and vasoconstriction in the kidney, skin and splanchnic area. Thus, the compensatory mechanism, the redistribution

of cardiac output, is similar in nature to that found in other types of stagnant hypoxia (GÖMÖRI and TAKÁCS 1960).

In alert rats both the cardiac output (SZABÓ et al.; in preparation) and its distribution (KÁLLAY and TAKÁCS 1961; SZABÓ et al.; in preparation) significantly differ from the similar parameters of anaesthetized animals. This has justified the reproduction of the experiments in alert rats. The excitation caused by restriction, the stressor effects substantially increase cardiac output and blood pressure. Vasoconstriction develops in the splanchnic area, and the blood supply to the carcass steeply rises. It is the kidney which reacts the most sensitively to restriction: the renal fraction of cardiac output and renal blood flow extremely decrease. Similar changes occur in alert-unrestricted animals (control related to anaesthetized controls), but these changes are less severe, for example the decrease of the renal fraction amounts to 2.6 per cent of the cardiac output, as compared with the 10 per cent in immobilized animals. As compared with the anaesthetized controls, in both groups vasodilatation occurs in the skin.

Dehydration brings about an impairment of systemic circulation. The decrease of cardiac output and the fall of blood pressure develop in alert animals, too. The results, however, differ from those of the anaesthetized group in the following features. The pulmonary fraction shows a significant increase, but the interpretation of this is limited, because the method employed does not offer the possibility of distinguishing bronchial from pulmonary flow (TAKÁCS et al. 1964). In the splanchnic area vasodilatation, in the carcass vasoconstriction, can be observed. The changes in the heart and the skin are unequivocal: like in the anaesthetized animals, vasodilatation and vasoconstriction, respectively, develop in these organs.

As compared with the anaesthetized-dehydrated animals, the most marked differences are found in renal circulation. In the alert-restricted group the renal fraction which had dropped as a result of the excitation caused by immobilization did not decrease further in response to dehydration; presumably, it could not have decreased further, because even in the anaesthetized animals dehydration caused a decrease amounting to about 3 per cents of the cardiac output. However, a tendency of the fraction to increase was found in the alert, unrestricted animals following dehydration. BÁLINT and STURCZ (1959), using a direct method studied the changes of renal circulation in pylorus-ligated, anaesthetized dogs and found no decrease of the renal fraction. It follows that in dehydration or in different conditions of shock more attention should be devoted to the effects of anaesthesia when appraising the deterioration of renal circulation: SAPIRSTEIN likewise found no decrease of the renal fraction in haemorrhagic shock induced in alert rats (SAPIRSTEIN et al. 1965).

Using SAPIRSTEIN's method, ROGER (1965) studied the circulation of anaesthetized dehydrated rats. Our results differ from his in several points.

In ROGER's investigations cardiac output in the anaesthetized controls was significantly higher (average: 43 ml/min/100 g) than the values given in the literature (SAPIRSTEIN et al. 1960; KÁLLAY and TAKÁCS 1961). ROGER found no significant change in the renal and skin fractions even in 48-hour dehydration, either. These differences in the experimental results appear to be due to superficial anaesthesia in ROGER's experiments.

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CRISES IN MYASTHENIA GRAVIS, I.

INCIDENCE, PATHOMECHANISM, SPECIAL FEATURES

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The character, pathomechanism, incidence, and clinical features of the crises associated with myasthenia gravis have been summed up on the basis of personal observations.

Since its earliest description (ERB 1878, GOLDFLAM 1893) myasthenia gravis has been a subject of permanent interest, and many of its theoretical and clinical aspects are being increasingly studied in our days. Knowledge of its clinical features and widening of therapeutic possibilities have made us aware of the occurrence of crises typical of this entity. It has been learned about these crises that they may decide the outcome of the original disease, and their diagnostic and therapeutic significance has been recognized to the degree of giving food for theoretical research of neurophysiology, but little is known about their true nature. As a matter of fact, in the literature the question has not received the attention it deserves. We have found no more than four papers on the subject in recent literature (TETHER 1955, ROWLAND et al. 1956, HERRMANN 1961, BLAUGRUND et al. 1964). It seems therefore appropriate to give an outline of our observations confronting them with published evidence on the basis of 80 patients in a period of 14 years whose management and follow-up have provided us with the opportunity of studying the crises in myasthenia at a closer range.

I. Definition and character

As to the definition of crisis, clinicians and neurophysiologists do not entirely agree, owing to their different angles of approach and the different interpretation of the phenomena involved. Greater importance has the clinical crisis. From the clinical aspect we must regard as a crisis every ventilatory disturbance or failure connected with myasthenia gravis. The crisis may be due to primary or secondary causes. The primary cause is a weakness of the respiratory muscles as the symptom of myasthenia, whereas every other cause or event indirectly related to it must be considered secondary. The most common secondary factors are hypoventilation pneumonia, atelectasis, aspiration,

mechanical obstruction in consequence of dysphagia, certain adverse effects of cholinergic drugs such as hypersecretion, hypersalivation, retention of secretion or of plugs in the bronchial tree owing to inefficient coughing. Moreover, certain additional factors may be triggered off and form the closing ring in the vitious circle which in the crisis frequently becomes manifest. Such factors may be cerebral hypoxia, confusion, weakness of the psychic energy, decreasing dynamism of the personality; strength of the patient eventually gives way and he suddenly finds himself at the end of his power to struggle for air; respiratory acidosis and cerebral hypoxia. The prevalence of the individual factors is variable, they often combine and intensify each other, so that it may be impossible to identify them though in some cases this may be easy to do.

After the knowledge of the beneficial effects of cholinergic drugs in myasthenia gravis (WALKER 1934) we got acquainted with the cholinergic crises or states (GROB et al. 1949). Most cholinergic drugs exert a muscarine- and nicotine-like activity which may give rise to cholinergic toxic states. Such crises involve considerable diagnostic difficulties as they bear close resemblance to actual myasthenic crises in respect to their main features. The *cholinergic symptoms* of the *muscarine type* include excessive salivation, lacrimation, sweating, nausea, belching, vomiting, dimness of vision, myosis, diarrhoea, intestinal colics, vesical tenesmus followed by urinary incontinence. Those of the *nicotine type* involve general progressive exhaustion, weakness, fasciculation with a tendency to generalization, persistent cramps, "thick tongue", dysarthria, dysmasesia, dysphagia, dyspnoe. The cholinergic effects on the CNS result in irritability, emotional lability, anxiety, vertigo, cephalea, confusion, drowsiness, coma and convulsions. According to WILSON et al. (1952) the highest incidence of such crises — termed by these authors as "cholinergic" — has been noted in case of alkyl phosphate treatment. OSSERMAN, KAPLAN and BESSON (1953) emphasize that the crises may result from coexisting myasthenic and cholinergic factors, later TETHER (1955) pointed out that in many instances the crises regarded as being myasthenic at a time when the existence of cholinergic crises was still unknown, were actually cholinergic in type. The best means of differentiating the two types is the intravenous administration of edrophonium chloride as suggested by OSSERMAN and KAPLAN (1953).

II. Pathomechanism of the crises

The clinician familiar with myasthenia is faced, outside the typical, on the whole, responsive type, and the stationary types also with cases which are practically unmanageable from the very outset, showing very little, if any, response to drugs and being liable to take at any time an unexpected turn to the worse with very little hope for remission. This resistant type is prevalent

in the age groups over 40 when it is generally associated with thymoma, but this malignant type fairly often affects younger age groups as well in the absence of any tumor of the thymus. None of the existing therapeutic measures either the thymectomy will brake the progress of the disease in such cases; latent or manifest ventilatory failure, ready to lead to more or less persistent crises, is an early sign. Moreover, even in cases which have shown satisfactory response or a seemingly permanent remission, there may be a sudden impairment, resistance to drugs may develop calling for higher doses which carry the risk of cholinergic crises. Extensive research work in this field documented by ample literature has failed to give a satisfactory explanation of these phenomena or to throw light on the causes of sudden death in myasthenia gravis if it is not caused by respiratory failure.

Let us throw a cursory glance on the theories put forward for the explanation of drug resistance, and of liability to myasthenic and cholinergic crises in myasthenia gravis.

The site of the basic myasthenic disorder is in the myoneural junction. We know, however, of no *structural abnormality* either of the terminal arborization or the synaptolemma or the subneural apparatus that was invariably demonstrable in every case of myasthenia gravis but in no other disease. Dysplasia, elongation of the motoric end-plate, want of terminal arborization have been described in other conditions as well (Coërs and Desmedt 1959, Bickerstaff, Evans and Woolf 1960, McDermot 1960) and are, therefore not pathognostic. Moreover, the microscopic findings in question have been derived from a mixed material in which not all the patients had died in crisis and in which resistant and responsive cases were represented. Therefore this casuistic could not be considered characteristic for the problem of crisis. In the same way, the autopsy and pathologic studies carried out by Genkins et al. (1961) in 31 cases of myasthenia failed to disclose any change accounting for either the liability to crises or the drug-resistance. The occurrence of lymphorrhages has not pathognostic significance, being an ubiquitary change which represents in our consideration a reactive phenomenon due to the unknown noxa of myasthenia.

The possible role of a *toxic damage to the terminal apparatus* has found no support either. Cholinesterase-inhibitors given in excessive doses are known to cause muscular weakness or paralysis even in healthy subjects, as a consequence of depolarization-block due to accumulation of acetylcholine. As pointed out by Herrmann (1961) the drugs applied in the treatment of myasthenia do not occur in the organism under normal conditions, the mechanism of their action is, therefore, of no substitutive character. Accumulation of intermediary products or of by-products originating from the breakdown of these "exogenous" substances may, however, result in toxic damage to the end-apparatus which in this manner becomes unresponsive to drugs.

The observations of CHURCHILL-DAVIDSON and RICHARDSON (1957) suggest the possibility of the reversible toxic lesion of the motoric end plate. Drug-resistant patients subjected by these authors to positive pressure respirator still under curarization for several days, were found more responsive at the end of this "resting process", assumedly in "consequence of an increased sensitivity to endogenous acetylcholine".

There are, however, plenty of arguments against the toxic theory. For instance, it has remained obscure to the present day why some cases become resistant, while some others remain responsive. Quite a number of patients under our observation have remained well-stabilized for years or even for decades without the slightest sign of toxicity or any suggestion of a cholinergic crisis. I followed up a female patient for years who had been myasthenic for 27 years and had responded fairly well to drugs; change-over from prostigmine to pyridostigmine was followed by a striking response. It is a further point of interest that increasing drug-resistance or a refractory condition may be limited to certain individual muscles or synergic muscle groups without involving the whole myasthenic musculature (HERRMANN 1961, SZOBOR 1964). Finally, there are patients who have been unmanageable or practically so from the very beginning, which contradicts the role of an accumulation of drugs or of their toxic breakdown products. Another argument pleading against a toxic origin is the observation that when drug-resistance has developed, drugs of the most diverse chemical structure fail to elicit any response, which would not be the case were a resistance to some special drug involved.

One of the possible theories of drug-resistance and of the crises links up these eventualities with the *synaptic structure*. The so-called vegetative synapses are easily re-modelled, owing to their "diffuse and collective nature" (SZENTÁGOTHAI 1957), whereas the neuromuscular synapses tend to preserve their structures in consequence of their "individual" character. The hermetic structure of the perisynaptic glia isolates it from external, particularly pharmacochemical, influences. It is well within the possibilities that this perisynaptic glial structure becomes the site of some abnormal process cutting off the pathways of response (which would account for the development of drug-resistance) or else that its close texture is *ab ovo* an unfavourable medium for pharmacological influences (which would provide the structural basis for originally drug-resistant cases). It is furthermore known that the functional structure of the neuromuscular junctions is not the same in the various muscles (CSILLIK 1965), and that the subneural apparatus of the synaptolemma has two functional patterns, i.e. the alpha- and the gamma-type. The neuromuscular junctions of the gamma-type are characterized by shallow junctional folds in contrast to the deep folds of large surface distinctive of the alpha-junctions. This restricts the space for ion-exchange in the gamma-junctions, thus reducing the post-junctional potentials and interfering with their trans-

mission. Depolarization is thus concentrated to a narrow area and transmitter-decay is accelerated. Our knowledge of the ratio between alpha- and gamma-junctions in the individual muscles is little more than guesswork. Nevertheless, we consider it possible that the dense structure of the motor synapses as well as their functional properties including a prevalence of subneural junctions of the gamma-type in certain individual muscles may well provide a sound functional-structural basis for the explanation of crises and of drug-resistance in myasthenia. To realize the difficulties of the problems involved by the mechanism of myasthenic or cholinergic crises we have only to remember that even normal synaptic transmission has many an uncleared or controversial point (PATON 1958).

Histo- and biochemical studies have also been inconclusive, yet they have shed some light on the problem. This particularly applies to the acetylcholine-insensitiv block described by KATZ and THESLEFF (1957). A block of this type involves two factors. The depolarizing drug, e.g. acetylcholine, effects depolarization as the first step of its action, and as the second, it makes the end-plates insensitive. Acetylcholine- or cholinesterase-inhibitors fail to elicit any response in this second stage. GROB and JOHNS (1961) ascribe a theory about the dual nature of the block-substance in myasthenia. In their view, the block, reversible to acetylcholine at first, becomes irreversible to this substance at a later stage, or insensitive. Accumulation of acetylcholine (or, possibly, choline) in the receptor area contributes to the irreversibility of the condition by its further desensibilizing effect. Confronting the problems involved by drug-resistance and by the occurrence of crises in apparently stabilized cases with the available bio- and histochemical evidence, we find the supposition of a dual nature of the neuromuscular block and possibly also of the myasthenic substance to be still the most acceptable hypothesis. In our view, this mechanism may not only account for the sudden shift of a crisis from the myasthenic to the cholinergic type but also for the combined occurrence of the two types, as we see it often enough in our clinical cases. We also believe, though there is little clinical evidence to this effect, that the two types may alternate and become inextricable, though from the therapeutic point of view it would be vital to identify the type. Such crises characterized by continual swings between myasthenic and cholinergic symptoms are best termed "fluctuating" or "oscillating" crises.

Shifts in the electrolyte environment also may determine the alternating character of the block-mechanism. It is known from the investigations of FATT and KATZ (1952) that even in the absence of any nervous impulse the membrane of the motoric end-plate is not completely inactive but exhibits "spontaneous miniature end-plate potential fluctuations", due perhaps to that the release of the acetylcholine molecules would proceed by quanta instead of following a rhythmic course. With normal nervous impulses such micropotentials do not

become perceptible, if, however, transmission of the nervous impulse is inhibited by changes in the electrolyte environment, these micropotentials did not disappear, and so there is never a quietness in the area of the motoric endplate. Release of acetylcholine molecules must, in all probability, run into thousands to cause such a fluctuation of micropotentials. It is unclear what part exactly these micropotentials play in nervous transmission under normal conditions though it is within the possibilities that they might be involved in the elementary mechanisms of the maintenance of muscle tone (SZOBOR 1964). In respect to the block-mechanism in question we may well ask whether by reducing and eventually using up the amounts of acetylcholine and by providing in this manner a continual exhaustive impulse, this fluctuation of micropotentials might not be one of the very factors of vulnerability responsible for the transmission disturbances in myasthenia gravis.

Among the other possible factors which have been made responsible for the mechanism of the crises, "primary asthenia of the vegetative centres" alleged by PASSOUANT (1956) or "impairment of drug utilization" suggested by COHEN and ZACKS (1958) appear to be of a certain, though minor, significance.

III. Incidence of the crises

As mentioned before, the literature on the subject is not in proportion with the theoretical and practical significance of the problems involved. An inquiry addressed by HERRMANN (1961) to the prominent experts on myasthenia (OSSERMAN, GAMMON, JOHNS, TETHER, SCHWAB, ROWLAND) shows that the problem has not received the attention it deserves. It emerged from the answers that no reliable data are available as regards incidence, frequency, cause and features of the crises. OSSERMAN (1958) had knowledge of 57 crises in 325 patients but found this number excessive. The crises were lethal in 47 per cent. He found a slight preponderance of myasthenic crises over the cholinergic ones, but it was not always possible to identify the type. TETHER (1955) recorded 20 crises in 186 patients with a 80 per cent mortality. GROB (1958) had 202 patients under his observation including 44 thymectomized subjects. There were 10 non-surgical, i.e. typically myasthenic, deaths. The most extensive patient material has been observed in Mount Sinai Hospital over 14 years and reviewed in the light of modern theraphy by BLAUGRUND et al. (1964). Crises occurred in 99, i.e. 15.8 per cent of 625 patients during the 14 years of study. This high incidence is in agreement with the records of HEAD (1964), ERBSLÖH and L'ALLEMAND (1965), HENSON, STERN and THOMPSON (1965) as well as with our own observations, crises having occurred, also repeatedly, in 24 out of our 80 cases.

When evaluating the antecedents from the point of view of crises, it must be kept in mind that some of the patients are admitted with an established

diagnosis of myasthenia and with the history of previous drug treatment, while in some others the disease is still unrecognized or, possibly, misinterpreted at the time of admission. Close questioning may offer valuable indications as to earlier occurrence of crises in which case further crises may be expected. Another point not to be neglected is the personal attitude of the observer. Much depends on what he considers to be a crisis as there are no established standards for the correct evaluation of respiratory performance in such cases.

Myasthenia gravis in infancy, childhood and also in senescence occupies a relatively small place in pertaining literature. Though crises doubtlessly do occur in these age groups, we have little information about them. One of our papers deals with myasthenia in childhood and infancy (SZOBOR [in press]). Crises occurring at any early age have been reported by ZWEYMÜLLER (1952), BIÉMOND and TROTSENBURG (1955), BÉLANGER and PLAMONDON (1957), MILLICHAP and DODGE (1960). TENG and OSSERMAN (1956) described a case of transitory myasthenia in the newborn infant of a myasthenic mother, and called attention to the weakness of respiration, sucking, crying, swallowing, as premonitory signs of a crisis. In other cases, weakness of sucking may be the only danger signal. We have observed a crisis of utmost severity in one of our myasthenic children.

The term "senile myasthenia" is reserved for the cases in which the first signs of the disease appear beyond the age of 55. One of our earlier papers (SZOBOR and KÖRNYEY 1965) deals with observations in elderly myasthenic patients, 3 out of 11 cases having been found liable to crises.

Crises thus affect early or advanced age groups in the same manner. In young age the crisis is practically always myasthenic in character as the disease is generally in the incipient stage or an unrecognized or untreated myasthenia leads to crisis. In the elderly, however, the possibility of a myasthenic or a cholinergic origin must always be considered.

IV. Questions of semiology

The crises in myasthenia gravis are viewed as an outcome of a process leading to respiratory failure. It is not possible to give here a full account of the whole syndrome, and only those of its features will be outlined which may be suggestive or diagnostic of a myasthenic respiratory crisis.

One of these special characteristics is the *sudden onset*, which may occur even in stabilized patients. Crises without any premonitory sign have been witnessed on few occasions in our own material. If the patient shows, however, the slightest hypoventilation, any attempt at a treatment at his home is unwarranted. He belongs to a specially equipped hospital unit. The problem why apparently stabilized patients are liable to unheralded respiratory crises has remained largely unanswered. Uneven involvement of the affected muscles

may, however, be an essential factor. As pointed out by HERRMANN (1961) weakness confined to a single muscle of major respiratory importance is sufficient to cause crisis. In one of his apparently well-stabilized patients, lethal outcome of a crisis of sudden onset could not be averted by any of the existing therapeutic measures. The factor responsible for the crisis had been a weakness of the laryngeal muscles.

ERBSLÖH and L'ALLEMAND (1965) have called attention to the latent hypoventilation preceding respiratory failure. This premonitory hypoventilation, recognizable by tachypnoea and tachycardia, may induce at any time, through respiratory acidosis, an actual respiratory crisis.

Myasthenic patients are particularly susceptible to affections of the respiratory tract. A slight bronchial infection, influenza or rhinitis may cause a sudden turn to the worse. Conversely, the majority of respiratory crises follow close upon some respiratory tract infection. Presumably, in myasthenia the respiratory equilibrium is delicate, ready to be upset by the slightest overcharge and give way to a crisis. The commonest causes of overcharge are aspiration, partial atelectasis, bronchial plugs, hypersalivation, superinfection, respiratory acidosis, or disturbances of central origin.

Another feature of myasthenic or cholinergic crises stressed by us in an earlier paper (1958) and discussed also by HERRMANN (1961) is the extreme prostration of the patient. The face is pathetic, it wears the expression of extreme distress, suspense and of appeal for help. This is characteristic with the usual appearance of the myasthenic patient which is that of dejection, weariness and anxiety. The very appearance of the patient may betray the presence of a crisis to the experienced eye even in unrecognized cases. This is the more important as cyanosis generally remain absent until the terminal stage. The pale and clammy face need not be connected with a cholinergic origin. If, however, the crisis is cholinergic in type, salivation, profuse sweating, lachrymation, bronchial discharge, fasciculation as well as other features of the muscarine or nicotine syndrome join the picture of general exhaustion. We must, however, admit that despite of innumerable observations and various valuable tests, we are often unable to identify the type of the crisis. This has its obvious grounds. First of all, the two types have several features in common, while several others are similar. Since the main characteristics of the cholinergic crisis as described by GROB and al. (1949) have been outlined above, only the outstanding features common to both types will now be stressed, above all the dyspnoea which accompanies the typical myasthenic crisis from beginning to end, while in cholinergic crises it may appear as an early sign of the muscarine-syndrome and is part of the nicotine-syndrome. Extreme exhaustion is also common to both types and so do dysarthria, dysphagia, and dysmasesia; latter signs in cholinergic crisis belong to the nicotine-syndrome. Signs, such as restlessness, anxiety, emotional lability, confusion are generally assigned

to the 3rd phase of the cholinergic syndrome. Personally, we do not believe in the possibility of such a sharp distinction. The foregoing psychical and psychomotor signs certainly do occur in cholinergic crises but they are no less liable to appear in typical myasthenic crises as well. Anxiety leads to motor restlessness which on its part requires surplus energy. When, however, extreme physical exhaustion makes practically every motor activity impossible, anxiety becomes existential. No contact worth speaking of can be established with the patient in this stage. In the desperate struggle for air the patient is continually thrown about between going down and coming up to the surface again. In the physical and mental distress, even if the patient has all that the modern techniques of surgery or laryngology can afford the presence of the attending neurologist is indispensable. Comfort and encouragement by the physician may help to tide the patient over by calling forth all that has remained of his psychic strength. Psychic care and attention of the myasthenic patient is still a neglected field. The papers of TETHER (1961) and HERRMANN (1961) are voices in the desert. As a psychiatrist, the present author has been specially aware of the importance of this aspect of myasthenia gravis despite of its organic character.

The difficulties in discerning myasthenic from cholinergic crises have been stressed by numerous authors. OSSERMAN and KAPLAN (1953), furthermore HERRMANN (1961) have referred to this point, the former authors in their study describing the edrophonium- (Tensilon-) test (1953). GROB (1958) emphasized that the two types overlap in many cases. Both may be associated with weakness of the respiratory muscles and both types may be present simultaneously.

Among the various tests and provocative procedures designed for the identification of the myasthenia, none but the edrophonium test is warranted during a crisis. By its triple effect (myasthenic type, adequate type, cholinergic type), this test not only has been of substantial help in differentiating the types and thus in prescribing the correct treatment, but also has contributed to the understanding of the pathomechanism of the crises.

Application of the test may, however, be frustrated by the sudden onset of the crisis, and in some cases its result may be inconclusive.

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CRISES IN MYASTHENIA GRAVIS, II

CAUSES, CLINICAL FEATURES, MANAGEMENT

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Crises associated with myasthenia gravis, have been studied under the following aspects:

Definition, theoretical and practical significance of the crises in myasthenia. Description of their types, possible mechanisms accounting for their production. Incidence. Semiologic questions. Distinctive features of the types. Personal observations of 24 cases among 86 myasthenic patients in a period of 14 years. Closer study of the most common causative factors. Crises after thymectomy. Types and features. Possible causes. Current theoretical and practical aspects of the management of crises. Preventive measures.

In a previous paper we have discussed the theoretical aspects of the crises associated with myasthenia gravis, particularly as regards their definition, character, incidence, possible pathomechanism and certain questions related to their semiology. The present paper gives a closer study of the crises observed by us in myasthenic patients.

I. Case material

Our observations have been made in 86 cases of myasthenia gravis during 14 years. Those 24 cases, i.e. nearly one third of the whole material, will be discussed in the present paper in which crises as defined in the previous paper have been encountered. The material to be reviewed has been collected regardless of thymic tumour or whether surgery had been performed. All of the data presented here are based on personal observations. Among the patients there were 12 males and 12 females. The general prevalence of females was equalized by the fact that 8 out of the 24 patients had malignant thymoma and 5 of them were males. Malignancy of the thymus gland is, however, known to be prevalent in males of higher age groups. Age distribution was as follows.

10 to 20 years	2 cases
20 to 30 years	10 cases
30 to 40 years	3 cases
40 to 50 years	4 cases
50 to 60 years	3 cases
60 to 70 years	2 cases

The earliest age at which the first crisis appeared was 14, the latest, 67 years. The duration of the disease until the occurrence of the first crisis ranged from 1 to 6 years, there were, however, cases in which the disease had been present for twenty years when the first crisis occurred. On the other hand, in three of our cases the crisis had been the very first manifestation of the disease. The original disease was generally mixed in type, in other words, the same case combined ocular, bulbar and skeletal localizations, while oculo-bulbar or skeleto-bulbar types were rarer. The crises were of the following types,

Myasthenic	16 cases
Cholinergic	2 cases
Combined	6 cases

The crisis is combined in type when both myasthenic and cholinergic features are present at the same time or when they alternate during the crisis, in which latter case we may speak of an "oscillating" crisis as we have termed it in the previous paper.

Several patients had repeated crises, 11 had two or more, a young female patient was seven times in a state of crisis. The crises were variable in duration. In a case for instance, it was lethal in the very first minutes, precluding any possibility of tracheotomy, on the other hand, a young female patient maintained on artificial respiration had been in a state of crisis for six months when first seen, without any noteworthy change in her condition. In the majority of cases the crisis lasted 3 to 12 days.

The cases displaying crises presented the following prognostic aspects.

Cured	2 patients
Remission "A"	8 patients
Remission "B"	5 patients
Remission "C"	3 patients
Died	6 patients

(By our standards, a patient may be regarded as cured if, with the exception of electromyographic changes, he has remained free of any myasthenic sign and symptom for five years without taking drugs and is active in his former sphere of work. Remission "A" means that the patient is able to continue his former activity free of any objective sign, without drugs or taking minimal maintenance doses. In remission "B" the patient depends on drugs to remain active and even if he is inactive he cannot do without minor maintenance doses. Remission "C" is equivalent with practically minor improvement. To simplify matters, we have included into this group 2 patients being in a permanent or stationary condition.)

Survival after severe crises is reflected by the following figures.

Survival over 10 years ..... 1 case
Survival from 5 to 10 years ... 11 cases
Survival from 1 to 5 years .... 3 cases
Survival less than one year ... 3 cases

Confrontation of the data related to survival or prognosis and type of remission failed to reveal any close correlation between the severity or duration of the crises and the tendency to remission. Even in the case of repeated crises, the condition may be satisfactory either in the intervals or when the crises have ceased altogether. There is evidence (HERRMANN 1961) that even the severest crisis, survived, may be followed by an excellent remission.

The crises associated with thymectomy deserve special comment. In our earlier studies (SZOBOR 1958, 1961) we found these crises to be "early" or "late" in onset. Though classification of these crises on the ground of timing has not been recorded thus far, we find it nevertheless necessary. This point will be discussed later.

29 out of a total of 86 patients had been subjected to thymectomy. Ten of them experienced crises. Their data are given below.

Table I

	Name	Age (years)	Myasthenic crisis		Cholinergic crisis	Outcome
			early	late	(combined)	
1	K. M.	♀ 18	+			Died
2	H. M.	♀ 20		+++		Cured
3	F. T.	♀ 17	++		+++	Remission "B"
4	S. M.	♀ 29		++	+	Remission "A"
5	K. G.	♀ 22	+++			Remission "B"
6	H. M.	♀ 44	+++			Remission "C"
7	C. I.	♀ 49		++		Remission "A"
8	M. A.	♂ 53		+++		Cured
9	A. A.	♂ 54	+++		+	Died
10	H. P.	♂ 67	+++			Died

"Early" = onset between the 1st and 3d days after thymectomy
"Late" = onset between the 8th and 11th day after thymectomy
+, ++, +++ = grades of severity of the crises.

II. Causes, responsible factors

In the light of our observations and of evidence recorded in literature it will be now attempted to outline the possible factors which, to our present knowledge, may be responsible for the production of crises. As emphasized in the previous paper, the pathomechanism of crises in myasthenia, apart from some essential aspects, is largely obscure, consequently our knowledge of the factors responsible for their production is also incomplete. We discuss the most common and, therefore, practically the most significant events that precede a crisis.

Our observations are in line with published evidence in placing *respiratory tract infections* in importance above all other conditions that may be responsible for the production of a crisis. Infections may either bring on a crisis or further impair the condition in an established crisis. GENKINS et al. (1961) reviewed the autopsy findings of 31 cases where death had been the direct consequence of a crisis. Some abnormality of the respiratory tract was invariably present in all of the cases, generally some pulmonary infection either focal or with a tendency to confluence or to generalization, often atelectasis, mucous plugs or distal infection resulting from inefficient coughing. Alveo-tracheal haemorrhage if present was associated with interventions of some kind (tracheotomy, bronchoscopy). Similarly to other observers, we also find respiratory failure to be the most common cause of death irrespective of the pattern of the crisis, i.e. whether myasthenic or cholinergic or postoperative in character.

Nasal, pharyngeal, tracheal or bronchial secretion associated with respiratory infections leads to rapid exhaustion of the patient, cough being often unproductive in consequence of muscular asthenia. The vain efforts to bring up discharge call for anticholinesterase treatment, often by the intravenous route, but the transitory benefit derived from these drugs is outweighed by their ill-effects on the respiratory process owing to salivation and bronchial hypersecretion to which they usually give rise. Renewed futile attempts at bringing up discharge by coughing leads to ventilatory failure and eventually respiratory acidosis. Aspiration of saliva, partial atelectasis, progressive insensitiveness of the respiratory centre in consequence of respiratory acidosis, confused mental state due to anoxia, failing morale of the patient are further factors resulting in a vitious circle, which is very often fatal because irreversible. Artificial respiration would offer the best solution were it not frustrated in many cases by massive atelectasis or confluent pneumonia causing a severe restriction of the respiratory surface.

In a full-blown crisis of such mechanism there is often no way of telling whether it is *myasthenic or cholinergic in character*, symptoms of the muscarine type being absent and the result of the Tensilon-test equivocal. In the presence of respiratory acidosis and hypoxia, it is almost certain that the patient's

morale spent in a vain struggle for air, will eventually give way. This surrender of the patient paves the way for further respiratory impairment. Persistent anoxia may be associated with epileptic convulsions of generalized or Jacksonian type, often with fatal outcome. Other incidental factors, particularly hypopotassaemia, add to the risks of the crisis by increasing muscular weakness.

Our own observations fully bear out the significance of respiratory infections, atelectasis, pneumonia or aspiration in the production or maintenance of crises. In 10 of our patients the crises followed upon a respiratory infection, this occurred repeatedly in 4, and almost currently in 2 of them. One of our female patients had had her first crisis at the age of 14, subsequent to a trivial accident sustained at school. Owing to bulbar signs and respiratory failure, tracheotomy, positive-pressure-respiration and, finally, thymectomy had been performed. At the age of 17, another mediastinotomy had been undertaken as the last resort owing to incessant crises and deterioration of myasthenia. On this occasion, aberrant thymus islets had been removed from firmly adherent fibro-adipose adjacent tissue. Repeated courses of radiotherapy had been given. In the course of the years she lapsed into a state of crisis on seven occasions. The pattern of the crises was generally myasthenic, but on two occasions cholinergic features were also present though drug treatment had not been modified. Before the second operation it had come to a severe respiratory failure in consequence of tracheo-bronchitis associated with aspiration pneumonia. Ever since her second operation followed by radiotherapy she has been in remission "B", in other words, she has been able to continue her studies but former experience has tought us to confine her to strict bed-rest on the slightest sign of respiratory infection which otherwise would inevitably bring on a state of crisis. — Another female patient, 35 years of age, with hysteroid features, given to anxiety, had experienced repeated crises in consequence of catarrhal respiratory tract infections. Owing to an increase of the doses on her own account without our knowledge it occurred on two occasions that the crises, originally myasthenic, assumed a cholinergic type and required hospitalization in order to be brought under control. In both cases we were able to witness the pathomechanism of the crises at close range and the vitious circle dealt with in the foregoing became manifest under our very eyes. In the second case we must ascribe a large part of it to the hysteroid personality of the patient, her lack of discipline and psychic exhaustion.

Other factors responsible for the crises may be related to menstruation, pregnancy, delivery, puerperium or lactation. This aspect of the question will form the subject of a separate study. Premenstrual impairment of myasthenia is fairly common, whereas bulbar signs response to bed-rest and drug treatment was favourable. A 22-year old patient experienced a severe crisis as the first manifestation of myasthenia, during lactation, after an uneventful pregnancy and smooth delivery. Intravenous neostigmine not only confirmed the presence

of myasthenia but controlled the crisis. Processes related to pregnancy are pointed out by HERRMANN (1961), too, among the possible factors of crises.

Crises may be produced by exhaustion. In respect to physical overexertion, this needs no further comment. It is, however, less recognized though a highly important issue of the question under study that psychic exhaustion, emotional strain or stress are common precipitating factors of a myasthenic crisis, conversion of which to cholinergic intoxication may be promoted by the patient's confusion and anguish. The psychic aspects of myasthenia have received little attention in literature; TETHER (1961) devoted a study comprising 40 patients to this problem. It is, however, obvious that successive realization of a disease with all of its life-threatening aspects, is bound to affect the patient's personality in some way or another, and his fate largely depends on the question whether he will be able to make the best of the adaptability of his mind and whether he has the gift of mobilizing his resources of energy. We are dealing with the psychic condition and the personality changes of the myasthenic patient in a separate study (SZOBOR 1966), here we allude to those points only which are of interest from the aspect of the crises, stressing the significance of psychosomatic factors in their production. In 3 of our patients the crisis had been elicited by physical exhaustion, in 4 by emotional stress or anxiety. GRINKER and BUCY (1951) have pointed to the crisis-inducing influence of laughing and crying through acute respiratory paralysis and draw attention to the danger of regarding the syndrome as hysterical and in applying "persuasive" treatment which may finally result in complete respiratory arrest through "emotional dyspnoea". This eventuality is rare but nevertheless a possibility to be kept in mind. In our experience one of the most common diagnostic errors in respect to myasthenia — actually the second or third in the order of frequency, — is its being misinterpreted as psychic in origin.

Postoperative crises may supervene both during the disease or occur as its first manifestation. Apart from those associated with thymectomy, postsurgical crises occurred in 2 of our cases. In the first, a 18-year old woman, the crisis was the first manifestation of myasthenia. The onset, closely following upon tonsillectomy, was marked by acute bulbar signs, aphonia, anarthria, dysmasesia and severe dyspnoea. In the other female patient with established myasthenia a crisis, short in duration but difficult to bring under control, was associated with appendectomy.

Injuries may lead to the manifestation of latent myasthenia in the same manner as surgical interventions. Our observations lend support to this experience. In one of our cases, a young female patient, the first symptoms associated with a slight cerebral concussion consecutive to a road accident, had been ascribed to a post-concussion syndrome of hysterical origin until on closer observation at our department they were identified as being due to a latent myasthenic crisis. The other patient whose first signs could be traced back to

an accident suffered in her school-days, is the same who has been mentioned earlier for another reason. In the production of crises by surgical or other injuries the part played by psychic factors should never be left out of consideration.

Among other factors or circumstances favouring the occurrence of crises, misinterpretation of the myasthenic symptoms occupies the first place, since it delays efficient management. Crises recognized for the first time in undiagnosed cases are, obviously, always myasthenic in type, cholinergic crises being invariably due to drug treatment. HERRMANN (1961) also ascribes a part to diagnostic errors in the production of crises. Our two cases where the crises had been identified for the first time have been quoted earlier in this paper. Crises may be furthermore brought on by various diagnostic tests, such as curare, decamethonium-iodide, guanidine, quinine, quinidine, over-exertion. It is interesting to note the changing attitude to curare. The curare-sensitivity-test was described and used for the first time by BENNETT and CASH (1942). Since responsiveness of myasthenia gravis to physostigmine and neostigmine was an established fact at that time (WALKER 1934) and the new treatment was rapidly gaining ground, the opposition to the curare-test in literature was fully justified. PELIKAN, TETHER and UNNA (1953) found the treshold-value in myasthenia to be one fifth of the normal figure. In recent years CHURCHILL-DAVIDSON and RICHARDSON (1957) have studied the diagnostic possibilities of d-tubocurarine. Later ROWLAND, ARANOW and HOEFER (1961) made a plea for the revival of this unnecessary and unsafe procedure, using it in 92 patients on the ground that the diagnosis in 60 of them was dubious. This ratio is excessively high, though the diagnostic difficulties may be considerable and suspect cases high in number. The dangers of the curare-test were pointed out by EATON in 1947, five years after its description. In some cases the diagnosis of myasthenia requires the additional evidence of specific tests, in others the diagnosis is based on this evidence alone and, occasionally, it is the result of the specific test which draws attention to the possibility of myasthenia. Such cases are, however, relatively rare and even if they do occur, we may rely today on highly specific and at the same time entirely safe tests. As pointed out earlier (1958, 1964), careful history-taking, close observation of the signs and symptoms in their fluctuation, evidence of exhaustion-tolerance test, neostigmine and edrophonium tests, Jolly's electric test, oculoelectromyography, electrolyte studies have led us to the correct diagnosis in all of our cases, therefore we see no point in reconsidering our attitude to curarization or to any other unsafe diagnostic procedure.

Crises appearing in the course of drug treatment eventually may have three causes. They will be dealt with in the following.

1. Inadequate dosage may produce the same situation as failure of diagnosis. Ventilatory deficit resulting in a crisis may occur in both eventual-

ities. The constellation is essentially the same if the type of myasthenia suddenly changes, for instance, if bulbar symptoms join the ocular pattern. In such cases the doses on which the patient did well before may become inadequate.

2. The factors and mechanisms inherent to the drug itself are far more complicated as concerns susceptibility to crises. The usual drugs make liable to crises to a variable extent. The most important of them, neostigmine, occupies the middle in this respect. Of recent drugs, pyridostigmine is alleged to be the safest, its margin of safety being the most favourable.

OSSERMAN stresses the advantages of pyridostigmine in the bulbar type of myasthenia where the danger of crisis is the highest, though, as he points out, if neostigmine fails to bring muscular weakness under control, then it is most unlikely that pyridostigmine will do so. We have been using pyridostigmine extensively in our material. Occasional cholinergic effects associated with its use were slight, and crises did not occur at all. Observations with ambenonium which has been found to be efficient in the skeletal type, are far less favourable. On the basis of observations including 60 patients, SCHWAB (1955) found it liable to induce cholinergic block. DESMEDT (1957) cautions against the use of the drug in view of its narrow margin of safety. WESTERBERG and MAGEE (1955) do not use it at all without atropine. GROB (1958) also points to the danger of crises associated with the use of this drug, as it frequently gives rise to cholinergic muscular weakness through a depolarization block-mechanism of the nicotin-type. In our cases unpleasant, persistent muscarine-type effects, such as nausea, diarrhoea, abdominal spasms, anorexia, also occurred. In a 60-year old male patient, on the third day of ambenoamine treatment, we witnessed the occurrence of a short but none the less severe respiratory crisis associated with stormy abdominal symptoms, psychomotor restlessness and anxiety.

The drugs which have been found up to now to carry the greatest danger of crises are those of the alkylphosphate group. OSSERMAN and KAPLAN (1953) stress the danger of crises involved by the use of organic phosphates owing to their considerable anticholinesterase activity. Combined use of these drugs with neostigmine particulary increases this risk (RIDER 1951). — We have long abandoned the use of organic phosphates, as we soon found out that their benefits did not outweigh their hazards. In consequence, we had no opportunity to study cholinergic crises of this kind. It has not been possible as yet to eliminate the toxicity of these drugs, accordingly the more recent complex phosphorus compounds (parathion, malathion, dematon, etc.) are still far from being harmless (RIDER and MOELLER 1961), this applies to alkylthiophosphocholine as well (OSSERMAN, COHEN and GENKINS 1961). Nevertheless, they are well worth studying as they might provide the "long-acting" drug long sought for. The BC substances evolved by KRAUPP et al. (1955) actually are long-acting, but in the course of clinical trials they have been found to

carry the risk of crises owing to their cholinergic property (PATEISKY 1959). The merits of ACTH suggested for the management of myasthenia by TORDA and WOLFF (1949) have been questioned from the very start. MILLIKAN and EATON (1951) have observed crises in association with such treatment, WESTERBERG and MAGEE (1955), OSSERMAN (1958), ERBSLÖH and L'ALLEMAND (1965) condemn it as being definitely dangerous whereas LAMARTINE DE ASSIS (1956, 1960) advocates the use of intravenous ACTH drip in severe cases resistant to other measures. We have made occasional attempts with ACTH in the absence of crises, without being able to obtain any proof of its efficiency.

None of the adjuvants used in myasthenia — i.e. vitamins, ephedrine, potassium — has been found to increase the risk of crises, but hypopotassaemia definitely does, therefore its control is essential (HERRMANN 1961, SZOBOR 1964). Sedatives used for the relief of psychic symptoms also may provoke crises, as observed in the case of a young male patient who was given inadvertently 0.50 g barbiturate after thymectomy at the surgical unit where the operation had been performed. The consequence was a transitory but none the less severe respiratory crisis. In our opinion (SZOBOR 1964) myasthenia precludes the use of barbiturate containing hypnotics while judicious application of minor tranquillizers may be beneficial.

3. Crises resulting from overdosage are cholinergic in character. As mentioned before, conversion of myasthenic into cholinergic crises or co-existence of both characters (combined or complicated crises) may similarly occur. In 2 of our patients the crises were cholinergic, in 6, they were combined. Overdosage was due in most cases to an increase of the doses by the patients without medical advice. Satisfactory response to adequate measures put into operation on admission, i.e. drug-withdrawal, injection of edrophonium, strict bed-rest and adjuvants, conclusively proved that the crisis had been cholinergic in character.

Crises due to overdosage are generally associated with symptoms of the muscarine- or nicotine-types, which are, however, rarely distinct enough to be of diagnostic value. According to TETHER (1955) and SCHWAB (1961) the importance of muscarine-symptoms lies in heralding a cholinergic condition. SKINØJ and PETERSEN (1957) have, however, shown by systematic spirographic study of a patient under artificial respiration that muscarine symptoms have no such ill-boding significance. They found a substantial lag between the appearance of muscarine-symptoms and the maximal antimyasthenic effect of cholinergic drugs. Increased tolerance to anticholinesterase drugs in myasthenia is a well-documented fact (OSSERMAN and KAPLAN 1953, SIMPSON 1960). VIETS (1961) believes excessive tolerance to massive doses of prostigmine to be diagnostic of myasthenia, since it occurs in no other condition. The tolerance to drugs, and consequently also their side effects, are, however,

variable depending apart from the actual state of the myasthenic process, on the patient's individual sensitivity, responsiveness and vegetative state. While in a number of patients the appearance of muscarine-like symptoms was largely a matter of vegetative and tissular responsiveness without any serious significance, we witnessed in others the onset of a cholinergic toxic condition unheralded by any sign of muscarinic or nicotinic character. By stressing the point of individual tolerance it was intended to illustrate the contradictory issues of the problem rather than to attack the predictive significance of muscarine-symptoms.

Our review of the various factors responsible for the production of crises in myasthenia closes with the discussion of the role of *thymectomy*. Thymectomy as a cure of myasthenia has found relatively much opposition, though evaluation of a substantial surgical material no longer permits to question its merits (KEYNES 1955, SIMPSON 1958, SCHWAB 1961, ROSS 1961, OOSTERHUIS 1963, HENSON, STERN and THOMPSON 1965, etc.). Reluctance to the operation may have various causes, first of all its relatively high mortality. The postoperative period being three weeks in this type, surgical deaths associated with thymectomy must be put at 8 per cent (SIMPSON 1958). ERBSLÖH and L'ALLEMAND (1965) have pointed out that thoracic surgery of other kind carries a 2 per cent mortality and they ascribe the 6 per cent difference to factors connected with the myasthenia itself, particularly the crises and the hazards of drug treatment. On the basis of our observations we fully subscribe to this view (SZOBOR [1958]). Our most powerful argument in favour of the operation spring from the knowledge that the danger lies in myasthenia, not in thymectomy.

On the other hand it must be recognized that thymectomy involves a considerable bodily and mental stress to the patient, and that it may bring on a myasthenic crisis or lead indirectly to a cholinergic one. CABO (1958) recorded instances of fatal postoperative crisis in his thymectomy material of 33 cases. PENNINGTON and EDWARDS (1960) registered postoperative crises in 4 instances with one death after 10 thymectomies. Occurrence of crises after thymectomy has been furthermore reported by WEIL et al. (1952), REID (1949), FISHER and CHILD (1955) and others. HENSON, STERN and THOMPSON (1965) encountered myasthenic as well as cholinergic crises in their material including 36 thymectomies, conversion of the former into the latter type having occurred under their very eyes without an intermediary stage with normal muscle power. The crises made their appearance 24 to 72 hours after surgery, 2 patients died, 3 could be saved by artificial respiration.

29 out of our 86 patients were subjected to thymectomy. This ratio largely corresponds to other statistics. Details of the after thymectomy crises have been presented in the foregoing Table. Classification into early and late crises does not occur in literature, we find it, however, justified, on the ground

of the typical double peak in their timing which we also found in earlier studies (SZOBOR 1958, 1962). Early crises occurred between the first and third postoperative days while late crises between the eighth and eleventh days. No crises were observed outside these periods, i.e. between the fourth and seventh days and beyond the eleventh day. Early and late crises were never seen in the same case. In 6 patients the crises were early, in 4, late, in onset. In 3 cases the myasthenic pattern was associated with features of cholinergic weakness, in one case there was a swing from the myasthenic to the cholinergic type without any transitory phase of normal muscle power or respiratory relief. In 5 cases of thymectomy associated with postoperative crises tumour of the thymus was found (patients No. 6 to 10 in Table I), in the other 5 there were no malignant changes. In one of the malignant cases the crisis, though appearing after reoperation for haemothorax instead of thymectomy, has been nevertheless included into this group. The late crisis occurring in the other case was associated with the removal of a mediastinal tumour believed to be a fibroma. Operation revealed a thymoma and led at the same time to the diagnosis of myasthenia. This case represents a rarity in literature (HEDRI, DROBNI and SZOBOR 1961, SZOBOR and KÖRNYEY 1965). Another remarkable case is that of a 49 year old woman who had myasthenia of combined localization. Operation, made necessary by the rapid progression of the process, revealed an encapsulated fist-sized tumour with histologic evidence of malignancy (SZEGEDY and SZOBOR 1961). The operation was followed by a late crisis which could be brought under control. At present, eleven years after the first symptoms and seven years after thymectomy, the patient works in her former occupation without needing any drug.

Postoperative crisis was the cause of death in 3 of our cases. In the first, a 54-year old male, mediastinotomy revealed carcinoma of the thymus occupying the anterior mediastinum and adhering to the left upper pulmonary lobe as well as to the large vessels. Haemothorax ensuing 24 hours after partial resection of the tumour made reoperation necessary. A few hours after this second operation a crisis of the oscillating type appeared, with alternating prevalence of myasthenic and cholinergic elements, and caused death within 18 minutes leaving no possibility or tracheotomy or artificial respiration. — In the second case, a 67-year old patient, a fulminant respiratory crisis appearing 24 hours after operation was associated with cardio-vascular involvement. In the third case, a 18-year old girl, there had been no malignancy of the thymus, operation had been smooth, the postoperative course entirely uneventful, until she died suddenly a few minutes after the onset of a respiratory failure, 36 hours after operation.

Apart from the factors have been discussed in our paper I., there are circumstances, such as surgical and psychic stress, anaesthesia, modification of drugs, etc. which may play a part in the production of postoperative crises.

There may be a particular factor which we have found to be of importance on closer analysis of the postoperative crises in our cases on the one hand, and on the grounds of the favourable results of postoperative radiotherapy on the other, namely the existence of aberrant thymus tissue or thymus-islets, transitory hyperfunction of which may be responsible for the postoperative crises. When the principal thymus gland has been removed, the remaining aberrant elements could take over its endocrine functions passing through a stage of transitory hypertrophy. It is well-known that normally functioning or even hyperactive thymus tissue may occur at anomalous sites. There exist numerous descriptions of aberrant thymus tissue, duplicate or multifocal thymus glands, functioning thymus tissue embedded in the thyroid, parathyroid, the sheath of the vagus nerve, wall of the small intestine, ovarial cyst, etc. The presence of aberrant thymus tissue may account in many a case for the failure of thymectomy to produce the expected result, and transitory hyperfunction of such tissue may be one of the possible factors of postoperative crises.

The present review is obviously incomplete owing to imperfect understanding of the mechanism involved. Further observations and collection of data will probably help to identify other possible primary or accessory factors which may have a part in the phenomena outlined.

III. Management of the crises

Principles and practice

The last fifteen years have brought a substantial change in the management and prognosis of crises in myasthenia. The change is due first of all to the fact that we have become acquainted with the nature of the cholinergic crises which allows to consider their possibility in every case of myasthenia liable to crises.

As pointed out before, the most common factor responsible for, or contributing to, crises is respiratory infection or obstruction. Ensuring a clear respiratory tract is therefore essential to their efficient management. Swabbing, or suction of saliva or mucus, pulling forward the atonic tongue, though being the simplest possible procedures, may nevertheless put an end to a crisis. Drainage with the patient in Trendelenburg's position makes clearing of the airways easier. In laryngeal asthenia HERRMANN (1961) found intubation maintained for a few hours to be beneficial. This must be followed, if necessary, by tracheotomy. In case of emergency however, he finds the introduction of a wide-bore, short-tipped cannula into the upper trachea preferable to the "pen-knife-tracheotomy". We can only subscribe to this advice having convinced ourselves of the adverse consequences of tracheotomy carried out in an inadequate manner.

Hypersecretion associated with the use of the current drugs in myasthenia raises the question of preventive dosage of atropine for the control of salivation. Literature agrees upon the necessity of this drug where there is a tendency to hypersecretion. OSSERMAN (1958) prescribes atropine routinely to myasthenic patients, directing them to use it in case of secondary effects. Symptoms of the muscarine-type or imminent cholinergic signs call for the use of atropine. It is known from the studies of SKINHØJ and PETERSEN (1957) that atropine controls most of the muscarine-symptoms without interfering with the antimyasthenic effect of the drug in use. HERRMANN (1961) while making use of the benefits of atropine points to its facilitating influence on the nicotine-syndrome and on depolarization-weakness. Viscosity of mucus due to atropine has its dangers which have been duly pointed out by DINNICK (1958), OSSERMAN (1961) and HERRMANN (1961). HARLAND and STEPHEN (1958) even protect from atropine in post-thymectomy sate. Personally we make use of the drug whenever there is a tendency to hypersecretion, prescribing atropine sulphate in doses of 0.2 to 0.4 mg to be taken with every second dose of neostigmine. In cholinergic conditions we find the use of atropine peremptory without any regard to the possible danger of nicotine-block pointed out by HERRMANN, the more so as this hazard is felt by us to be more a theoretical than a real possibility.

For the control of cholinergic crises HERRMANN (1961) as well as STORM-MATHISEN (1961) advocate the use of edrophonium given at close intervals.

The policy of preventive tracheotomy finds increasing favour in recent literature. HERRMANN (1961) advocates its use in threatening crises instead of waiting until the crises become manifest when the circumstances are less favourable. OSSERMAN (1961), HEAD (1964), SCHWAB et al. (1964), BLAUGRUND et al. (1964) stress the necessity of early tracheotomy which, in the experience of these authors, contributes to low surgical mortality. All of these claims are supported by figures which make the merits of the intervention unquestionable and may possibly lead us to reconsider our attitude, since, in agreement with many other clinicians dealing with a larger myasthenic patient material, we have not made current use of preventive tracheotomy up to now. And we may well ask whether there is any justification of preventive tracheotomy in a patient who has never had any respiratory or bulbar symptoms or whether, when the crisis is not yet manifest, it will not become so under the somatic and psychic stress involved by the intervention. We therefore believe that the question should be decided on individual grounds. Bulbar symptoms, or actual respiratory failure, make preventive tracheotomy justified, while in the absence of bulbar symptoms its necessity is questionable. This view is shared by the surgeon engaged in operations for myasthenia, in view of the danger of mediastinitis (DROBNI 1965).

The most efficient weapon in the management of crises is artificial re-

spiration. Nursing and supervision of the patient needs a specially trained staff There is still a great deal to be done in this respect. OSSERMAN (1958, 1961) finds positive-pressure respiration preferable in the interest of nursing care. This was applied in 5 of our cases; in 4 of them the crisis was brought under permanent control, one patient has been, however, in a state of crisis for six months at the time of the present study. While the patient under artificial breathing, we abstain, if possible, from the use of any anticholinesterase drug so as to provide for the "resting process" pointed out by CHURCHILL-DAVIDSON and RICHARDSON (1957). On the basis of what has been said earlier, we never use curarizing agents in controlled respiration. Release of the patient must be successive. By the end of this period he is usually as responsive to drugs as he originally was when first receiving them in the initial stage of the disease. After successful control of a crisis the patient still requires careful supervision, management and care in a specialized unit with due regard to his psychic condition owing to the profound mental stress which, obviously, never fails to affect his psychic state.

More recently, the use of oxim-compounds has been advocated for the management of cholinergic crises (GROB and JOHNS 1958, OSSERMAN 1961) but they have proved, on the whole, disappointing (HERRMANN 1961).

Management of combined crises is a complicated and often futile task. All we can do is to adjust the drug to the prevalent symptoms with all the perils of therapeutic inconsequence to the patient. In view of the fact that the antimuscarine effect of atropine does not interfere with the antimyasthenic properties of the necessary drugs, it may be safely given in combined crises. Clear airways, regulation of electrolyte- and fluid balance, administration of adjuvant drugs are additional factors which may help to overcome the crisis, while long-acting drugs, BC-compounds, ACTH should not be prescribed.

Prevention of crises is no simple task. Supervision and guidance of the patient embracing every possible aspect of his disease, special care in increased exposure to crises such as respiratory infections and pregnancy, further adequate education and a suitable regimen may be of help. These are very arduous tasks, yet the physician entrusted with the management of myasthenic patients, has to face them.

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EPILEPTIFORM EEG CHANGES IN THE SYNDROME OF PERIODIC ABDOMINAL PAIN*

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EEG recordings made in the waking state and during hexobarbital anaesthesia have been studied in 38 children and adolescents with recurrent paroxysms of abdominal pain.

A total of 56.7 per cent of the waking records revealed spontaneous abnormal slow rhythms and 13.5 per cent, epileptiform abnormalities. Typical spikes were demonstrable in 83.3 per cent of the tracings recorded during induced sleep. In 64 per cent, the spike discharges appeared in the rolandic area or at the vertex over one or both hemispheres; in the remaining cases, the site of origin of the discharges was the frontal area; in one case an antero-basotemporal spike focus was localized, in another, a generalized 3 c/sec spike-and-wave activity could be evoked. Plurifocal epileptiform activity was likewise not infrequent.

In 31 per cent of the cases the findings suggested the possibility of an epileptogenic brain lesion, for which reason these patients are considered epileptics while remaining 42 per cent "borderline cases of epilepsy" the findings being ascribed to some benign developmental abnormality (immaturity) of the brain.

Evidences revealed by the research of higher neural gastrointestinal regulation, further, clinical observations on epileptic patients, are suggestive of the assumption that the rolandic and vertex spikes may represent the convulsoid hyperexcitability of the non-specific visceromotor structures functioning in the sensorimotor cortical areas, and therefore the prescription of anticonvulsants is recommended in the syndrome of recurrent abdominal pain.

Some ten years ago, one of the present authors [14] has pointed out on the grounds of statistical evidence that certain signs of visceral excitation, particularly in the areas of abdominal, cardial, respiratory, vaso- and sudomotor innervation, not only belonged to the conspicuous manifestations of temporal lobe epilepsy but that the abdominal syndrome was in occasional cases the most prominent feature of the seizure.

Abdominal pain is a common complaint in childhood. HARNACK [13] found it to occur in 7 per cent of school age children. Since complaints of this kind are generally of little significance, parents learn to disregard them. There are, however, brief spells of violent abdominal pain with a tendency to unexpected periodic recurrence, surprising the child not infrequently when engaged in some activity he enjoys. The pain which usually starts periumbilically and spreads to the epigastric region may be associated with headache as well as with other autonomic dysfunctions, often with clouding or even loss of con-

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sciousness. The autonomous character of the objective signs bears evidence to the essentially biologic source of the subjective symptoms. The seizure usually appears at daytime when the child is fully conscious but in case of nocturnal onset, it may awake the child [17].

The abdominal pain, headache and episodic autonomic symptoms were grouped together into an entity by WYLIE and SCHLESINGER [25] and called by them "the periodic syndrome"; they believed that the attacks were a manifestation of central nervous system disturbance.

LIVINGSTON [19] resumed the EEG findings of 20 children observed and treated by him for recurrent spells of abdominal pain, as follows: "Electroencephalographic studies revealed definite abnormalities in 16 cases ... abnormally slow activity in seven cases, abnormally fast activity in five, bursts of spikes in two, and spike and wave forms of the petit mal variant type in two patients."

In his monograph entitled "Masked epilepsy", WALLIS [26] described eight children with recurrent abdominal crises. With exception of one the waking EEG showed gross abnormalities judged by the author to be consistent with, or suggestive of, the bioelectrical pattern of epilepsy, the chief sites of abnormality being the parieto-temporal, the temporal or the temporo-occipital regions.

APLEY [1] devoted a short monograph to the diagnosis and therapy of abdominal pain in childhood. His studies comprise a child population under hospital care, as compared with a population of school-children. Fifty per cent of the children complained of slight, 25 per cent of severe abdominal pain. The waking EEG displayed an epileptiform activity with spike bursts in 19 per cent of the hospital population (93 children) as against 10 per cent of the 97 school-children. The difference of the percentages correlated with the electroencephalographic findings obtained in the group without abdominal pain was, however, not significant.

KELLAWAY, CRAWLEY and KAGAWA [17] resumed their findings as follows: "Approximately 86 per cent of the 459 cases which have been seen with episodic abdominal pain and/or headache ... showed the 14 and 6/sec positive spike abnormality in sleep. The other 14 per cent had various other types of electroencephalographic abnormalities (temporal spike foci, spike and wave activity, paroxysmal slow bursts, etc.) or showed normal electroencephalograms ... the 14 and 6/sec positive spike pattern is not seen during an attack, instead paroxysmal generalized slow bursts are seen at such times. ..."

SCHADE and GOFMAN [23] had 46 children with "abdominal epilepsy" under their observation. According to their statement, "The electroencephalograms displayed a temporal focus in association with the bouts of abdominal pain in 22 cases; generalized dysrhythmia, no focus in 16, higher potentials over left hemisphere in 1 and normal record in 7 cases were found."

SHEEHY, LITTLE and STONE [24] studied the EEG in sleep in 19 children with abdominal epilepsy with the following result: "A summary of the EEG changes shows that (1) the majority of these patients had either borderline or definitely abnormal electroencephalograms and (2) the most frequent changes (42 per cent) consisted of rhythmical rectified spikes (14 and 6 per second dysrhythmia)."

DAGENAIS-PÉRUSSE et al. [3] reviewed 6 cases of "abdominal epilepsy". The EEG pattern in the resting waking state or upon photic stimuli, was invariably abnormal. In five cases, a paroxysmal or epileptiform activity was demonstrable in the temporal or occipital area, and in one case centroencephalic myoclonic convulsions were present.

Patients with pains due to abdominal changes have been excluded from the case material of the above authors.

Material and method

EEG recordings were made during the interictal intervals in 38 children and adolescents whose symptoms were consistent with the syndrome of paroxysmal periodic abdominal pain. During the pain paroxysm we could obtain no more than two records. The age of onset varied between 4 and 14 years, largely corresponding thus to the age distribution recorded by the foregoing authors. With a few exceptions, the children were subjected to complete clinical and serological studies, comprizing blood sugar tolerance tests, careful examination of the abdominal organs and search for intestinal parasites [16].

The EEG recordings were made with a 1955/56-type 8-channel Kaiser apparatus, and recently with a 16-channel Galileo apparatus. Anterior frontal, central, parietal, antero-, medio- and postero-temporal electrodes were placed symmetrically in identical sites as well as in the midline to the vertex forming sagittal and coronal chains. When a temporo-basal focus was suspected, nasopharyngeal electrodes were also used.

Routine recording in the resting waking state included two 3-minute continuous tracings interrupted by a 3-minute period of hyperventilation and terminated by photic stimulation by means of white light with flashes at various frequencies. In 33 cases the waking EEG tracings were completed with continuous records under hexobarbital anaesthesia, reflecting every phase of the barbiturate effect. This was associated with sensory activation, namely auditive stimuli (electric buzzer, calling by name) and nociceptive stimuli (pricking, pinching). Hexobarbital was injected intravenously at a rate of 25 mg/10 sec, occasionally 25 mg/15 sec, up to a total dose of 250 to 500 mg adapted to age and individual tolerance, until continuous delta-activity had been developed.

Results

1. The number of girls greatly exceeded that of boys, their ratio being 71 per cent (27 cases) against 29 per cent (11 cases). Together with the material of SCHADE and GOFMAN (26 boys, 20 girls), WALLIS (4 boys, 4 girls), DAGENAIS-PÉRUSSE et al. (6 girls), this makes a total of 66 per cent (72) girls against 36 per cent (41) boys. In APLEY's material the ratio of girls was 22.7 per cent higher than that of boys. No reference to sex distribution is found in the papers of LIVINGSTON, KELLAWAY et al., or of SHEEHY et al.

The above figures point to a prevalence of the syndrome in females.

In all cases but one the recurrent abdominal crises were associated with violent pain. The incidence of the various elements of these crises are presented

Table I

Incidence of seizure-elements in 38 cases of periodic abdominal syndrome

	Number	Per cent
Abdominal pain	38	100.0
Nausea, vomiting	18	47.4
Signs of intestinal excitement	6	15.9
Headache (vascular)	21	55.2
Precordial pain	3	7.8
Pallor	12	31.5
Flushing	3	7.8
Sweating	6	15.6
Salivation, lachrymation	2	5.2
Fever	2	5.2
Loss of urine	1	2.6
Fainting, dizziness	12	31.8
Initial focal sensory crisis (face, leg)	2	5.2
Transient clouding	2	5.2
Terminal sleep, collapse	23	60.5

in Table I, pyretic origin has been reported by the parents of 6 children (15.6 per cent).

The prevalent changes revealed by the sleep records of patients were spike potentials appearing in the central region. In Table II the various elements of seizures occurring in 43 epileptic subjects as a consequence of discharge of epileptogenic foci in the central region are presented for comparative purposes.

2. No physical or serological abnormality accounting for the abdominal crises was demonstrable in any of the cases.

3. Normality or abnormality of the background activity of the resting waking EEG was judged by the standards of GIBBS and GIBBS' Atlas [12] and FOIS-LOW's The Electroencephalogram of the Normal Child. [11]. Over five years of age, high-voltage spontaneous delta-groups are no longer considered normal, the less so the older the child and the greater the prevalence of this activity over other spontaneous rhythms. The appearance of 5 to 7 c/sec rhythm is considered physiological up to the age of 7 years, between 7 and 10 years as a moderate slowing, a sign of immaturity, beyond that age as a medium-degree slowing.

(a) By these standards, we found abnormal rhythms and epileptiform patterns or convulsive potentials in more than 50 per cent of the cases.

In 43.3 per cent of the cases, i.e. 16 patients, the resting waking records showed no abnormality whatsoever, whereas in 56.7 per cent, i.e. 21 patients,

Table II

Incidence of seizure-elements in sensorimotor attacks
(46 epileptic patients)

	Number	Per cent
Abdominal pain	0	0.0
Nausea, vomiting	4	7.6
Signs of intestinal excitement	1	2.1
Headache	0	0.0
Precordial pain	2	4.2
Pallor, dizziness	2	4.2
Flushing, sensation of heat	2	4.2
Sweating	1	2.1
Salivation	2	4.2
Pyrexia	0	0.0
Loss of urine, urge of micturition	6	13.0
Focal sensori-motor seizure (cranial)	23	50.0
Speech arrest, motor aphasia	6	13.0
Focal sensori-motor seizure (upper extremity)	25	54.3
Focal sensori-motor seizure (lower extremity)	7	15.3
Generalized seizures	2	4.2

abnormally slow rhythms in relation to age were demonstrable, mostly over the rostral or posterior regions on both sides. Slowing was associated with a variable increase in voltage. Definite slowing of spontaneous ground activity was seen in 13.5 per cent (5 cases), moderate slowing in 43.2 per cent (16 cases), while in one case the EEG was inconclusive.

Spontaneous appearance of interictal seizure potentials, spikes or sharp waves in the waking state, were registered in 13.5 per cent (Fig. 1), spontaneous bursts of high slow waves over the centrofrontal region of one side, in 2.7 per cent. An epileptiform discharge was evoked by hyperventilation in one case, photosensitivity became manifest on rhythmic flashes in two cases; in one case a 3 c/sec spike and wave pattern was induced by the photopentetrazole method. KELLAWAY et al. failed to elicit an abnormal pattern by flickering light in a large material. PRECHTL [22] induced paroxysmal waves over the temporal region by intermittent acoustic stimuli applied to two waking patients whose chief complaints were recurrent bouts of abdominal pain. Auditory stimulation was consistently applied by us during induced sleep but seizure potentials ascribable to the stimuli occurred in three cases, i.e. 8.1 per cent, only.

The EEG records taken during the spontaneous abdominal crises were characterized by a high-voltage 6—3 c/sec activity and the absence of seizure

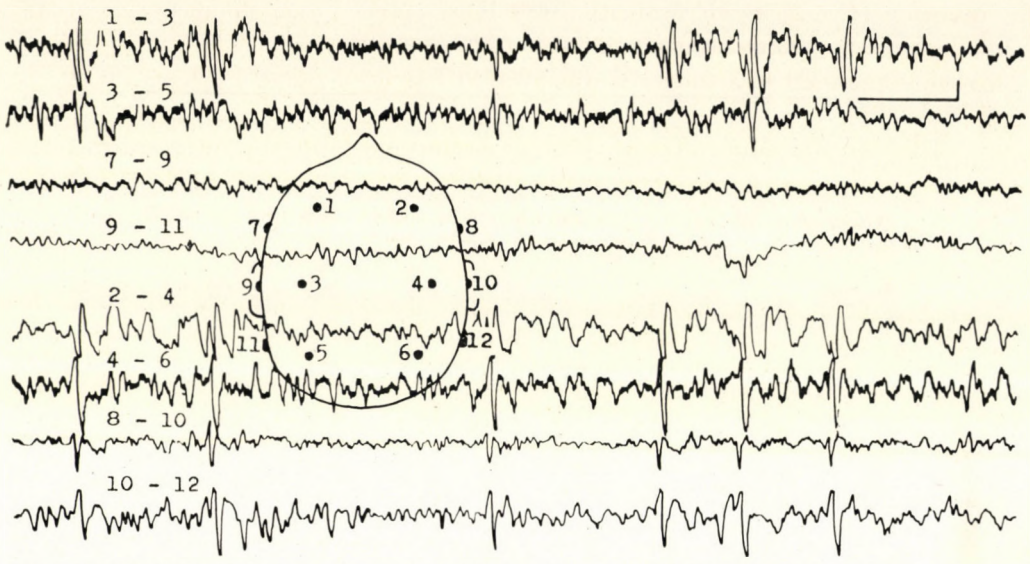

Fig. 1. No 252/64. Waking spontaneous EEG, calibration 50 μV and 1 sec. Explications in reference to this and the other figures see under 3 of the section "Results". — 6-year-old girl who had suffered a cerebral lesion at birth. No oligophreny. The abdominal crisis is ushered in by clonic twitchings of the right facial musculature, followed by abdominal pain, headache, salivation

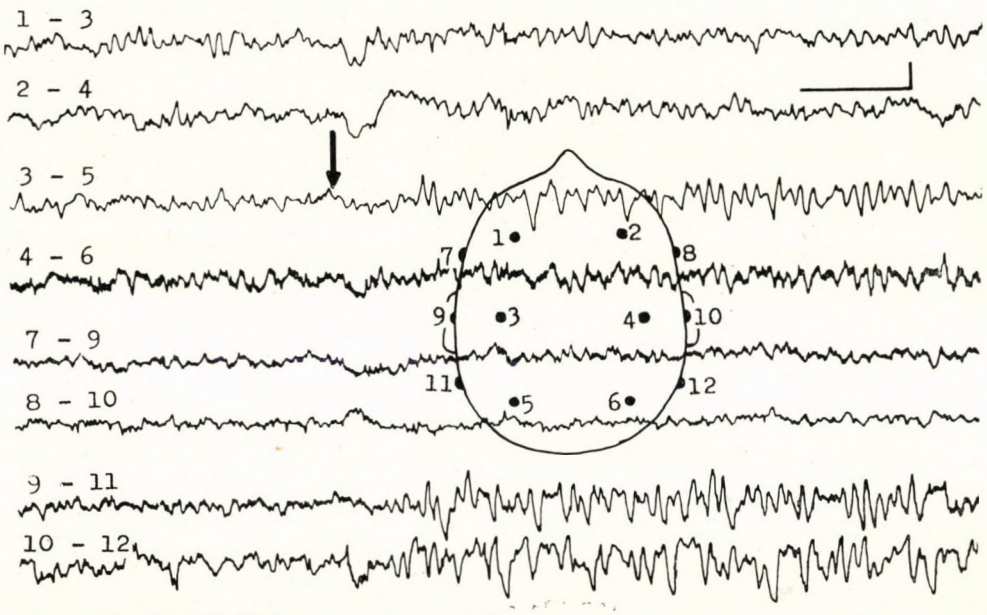

Fig. 2. No. 1719/60. Waking spontaneous EEG; the arrow marks the onset of abdominal attack during which a high-voltage slow-activity appeared over both hemispheres with postero-temporal prevalence. 10-year-old girl, in the history no data referring to cerebral lesion

potentials (Fig. 2). Synchronization was most marked over the posterior areas of the temporal lobes, occasionally over the frontal region. A slow frequency, high-voltage ictal EEG-pattern was all that has been found by KELLAWAY et al., too (Fig. 3).

(b) Hexobarbital anaesthesia was performed with the purpose of activation in 33 cases. Four patients had EEG records taken during sleep on two or three occasions. In one case recording was made during natural sleep. Fail-

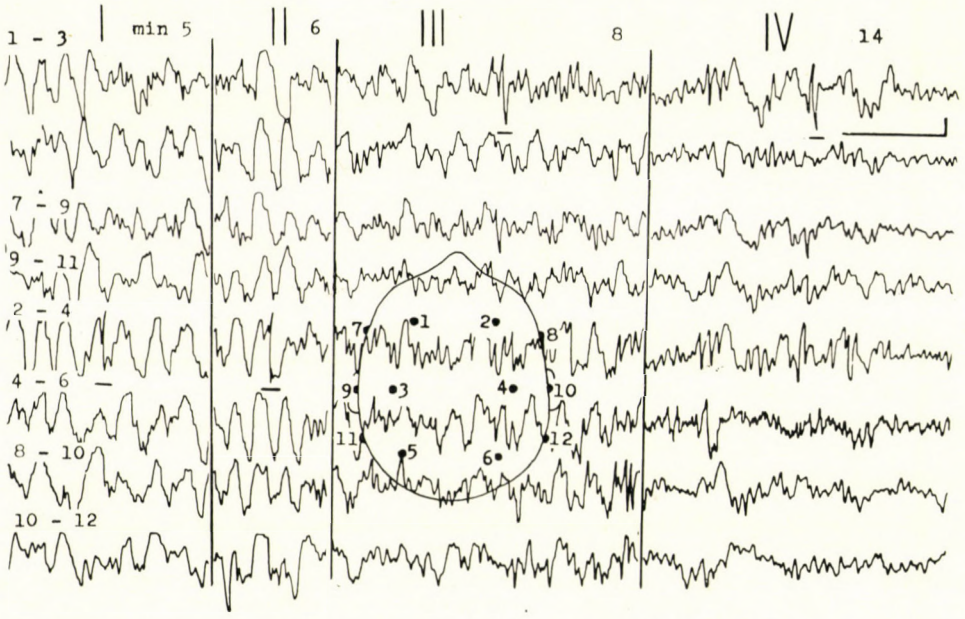

Fig. 3. EEG of the same girl recorded three years later during induced sleep. Segments I—IV mark the four sections of the recording; the numerals 1—4 mark the minute of recording from the beginning of hexobarbital injection. I—II have been recorded in the phase of deepening anaesthesia, III during the first phase of its relief, IV in the phase before awakening. The short horizontal lines mark the spike bursts, this applies to the other Figures as well

lure of induced sleep to precipitate epileptiform activity was noted in 3 cases only, in one of these the effect of the barbiturate resulted in the disappearance of spontaneous discharges demonstrable in the waking state. Hexobarbital was found to activate seizure potentials in patients with abdominal syndrome in the same manner as it did in established epilepsy [15]. The morphology of the abnormal bioelectric transients registered under barbiturate effect shows the following distribution in the 30 cases (Table III).

The spike discharges were counted in the EEG records obtained from 23 patients during artificial sleep. In all of these, epileptiform potentials showing central or anterior frontal voltage peaks occurred independently from each other. The spikes reached the 1/min or greater frequency in 13 cases. The nu-

Table III

	Number	Per cent
Spikes in central and/or frontal region	24	80.0
Spikes in the basotemporal region	1	3.3
Frontal negative sharp waves	2	6.7
Convulsive "mittens" in the frontal region	3	10·0
Total	30	100.0

merical preponderance of central spikes was found in 11, predominance of frontal spiking in 6 records. The number of asynchronous central and anterior frontal discharges was nearly identical in 6 other records. A total of 445 spike discharges was identified, 287 out of them (64.4 per cent) with central voltage peaks (Fig. 4).

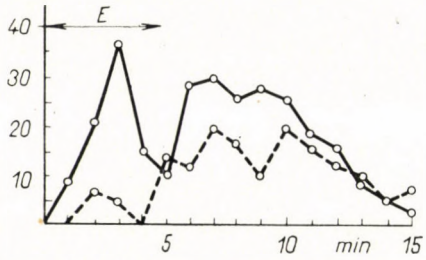

Fig. 4. sums up the number of spikes per minute of 23 records made during hexobarbital anaesthesia. The continuous line represents the number of vertex and rolandic spikes, the broken line that of frontal spikes; the numerals on the abscissa mark the minutes from the start of hexobarbital administration, those on the ordinate, the per minute discharges; the horizontal arrow shows the average duration of hexobarbital injection calculated from the data of 23 records

The most common discharge pattern during induced sleep was thus made up of solitary, occasionally serial, spikes in the central area, duration of which beyond 80 m/sec was exceptional. The discharges emerged at irregular intervals, mostly over one hemisphere, often shifting between the two sides, occasionally over homologous areas of the two hemispheres (Fig. 5). In contrast to the waking seizure potentials, hexobarbital did not significantly change the shape of the epileptiform potentials, it only reduced slightly their duration. The number of negative spike potentials prominent in the central region increased in the early stage, i.e. in the second or third minute of barbiturate effect, however it fell suddenly as anaesthesia became deeper, in the fourth and fifth minute. The rapid subsidence of the barbiturate effect allowed another peak of activation of flattened and more protracted character to manifest itself during the interval sixth to tenth minute; after this period, the fre-

quency of central spikes decreased successively and disappeared in a few minutes (Fig. 4).

The negative spikes in the antero-frontal region were scarcely elicited by the initial barbiturate effect, therefore their activation was somewhat delayed. However, from the fifth minute on, the number of frontal spikes showed a distinct rise, the curve of frequency running parallel with that of central spikes, though with a definitely lower rate per minute.

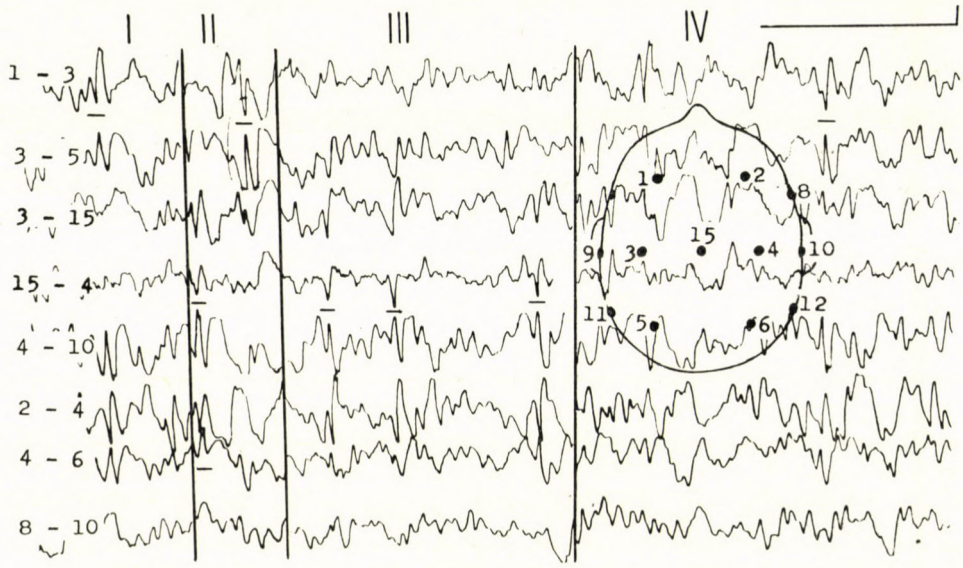

Fig. 5. Sections from record No. 600/64 of a 9-year-old girl who had minor epileptic seizures provoked by febrile state at the age of 6 months. Record made in the 4th–5th minute of hexobarbital injection, at a paper speed of 60 mm/sec. Sections I and IV clearly show an epileptiform discharge culminating over the vertex with broad extension to both central areas. On segments II—III a bilateral rolandic discharge, independent from the former is seen. The warning sign of the crisis was a numbness of lips, face and leg at the left side, this was followed by violent abdominal pain, palpitation, precordial pain, finally by clouding

4. General anaesthesia allowed a careful study of epileptiform discharges, as regards site and extent of the potentials, and the shifts of their epicentres. These latters were detected firstly on the basis of phase alternations recorded by the bipolar technique. It may be seen in the Figures that, in the sagittal rows, electrodes were connected in the rostro-caudal direction, while those in the coronal rows, from the left to the right side. In the chains of identical direction bridging over homologous areas, the electrodes were spaced at equal constant distances.

Morphologic analysis of the spikes seen in the bipolar leads resulted in the inference that cerebral electrogenesis of the same person may produce epileptiform discharges with simultaneous or independent appearance in different areas of the most variable extent above the one or both hemispheres.

Fig. 5 (segments I and IV) shows the seizure potentials picked up by both the fronto-central and centro-parietal electrode pairs simultaneously from both hemispheres. Phase reversal at the central electrodes clearly indicates that the epicentre of epileptiform discharge is localized bilaterally below the central electrode or in its close vicinity. At the same time, the coronal electrode chain fails to record any spike at the central electrode pairs connected with the mid-line electrode, but a negative spike discharge emerges at the right centro-temporal electrode pairs. These phase- and voltage relationships show that the midline electrode together with both lateral central electrodes is situated within an equipotential area, namely in the field of an extensive epileptiform discharge occupying symmetrical areas on both sides of the vertex. Fig. 5., segment III shows serial spike discharges of relative slight extent whose epicentrum appears at the right central electrode as shown by the phase reversal. In segment II of Fig. 5, there is a minor discharge on each side, independent of each other.

In segments I and II of Fig. 6, phase reversal between the fronto-central and centro-parietal electrode pairs points to the epicentre of discharge being situated at the left central electrode. The discharge does not extend to the temporal lobe. It shows a central voltage maximum in the coronal chain as well. The phase and potential characters of the spikes recorded by the coronal chain also permit to locate the central discharge to the area situated between the left midtemporal and the mid-line electrodes with a slight marginal overlap to the right bank of the longitudinal fissure. On the other hand, the discharge seen in section III of Fig. 6, the potential of which culminates in the antero-frontal region, extends to the antero-temporal region on the left side, though with a lower voltage. The electrical field does not spread to the right hemisphere and its voltage declines in the caudal direction.

Fig. 7 also shows a plurifocal epileptiform activity. Section I: synchronous burst of a left fronto-central and right antero-frontal negative discharge. Section II: rolandic discharge on the right side with phase reversal at the central electrode. Section III: the coronal electrode chain records a spike of minor extent emitted by the region of the vertex, its epicentre being close to the longitudinal fissure.

5. Some cerebral damage suffered years before the onset of the abdominal symptoms was mentioned in the history of 13 patients. There had been birth injury in 3 cases, meningoencephalitis in one case, hyperpyretic eclampsia, i.e. convulsions elicited by fever, in the early childhood, had been witnessed by the parents of 6 children. In 5 cases there was a history of typical epileptic fits associated as often as not with the abdominal crises. SHEEHY et al. identified some convulsive episode, mainly of hyperpyretic origin, in the history of 31 per cent of their cases, while SCHADE and GOFMAN noted the occurrence during febrile episodes of epileptic seizures in 54 per cent of their material.

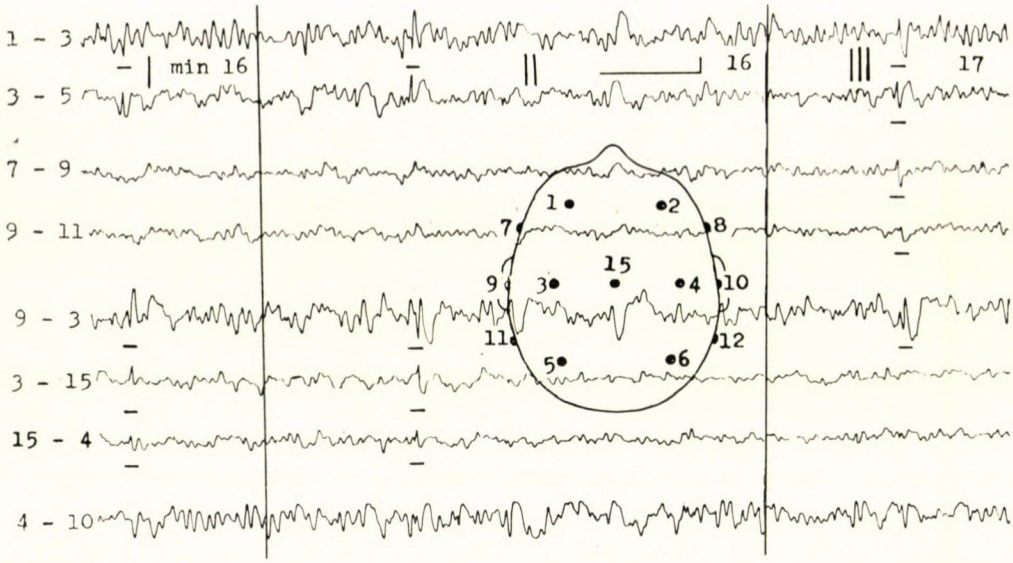

Fig. 6. No. 564/64. Patient of 10 years who had suffered cerebral injury at birth, and had recurrent crises of violent abdominal pain and headache from the age of 3 years. Recording made during the last, protracted phase of hexobarbital anaesthesia. Sections I—II show left rolandic spikes, section III an epileptiform discharge with a frontal epicentre and spread over the entire left hemisphere

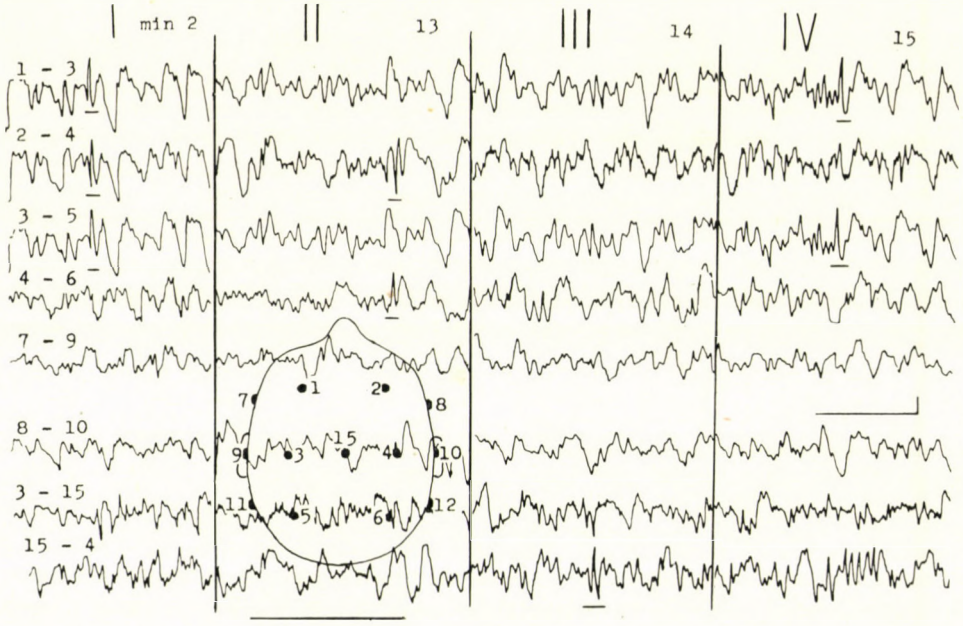

Fig. 7. No. 287/65. Record made under hexobarbital anaesthesia. Sections I and IV show simultaneous left frontocentral and right anterofrontal epileptiform discharges. Section II: right rolandic spike which seems to be precipitated by auditory stimuli (electrical buzzer); Section III shows vertex spike of minor electrical field

Discussion

The abdominal paroxysms of our patients had an epileptiform onset but their course was more protracted than that of either the focal sensory and motor seizures or of the generalized convulsions. During the seizure the attitude of the patients expressed intense discomfort due to violent pain and to other autonomous factors of the paroxysm. The crises were on the whole of greater severity in our material than in the cases reviewed by APLEY.

There were, furthermore, typical epileptic seizures in the history of a considerable number of our patients. 83.3 per cent of the EEG tracings recorded under barbiturate anaesthesia showed a focal or plurifocal spike activity. The epicentre of the discharges was found prevalently over the middle third of the rolandic fissure, less frequently over the vertex. The number of antero-frontal spikes was considerably less than that of rolandic spike potentials. The electromorphology of the epileptiform discharges was found to correspond to that of the spike pattern of the central or frontal epileptogenic lesions recorded under the effect of hexobarbital [15].

On the basis of this evidence, we feel justified in regarding the patients reviewed in the foregoing as "borderline cases of epilepsy", or even as "genuine epilepsy" if regular seizures also have occurred. In opposition to this, APLEY [1] found no higher proportion of epileptiform or other abnormal EEG-patterns in a group of children with abdominal pains as contrasted to a control group. His EEG findings contain, however, no satisfactory details as to localization or electromorphologic features. APPLEY came to the conclusion that there was hardly ever any connection to be found between epilepsy and recurrent abdominal pain. He regarded the majority of his patients as psychiatric cases and achieved success with psychotherapy. This would seem to suggest that APPLEY included in his material all patients with recurrent abdominal syndrome without any attempt at closer identification of the origin.

As to the pathophysiologic significance of the rolandic, vertex and antero-frontal spikes registered by us, having analysed over 4000 recordings made under hexobarbital anaesthesia we may safely claim that rolandic or vertex spikes are hardly ever encountered in adults who never had focal sensory, motor or sensorimotor seizures. On the other hand, the epileptogenic foci of the central areas rarely cause partial seizures associated with abdominal sensations (Table II). Again, in the syndrome of abdominal pain, sensory or motor paroxysmal elements are usually lacking (Table I). Discharge of minor epileptogenic lesions of the frontal pole may occasionally generate initial signs of abdominal excitation, and in seizures originating from discrete epileptogenic foci within the cortical zone making up the walls of the longitudinal fissure signs of abdominal, genital or other visceral excitation are common but they are usually associated with tonic and postural motor phenomena [18].

Apart from a few cases, the seizures occurring in our material were of a visceral autonomic type. It is, therefore, most unlikely that the interictal negative spikes appearing over the central areas and the vertex should have the specific somatosensory or somatomotor cortical nerve cells as their site of origin. On the other hand, the sensorimotor cortical areas may well contain non-specific structures emitting stimulatory impulses transmitted through the hypothalamic route to the gastrointestinal musculature; occasional observation of borborygmi through the abdominal wall during a crise of abdominal pain is suggestive of such a mechanism. The rolandic and vertex spikes may signify an abnormal excitability to the degree of focal activity of these non-specific structures.

In animals, the somatosensory cortex has been shown to contain splanchnic projection areas [2, 4, 5]. There is evidence suggesting that the sensorimotor cortex may influence gastrointestinal motility through the reticular formation [9]. The sensorimotor cortex belongs to those cortical areas which have been shown by FRENCH et al. [10]. to send projections to the reticular systems of the brain stem. The stimulatory effect is no conclusive evidence of any involvement of these specific cortical areas in gastrointestinal regulation. This seems to be in line with the fact that PENFIELD [21] failed to elicit abdominal visceral reactions in epileptic subjects by electrostimulation of the central cortical surface. "Seizures in which the manifestations are limited to the alimentary system are quite rare . . . The tissue abnormality and the actual electrographic abnormality, as well as the stimulation focus, seem to be most often in the cortex of the insula . . . fusiform gyrus, hippocampal gyrus, amygdaloid nucleus, and uncus . . . as well as in the circuminsular cortex."

ELIASSON [9] does not, however, reject the possibility of their being afferent as well as efferent pathways to the alimentary tract through hypothalamic transmission. DELL and OLSON [6] demonstrated vagal projections to the amygdaloid region in animals; this might account for the occurrence of an unilateral basotemporal focus associated with epileptiform abdominal crises.

In the last decade the "rolandic spikes" have been studied by numerous authors. VIZIOLI [27] gives a brief summary of the subjects, stressing that this finding is interpreted as the expression of "a reactive feature of the central regions peculiar to childhood or to a certain maturation stage". In the child, the pre-rolandic regions are preferential structures which may display an unusually high irritability. The rolandic spikes "should be considered as the interictal equivalent of the morpheic attacks of the child usually benign ones . . . EEG alterations, in fact, will often last many years after clinical recovery has occurred."

It emerges from the present studies as well as from other investigations outlined in the foregoing that the central and vertex spike activity during

spontaneous or induced sleep studied by us as well as the 14—6 c/sec positive spike pattern described by KELLAWAY, CRAWLEY and KAGAWA [17] belong to the characteristic biophysical features of the abdominal pain syndrome. The rolandic and vertex spikes reflect in all likelihood the convulsoid hyperexcitability of the non-specific visceromotor cerebro-cortical structures while runs of positive spikes at 14—6 c/sec are a sign of excessive hypothalamic irritability. This hyperexcitability may be responsible for the recurrent abdominal syndrome which also greatly depends on age. LITTLE and BEVILACQUA [20] were able to show that a 14—6 c/sec positive spike dysrhythmia was associated with the compression caused by a tumour in the region of the hypothalamus and ventral midbrain.

It must be stressed that among the above quoted authors it was only KENNEDY [18] who demonstrated conclusively the connection between vertex spikes and epileptogenic foci responsible for the excitatory symptoms arising in the alimentary tract, while VIZIOLI merely pointed to the possibility of such a connection.

The history was suggestive of cerebral injury during delivery or due to other causes, or of encephalitis in 54 per cent of the cases of SCHADE and GOFMAN [23], in 37 per cent of those of KELLAWAY et al. [17], in 26 per cent of those of SHEEHY et al. [24] and in 34 per cent in our own material.

Though a characteristic interictal spike activity was demonstrable in the overwhelming majority of our cases, and response to anticonvulsants (phenytoin, mephenytoin, barbiturates) was on the whole satisfactory, we nevertheless prefer to speak of a "syndrome of periodic abdominal pain" owing to its benign character, instead of using the term "abdominal epilepsy". We reserve this latter term to cases where focal sensorimotor seizure elements precede or join the predominant abdominal pain, or, apart from visceral crises generalized seizures have occurred at an early age, or when the attacks continue after sexual maturity has been reached.

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ÜBER DIE AKUTE WIRKUNG DES NIKOTINS AUF DEN EXTREMITÄTENKREISLAUF

Von

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I. MEDIZINISCHE KLINIK (DIREKTOR: PROF. DR. I. MAGYAR) DER MEDIZINISCHEN UNIVERSITÄT BUDAPEST

(Eingegangen am 9. März 1966)

Die auf i. v. Verabfolgung von 1,0 mg Nikotintartarat zustandekommenden Kreislaufveränderungen wurden an 21 Kranken, bei denen kompensierte Herztätigkeit und keine periphere Gefäßveränderungen vorlagen, untersucht. Die Extremitätendurchblutung wurde in 12 Fällen mit der Stickoxydul-Methode und in 9 Fällen mit der sich zur Messung der gesamten Extremitätendurchblutung eignenden venösen Isotopen-Dilutionsmethode bestimmt. Ergänzungshalber wurden Haut- und Muskeltemperatur registriert und die radioaktive „Muskel-Clearance" bestimmt. Während die akute Nikotinwirkung eine mäßige Erhöhung des Minutenvolumens und eine bedeutende Abnahme der Hauttemperatur zur Folge hatte, blieben die Muskeltemperatur- und Muskel-Clearance-Werte (die Zeitdauer, in der das ^{131}J aus dem Muskel verschwindet) unverändert. Extremitätendurchblutung, Gefäßwiderstand und Minutenvolumenfraktion der Extremitäten wiesen keine signifikante Veränderungen auf.

Nach Nikotinverabfolgung verminderte sich die Sauerstoffaufnahme der Extremitäten bedeutend und der Venendruck in der unteren Extremität stieg an. In diesem Zusammenhang wird der Pathomechanismus der durch Nikotinverabfolgung bedingten Extremitätendurchblutungsveränderungen erörtert.

Während die pathogenetische Wirkung des Rauchens auf die mit peripheren Gefäßveränderungen einhergehenden Erkrankungen allgemein bekannt ist, sind die Ansichten bezüglich der Wirkung des Nikotins auf den Extremitätenkreislauf und auf den Stoffwechsel nicht einheitlich. Ein Teil der Verfasser vertritt die Meinung, daß das Nikotin die Gefäßdurchblutung akut vermindert, andere behaupten dagegen, daß es den peripheren Widerstand nicht beeinflußt.

In vorliegender Arbeit wurde der auf den Extremitätenkreislauf und auf den Stoffwechsel ausgeübte akute Effekt des Nikotins untersucht.

Methodik

Vorversuch. Die akute Nikotinwirkung wurde an 8 Hunden (12—15 kg wiegende Bastarde beiderlei Geschlechts) untersucht. Den Tieren wurde 0,50 mg Nikotintartarat intravenös verabfolgt. Die Durchblutungsveränderungen der A. carotis communis, des Ramus descendens der linken Koronararterie und der A. iliaca externa wurden mit Hilfe eines Schwimmrotameters registriert. Der Blutdruck in der A. femoralis wurde mit einem Quecksilbermanometer fortlaufend gemessen.

Die weiteren Untersuchungen wurden an 21 Patienten vorgenommen und zwar handelte es sich dabei um Kranken mit kompensierten Herzzustand, bei denen keine periphere Gefäßverengung vorlag; die Patienten waren zum Teil Raucher.

Im ersten Teil dieser Experimente (12 Kranken) wurde zwecks Registrierung des Extremitätenkreislaufs das Stickoxydul-Prinzip angewandt. Um die Haut- und Muskeldurchblu-

tungsveränderungen feststellen zu können, wurden einerseits die Temperaturveränderungen der Haut und der Muskeln (im Gebiet des M. quadriceps femoris) registriert anderseits die KETYsche „Muskel-Clearance" (Bestimmung der Zeitperiode, in der das $Na^{131}J$ vom Gebiet des M. quadriceps femoris verschwindet) bestimmt. Die Methode wurde vorangehend ausführlich bekanntgegeben [1].

Im zweiten Teil der Experimente (9 Kranken) wurden die Veränderungen der Minutenvolumenfraktion und der Gesamtdurchblutung der Extremitäten untersucht. In dieser Versuchsserie kam zwecks Bestimmung der Gesamtdurchblutung der Extremitäten das von uns bearbeitete venöse Isotopen-Dilutionsverfahren zur Anwendung [2]. Das Minutenvolumen wurde nach Evans-Blau-Verabfolgung anhand des HAMILTON-STEWARTschen Prinzips bestimmt. Zur fortlaufenden Messung des Venendrucks der unteren Extremitäten diente ein MORITZ-TABORAscher-Apparat. Der Sauerstoffgehalt im Blut der A. und V. femoralis wurde mit dem KIPPschen Oxymeter bestimmt. Die statistischen Analysen wurden mit dem STUDENTschen t-Verfahren ermittelt.

Tabelle I

Wirkung von Nikotin auf das Gefäßgebiet der A. carotis, A. coronaria und A. iliaca
(Hundeexperimente)

	P (mmHg)	Blutströmung (ml/min)		
		in der A. carotis comm. (ml/min)	im R. descendens A. coronariae (ml/min)	in der A. iliaca ext. (ml/min)
Nr.	8	8	8	8
A	110 / 80—130	106 / 70—150	70 / 50—95	51 / 30—95
B	120 / 90—150	115 / 70—170	68 / 50—90	59 / 35—100
C	120 / 85—150	110 / 75—150	75 / 60—100	55 / 40—95

A = Vorperiode
B = 5 Min nach i. v. Verabreichung von 0.5 mg Nikotintartarat
C = 20 Min nach i. v. Verabreichung von 0.5 mg Nikotintartarat

Ergebnisse

Die Ergebnisse der an 12 Kranken mit der Stickoxydul-Methode durchgeführten Untersuchungen bezüglich der Extremitätendurchblutung sowie die Haut- und Muskeltemperatur und die Muskel-Clearance veranschaulicht Tabelle II. Wie ersichtlich, waren 10 Minuten nach der intravenösen Verabfolgung von 1,0 mg Nikotintartarat signifikante Veränderungen festzustellen: Während sich die Sauerstoffaufnahme der Extremitäten und die Hauttemperatur in beudeutendem Maße verminderte, stieg der Druck der V. femoralis an. Durchblutung und Widerstand der Extremitäten wiesen keine statistisch signifikante Veränderungen auf. Unter den Parametern der Muskeldurchblutung blieben auf Nikotinwirkung sowohl Muskelclearance wie Muskeltemperatur unverändert. Blutdruck und Minutenvolumen stiegen — wie das voraus-

zusehen war — nach der Nikotinverabfolgung im allgemeinen an. In Tabelle III haben wir die Resultate der mit unserer Isotopen-Dilutionsmethode durchgeführten Untersuchungen angeführt. Wie ersichtlich, gestalteten sich die Ergebnisse ähnlich wie bei der ersten Versuchsserie (Stickoxydulmethode). Die akute Nikotinwirkung hatte keine wesentliche Änderung der Gesamtdurchblutung der unteren Extremitäten zur Folge: Parallel mit dem Anstieg des Blutdrucks und des Minutenvolumens waren in allgemeinen etwas erhöhte Durchblutungswerte zu verzeichnen. Sowohl der vaskuläre Widerstand der unteren Extremität als auch das zwischen dem Minutenvolumen und der Extremitätendurchblutung bestehende Verhältnis — die Minutenvolumenfraktion der Extremitätendurchblutung — blieben im wesentlichen unverändert Die Verminderung der Sauerstoffaufnahme und den Anstieg des venösen Drucks der Extremitäten konnten wir auch in dieser Versuchsserie beobachten (die beiden letzterwähnten Veränderungen waren signifikant: Venendruckanstieg: $p < 0{,}1\%$, Verminderung der Sauerstoffaufnahme: $p < 1\%$).

Besprechung

Die Resultate der sich mit der Einwirkung des Nikotins auf den peripheren Kreislauf befassenden Untersuchungen sind stark abweichend. Nach Maddock und Collier [3], Lampson [4], Rottenstein und Mitarb. [5] sowie Burch und DePasquale [6] vermindert sich die Extremitätendurchblutung auf akute Nikotinwirkung. Die Experimente von Rappaport und Mitarb. [7] sowie Strombald [8] ergaben, daß das Nikotin — vornehmlich durch Vermittlung des sympathischen Nervensystems — periphere Gefäßverengung verursacht. Hines [9] und Burn [10] vertraten die Ansicht, daß das auf Nikotinwirkung aus der Gefäßwand freiwerdende Noradrenalin und Adrenalin in den Haut- und Muskelgefäßen eine Vasokonstriktion herbeiführe. Barker [11] sowie Roth und Sheard [12] fanden, daß nach Nikotinverabfolgung die Hauttemperatur abnimmt. Freund und Ward [13] sowie Abramson und Mitarb. [14] nahmen an, daß auf Wirkung von Nikotin in der Haut Vasokonstriktion entsteht, während die Gesamtdurchblutung der Extremitäten unverändert bleibt. Nach den Untersuchungen von Rueff und Mitarb. [15], Coffman und Javett [16] sowie Bremer [17] bewirkt das Nikotin in den Hautgefäßen der Extremitäten zwar eine Vasokonstriktion, da es jedoch gleichzeitig die Muskelgefäße erweitert, bleibt die Extremitätendurchblutung unverändert.

Unsere Experimente unterstützen die Annahme, daß das Nikotin lediglich die Vasokonstriktion der Hautgefäße herbeiführt und den peripheren Kreislauf nicht beeinflußt. Laut unserer, orientierungshalber durchgeführten Vorversuche verursacht das Nikotin auf dem Gefäßgebiet der A. femoralis keine Durchblutungsveränderungen. Die an 12 Kranken mit der Stickoxydul-

Tabelle II

Wirkung von Nikotin auf den
(Ergebnisse der Extremitätenkreislaufmessungen

Nr.	Name, Alter (Jahre), Geschlecht, Diagnose	Periode	Blutdruck mmHg	CO 1/min	MBF ml(min) 100 g Gewebe
1.	A. T. 40 ♂ Dystrophia musc. progr.	A	150/80	—	6,5
		B	150/95	—	9,0
2.*	S. P. 39 ♀ Dystrophia musc. progr.	A	180/110	3,1	5,4
		B	150/90	3,4	4,9
3.*	Sz. B. 61 ♂ Atherosklerose	A	160/100	4,4	8,9
		B	160/90	4,8	8,0
4.	M. F. 44 ♀ Neurose	A	130/85	—	10,0
		B	140/95	—	9,6
5.*	T. T. 28 ♂ Duodenalgeschwür	A	120/80	5,6	11,0
		B	125/80	5,9	10,6
6.	J. M. 36 ♂ Magengeschwür	A	125/80	—	8,0
		B	135/80	—	7,6
7.	K. L. 29 ♂ Colitis	A	100/80	—	8,8
		B	120/85	5,0	8,0
8.*	R. É. 34 ♀ Neurose	A	115/85	5,0	7,0
		B	120/90	7,4	7,5
9.	V. I. 54 ♂ Hypertonie	A	170/110	5,0	9,0
		B	180/110	5,9	8,2
10.	E. J. 49 ♀ Klimax	A	140/80	—	8,1
		B	140/80	5,4	7,9
11.*	D. I. 40 ♂ Hypertonie	A	180/100	—	7,6
		B	190/110	6,0	8,0
12.*	Cs. K. 41 ♀ Colitis	A	130/85	5,0	8,4
		B	140/85	6,0	7,9

Perioden: A = vor dem Experiment
B = nach Nikotininjektion (1.0 mg Nikotintartarat)
CO = Minutenvolumen l/min
MBF = Extremitätendurchblutung ml/min/100 g Gewebe

Extremitätenkreislauf
mit der Stickoxydul-Methode)

MVR	Sauerstoff-Verbrauch der Extremität (ml/min)	^{131}J-Halbierungszeit (sec)	Hauttemperatur C°	Muskeltemperatur C°	Druck in der V. femoralis mmH_2O
15,8	—	16	22,0	32,0	90
12,6	—	15	18,5	33,0	95
23,4	0,21	15	22,4	30,4	180
22,5	0,19	15	17,8	29,9	200
13,4	0,50	11	25,4	33,4	25
14,2	0,52	12	17,5	32,8	70
10,0	0,30	12	25,5	33,0	45
11,4	0,24	12	19,0	32,0	65
8,4	0,38	13	24,4	31,0	50
8,9	0,30	11	17,3	31,5	66
11,8	0,32	—	—	—	60
13,0	0,33	—	—	—	80
10,1	0,40	14	25,0	30,8	55
10,2	0,31	14	17,0	30,0	75
13,6	0,44	—	23,4	29,8	—
13,1	0,38	—	16,4	30,4	—
14,4	0,35	13	25,0	31,0	60
16,2	0,32	14	17,5	30,0	82
12,3	0,32	—	24,5	33,0	80
12,6	0,36	—	18,0	31,0	92
16,6	0,28	15	24,8	31,0	90
17,1	0,25	16	17,8	30,0	98
11,8	0,30	13	25,0	31,8	80
13,1	0,26	13	18,0	30,4	98

MVR = Gefäßwiderstand in den Extremitäten (arterieller Mitteldruck/Extremitätendurchblutung)
Sauerstoffverbrauch in den Extremitäten: Sauerstoff ml/min/100 g Gewebe
* = Raucher.

Tabelle III

Akute Nikotinwirkung auf Blutkreislauf und Minutenvolumenfraktion der Extremitäten
(Venöse Isotopen-Dilutionsmethode)

Nr.	Name, Alter (Jahre), Geschlecht, Diagnose	Periode	Blutdruck mmHg	CO l/min	TPR dyn.sec.cm^{-5}	LBF ml/min	LVR dyn.sec.cm^{-5}	LO_2C ml/min	V_f mmH_2O	LCOF %
1.*	G. H. 48 ♂ Extremitäten-varikosität	A	140/80	5,3	1,13×1332	360	16,6×1332	15,0	80	6,7
		B	150/90	6,0	1,10×1332	400	16,5×1332	12,0	90	6,6
2.	E. M. 26 ♀ Neurose	A	130/85	6,0	1,00×1332	450	13,3×1332	18,0	75	7,5
		B	140/95	6,0	1,10×1332	480	13,7×1332	16,0	80	8,0
3.*	I. T. 36 ♂ Hypertonie	A	160/100	5,0	1,42×1332	350	22,5×1332	14,0	88	7,0
		B	160/100	5,8	1,24×1332	330	21,7×1332	11,8	98	5,7
4.	M. N. 34 ♀ Hypertonie	A	170/110	4,9	1,59×1332	380	20,5×1332	16,0	72	7,8
		B	180/120	5,8	1,45×1332	410	20,5×1332	13,5	80	7,0
5.*	K. I. 41 ♀ Extremitäten-varikosität	A	135/80	4,6	1,28×1332	300	19,6×1332	12,0	75	6,6
		B	150/100	5,0	1,40×1332	340	20,5×1332	10,5	65	6,7
6.*	T. T. 50 ♀ Klimax	A	140/80	4,5	1,33×1332	330	18,2×1332	15,0	84	7,6
		B	160/100	5,2	1,38×1332	360	20,0×1332	15,0	90	6,9

7.	P. Z. 48 ♂ Extremitäten-varikosität	A	140/80	5,5	1,09×1332	360	16,4×1332	20,0	78	6,5
		B	140/80	5,9	1,01×1332	360	16,4×1332	17,5	85	6,1
8.	S. J. 55 ♂ Hypertonie	A	160/100	4,9	1,47×1332	380	18,9 ×1332	19,0	74	7,7
		B	190/130	5,9	1,52×1332	450	17,0×1332	17,0	88	7,6
9.*	Zs. H. 50 ♀ Extremitäten-varikosität	A	130/85	4,0	1,50×1332	310	19,3×1332	12,0	100	7,7
		B	150/90	4,5	1,47×1332	335	19,6×1332	10,0	110	7,6

TPR = Totalgefäßwiderstand im großen Kreislauf
LVR = Totalgefäßwiderstand in der Extremität
LCOF = Minutenvolumenfraktion in einer Extremität
LBF = Totaldurchblutung in der Extremität
V_f = Druck in der V. femoralis (auf die rechte. Vorhofhöhe gerechnet)
LO_2C = Totale Sauerstoffaufnahme in der Extremität
A : vor dem Experiment
Perioden:
B : nach Nikotininjektion (1.0 mg Nikotintartarat).

methode durchgeführten Untersuchungen ergaben ebenfalls, daß nach Nikotinverabfolgung sowohl die Durchblutung als auch die vaskuläre Resistenz der Extremitäten im wesentlichen unverändert blieben. Die gleichzeitig beobachtbare, auf Nikotinwirkung zustandekommende Abnahme der Hauttemperatur weist auf die Vasokonstriktion der Hautgefäße hin. Im Gegensatz zur Hautdurchblutung blieb die Muskeldurchblutung unter akuter Nikotinwirkung unverändert (nach der Nikotinverabfolgung änderte sich weder die Muskeltemperatur noch die Muskel-Clearance). Unsere mit der Isotopen-Dilutionsmethode an 9 Kranken durchgeführten Experimente führten zu ähnlichen Resultaten. Das Nikotin bewirkte weder die Verminderung der Extremitätendurchblutung noch den Anstieg des Gefäßwiderstandes der unteren Extremitäten, noch die Veränderung der Minutenvolumenfraktion der Extremitätendurchblutung. In beiden Versuchsserien (Stickstoffoxydul-Gruppe bzw. Isotopen-Dilutions-Gruppe) waren nach Nikotinverabreichung zwei konstante Veränderungen zu beobachten: Verminderung des Sauerstoffverbrauchs der Extremitäten und Anstieg des Drucks in der V. femoralis.

Die Ursache der Verminderung des Sauerstoffverbrauchs der Extremitäten kennen wir nicht. Es kann angenommen werden, daß sich infolge der Vasokonstriktion der Hautgefäße die Sauerstoffaufnahme der Haut vermindert. Die Steigerung des venösen Drucks der unteren Extremität kann mit der den venösen Tonus steigernden Wirkung des Nikotins erklärt werden; nach unseren an isolierten Venenabschnitten durchgeführten Untersuchungen [18] entsteht nach Nikotinverabfolgung Venokonstriktion.

Bekanntlich erhöhen sich nach Nikotinzufuhr das Minutenvolumen und der Blutdruck. Für den Umstand, daß sich die Extremitätendurchblutung, trotz der in den Hautgefäßen entstandener Vasokonstriktion nicht vermindert, können eventuell die erwähnten Erscheinungen verantwortlich sein.

Aus den Ergebnissen der akuten Versuche können und dürfen bezüglich der Entwicklung der durch Rauchen verursachten peripheren Kreislaufstörungen keine entscheidenden Folgerungen gezogen werden. Die akuten Nikotinversuche sprechen jedenfalls dafür, daß — mit Ausnahme der Vasokonstriktion der Hautgefäße — die Extremitätendurchblutung keinen Schaden erleidet und der periphere vaskuläre Widerstand nicht ansteigt. Unsere Untersuchungen bei einer großen Anzahl von Kranken — bei denen keine periphere Gefäßverengung vorlag — ergaben, daß zwischen den Extremitätendurchblutungswerten von Rauchern und Nichtrauchern kein signifikanter Unterschied besteht. Obwohl diese Angaben allein keinen entscheidenden Beweis darstellen, unterstützen sie unzweifelhaft die neustens immer ausdrücklicher betonte Auffassung, nach der die durch das Rauchen bedingten peripheren vaskulären Schädigungen bei empfindlichen Personen vornehmlich durch allergische Mechanismen und nicht durch sich wiederholende periphere Kreislaufverschlechterung zustandekommen.

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THE ROLE OF THE CENTRAL NERVOUS SYSTEM IN THE PATHOMECHANISM OF EPINEPHRINE-INDUCED PULMONARY OEDEMA

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The part played by neurogenic factors in epinephrine-induced pulmonary oedema has been investigated. In 41 crossed circulation experiments, epinephrine was administered partly into the circulation of the head, partly into that of the trunk. Intracranial administration of epinephrine caused pulmonary oedema of a more severe degree and in a higher percentage of animals than by the other route. The mechanism involved is believed to be neurohaemodynamic, owing to the increase in the values of pulmonary pressure and the slight reduction in colloid osmotic pressure in the course of the experiments. Capillary permeability showed a slight increase.

Epinephrine is the most widely used chemical agent for the experimental production of pulmonary oedema. Though nearly half a century has elapsed since the fundamental experiments of AUER and GATES [1], the manner in which the drug induces oedema has remained a controversial issue. According to one of the current views, the mechanism is peripheral and left heart failure would be due to the hypertension caused by the increase of peripheral resistance [2, 3]. Recently, however, the claim that this mechanism takes effect through the agency of the central nervous system, has been receiving increasing support [4, 5, 6 7].

The present studies had the aim to clarify the questions

1. whether, as concerns the induction of pulmonary oedema, the site of action of epinephrine is central or peripheral; and,

2. if it is central, to ascertain the changes

(a) in the haemodynamic conditions;

(b) in capillary permeability.

Method

The method of crossed circulation seemed to be best suited for our studies [8]. In the acceptor dog, the circulation of the head was completely isolated from that of the trunk, in a manner that the vertebral arteries were ligated at the neck, this was followed, after previous laminectomy, by ligation of the venous plexus running in front of the spinal cord. Cranial blood supply was provided from the donor dog by carotid-to-carotid, and jugular-to-jugular anastomoses. The acceptor's head was thus completely isolated from his trunk in respect to blood circulation, the nervous connections were, however, spared. Complete isolation was checked by repeated dye tests.

The experiments were carried out in dogs of both sexes under chloralose anaesthesia. Blood clotting was inhibited by 500 IU/kg of heparin. 57.0 μg/kg/min epinephrine + 2 ml/kg/min physiological saline were infused during 35 minutes into the cephalic circulation in 28, and into that of the trunk in 13 instances. The use of such large doses was due to the failure of lower ones to elicit pulmonary oedema in preliminary experiments. Similar doses have been used by other investigators [5, 7, 9, 10]. Infusion into the acceptor's head was done either directly through the carotid, or through the donor's trunk circulation. Control experiments with physiological saline were carried out in four instances. Blood pressure was recorded in both dogs by means of a mercury manometer. Left intraatrial pressure was measured with water manometer, the atrial lumen having been reached by transbronchial puncture. Pulmonary capillary pressure was measured by means of a wedged catheter introduced by the transjugular route under X-ray control.

The lungs of the animals were removed immediately after death and the ratio of lung weight per total body weight was determined. This is, according to recorded evidence, around 1.0, the upper limit of the normal range being 1.25 [11]. Specimens of the lung were submitted to histological study.

Colloid osmotic pressure of the plasma was calculated on the basis of MEYER'S formula [12], protein in blood serum and in oedema fluid was determined by the biuret method, the serum protein pattern by electrophoresis. For estimation of serum sodium, potassium, and calcium levels a Zeiss-type flame photometer was used.

Results

In 28 out of 41 experiments involving crossed circulation, epinephrine was introduced into the circulation of the head, in 13 into that of the trunk.

In case of intracranial perfusion, the lung weight per total body weight index of the acceptor dogs averaged 1.62, which is highly significant (p = 0.5 per cent) as opposed to normal dogs; that of the donors was 1.32 (p = 15 per cent) (Fig. 1).

The lung weight per total body weight index was outside the normal range in 61 per cent of the acceptors, i.e. 17 dogs as against 32 per cent of the donors, i.e. 9 dogs.

In case of epinephrine-perfusion into the trunk circulation, the lung weight per total body weight index averaged 1.35 (p = 20 per cent) as against 1.16 (normal values) in the donors. The lung weight per total body weight index was elevated in 54 per cent of the acceptors, i.e. 7 dogs, as against 23 per cent of the donors, i.e. 3 dogs.

In 10 instances (36 per cent) of intracranial infusion of epinephrine, pulmonary oedema was of such severity that oedema fluid was flowing from the tracheal cannula. This never occurred when epinephrine had been administered into the trunk circulation.

We also studied the haemodynamic conditions prevailing in the trunk under the influence of intracranial epinephrine infusion (Fig. 2).

Mean arterial pressure remained essentially unchanged during the experiment, the initial value of 129 mm Hg rising successively to 134 mm Hg. Left intraatrial pressure (10 tests) averaged 3.8 mm Hg prior to epinephrine perfusion, and 20.7 mm Hg during perfusion. Pulmonary capillary pressure rose from the initial value of 4.7 mm Hg to 19.8 mm Hg during perfusion (4 tests).

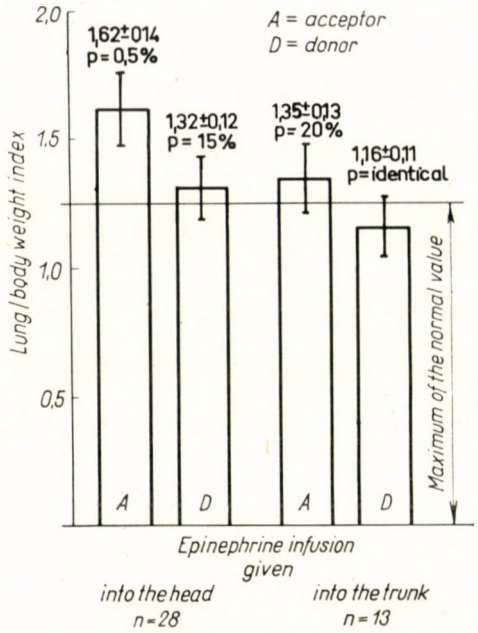

Fig. 1. Influence of epinephrine perfusion into the circulation of the head and of the trunk, respectively, on lung weight per total body weight index

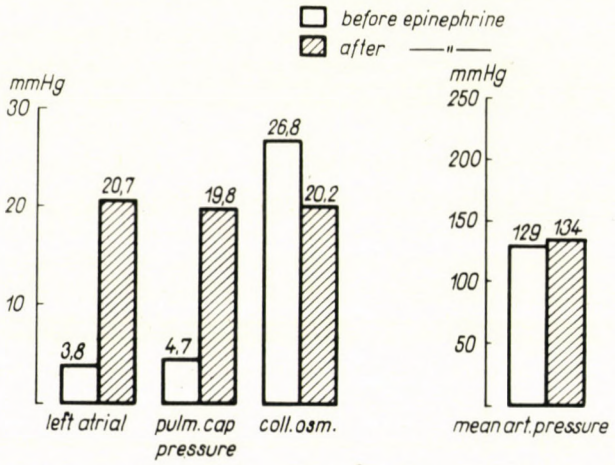

Fig. 2. Influence of intracranial perfusion of epinephrine on haemodynamics and colloid osmotic pressure in the acceptor's trunk

Colloid osmotic pressure was investigated in five instances. By the end of the experiment, the average osmotic pressure in the acceptors showed a slight reduction (from 26.8 mm Hg to 20.2 mm Hg). Mean arterial pressure in the donor dogs averaged 120 mm Hg at the start of the experiment, and 222 mm Hg upon the effect of epinephrine. Reduction of the osmotic pressure

from 29.3 mm Hg to 7.7 mm Hg in the donors was consistent with the volume of infused fluid.

The serum sodium, potassium, and calcium levels in the acceptors remained practically unchanged during the entire experiment. In the donors the sodium level showed a slight increase (from 146.0 mEq per litre to 155.0 mEq per litre) while the calcium level fell from 4.5 mEq per litre to 2.4 mEq per litre and the potassium level remained unchanged (5.0 against 4.8 mEq per litre). Serum total protein showed a slight reduction in the acceptor (from 5.5 g per 100 ml to 4.8 g per 100 ml), while in the donor it fell by 50 per cent as did calcium (from 5.3 g per 100 ml to 2.5 g per 100 ml). Protein concentration in the oedema fluid was relatively high, i.e. 4.5 per cent, and the fractions were of the same pattern as in the blood serum.

Control experiments with 2 ml/kg/min physiological saline infused intracranially failed to induce pulmonary oedema in none of the four instances. The lung weight per total body weight index averaged 1.1 in the donors and 1.0 in the acceptors, both values were within the normal range. In one instance the animals were alive by the end of 105 minutes and were sacrificed by bleeding.

Discussion

The existence of a neurogenic pulmonary oedema is a well-documented fact [6, 7, 13, 14], and there are data pointing to the neurogenic nature of the epinephrine-induced oedema, too. CASSEN et al. [5] failed to induce pulmonary oedema after spinal transsection in rats with the same doses of epinephrine which had invariably led to pulmonary oedema in the controls. Massive doses of epinephrine, even in the lethal range, do not cause pulmonary oedema in the cat, except when injected into the hypothalamic region. GLASS et al. [7] could influence the behaviour of epinephrine-induced pulmonary oedema by exstirpation or puncture of certain cerebral regions, furthermore by transsection of the sympathetic below the first cervical ganglion. Other investigators [15] were able to avert epinephrine-induced pulmonary oedema by decerebration or decortication. LUISADA [16] established crossed circulation between rabbit and dog, being thus able to produce under such conditions pulmonary oedema by the intracerebral administration of epinephrine. This evidence was, however, subsequently disproved by the author himself [17], because isolation of the circulation of the head from that of the trunk had not been complete, and second, because it was possible to induce pulmonary oedema by rapid intracarotid injection of physiologic saline as well. Both these sources of error have been eliminated from the present experiments, complete isolation having been ascertained by dye tests, and having failed to produce pulmonary oedema by identical amounts of physiological saline given under the same experimental conditions.

Two facts emerging from the present investigations may be considered evidence in favour of a neurogenic mechanism of epinephrine-induced pulmonary oedema. The first is the significant rise of the average value of the pulmonary index in the acceptor dogs as opposed to the controls after intracranial perfusion of epinephrine in 28 instances, and the second, the onset of fulminating pulmonary oedema in about one third of the cases.

SARNOFF and BERGLUND [6] have produced hypertension in both the systemic and the pulmonary circulation by intracysternal administration of fibrin [6]. Pulmonary oedema induced in this manner has been termed "neurohaemodynamic" by these authors. Further studies into the possible haemodynamic changes in the circulation of the trunk were carried out in order to decide whether epinephrine-induced pulmonary oedema is "haemodynamic" in this respect. A nearly 100 per cent increase in the blood pressure of the donor dogs was registered during the procedure, in contrast to that of the acceptors which remained unchanged. Left atrial pressure showed a very substantial rise in the acceptors, parallel with the pulmonary capillary pressure which was found to multiply its original value.

Colloid osmotic pressure diminished significantly in the donors, while only slightly in the acceptors. The changes in pulmonary capillary and colloid osmotic pressures thus provided conditions favouring the production of pulmonary oedema. Actually, the absolute values for the two pressures are very close to one another.

A significant reduction of serum proteins was found in the donors in consequence of the saline perfusions, while reduction in the acceptors was slight. Electrolyte concentrations remained practically unchanged in the acceptors. The lowering of the calcium level in the donors was related to dilution by the perfusion fluid, while the rise in the sodium level was due to the administration of saline.

The excess serum potassium in the donors takes its origin, in all likelihood, from the extravasal compartment.

The protein content of the oedema fluid was as high as 4.5 g per cent, suggesting an increased capillary permeability. In agreement with the findings of FREEMAN and JOEKES [18] the totality of protein fractions was demonstrable in the oedema fluid.

The foregoing results suggest that epinephrine-induced pulmonary oedema is neurohaemodynamic in origin, while the increased capillary permeability is thought to be of minor significance. LUISADA's claim [16] that epinephrine causes pulmonary oedema by affecting the regulatory centres of pulmonary circulation and of capillary permeability in the brain, is supported by the present findings.

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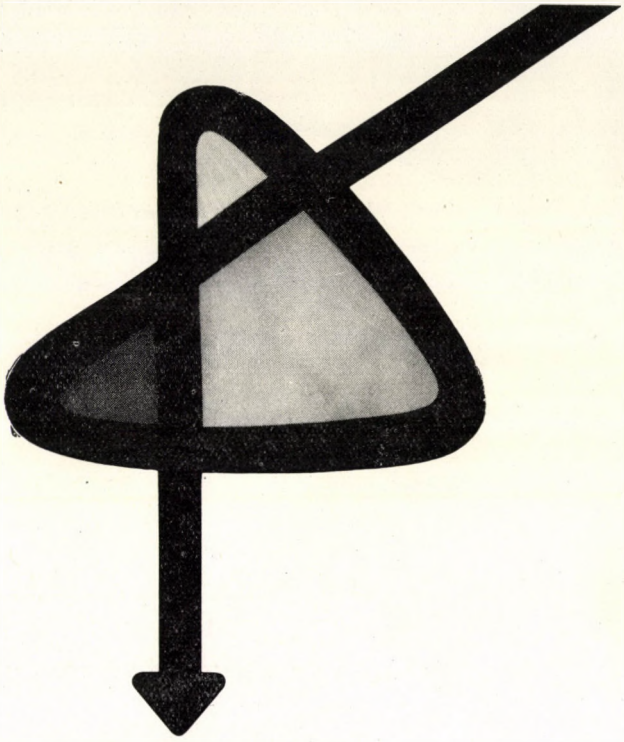

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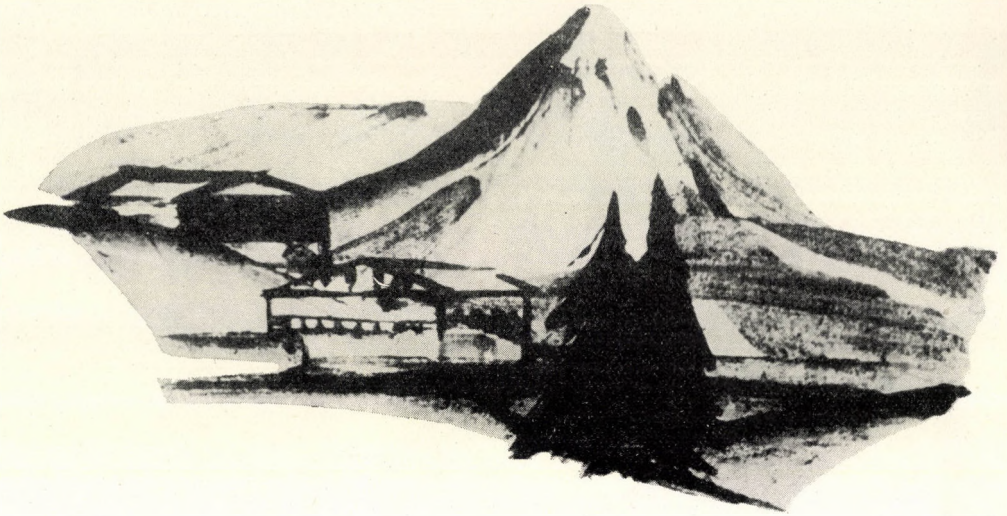

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РЕЗЮМЕ

СИНДРОМ ГЕМОРРАГИЧЕСКОЙ ТРОМБОЦИТЕМИИ (КОАГУЛОПАТИЯ ИЛИ ПЕРВИЧНОЕ РАССТРОЙСТВО ГЕМОСТАЗА, ВЫЗВАННОЕ ТРОМБОЦИТАМИ)

К. РАК, Л. ЛАКАТОШ и Р. САБО

У двух больных синдромом геморрагической тромбоцитемии авторы подробно исследовали условия свертываемости крови и у четырех больных также тромбоциты. Число тромбоцитов было у всех четырех больных выше 1 000 000, время кровотечения было удлиненным или достигло верхнего предела нормы. В одном случае результат генерационного теста тромбопластином (ГТТ) указал на расстройство плазматической профазы, вернее, на изменение, указавшее на уменьшение РТА. Компонент тромбоцитов больных оказал в случае высокой концентрации тромбоцитов тормозящее действие на ГТТ. Согласно исследованию «адгезии тромбоцитов in vivo» по Борхгревнику в строении тромбоцитного тромба (platelet plug) участвовало количество тромбоцитов, превышающее их количество в норме.

Среди миелопролиферативных синдромов можно клинически выделить синдром геморрагической тромбоцитемии, однако, его патогенетическое разделение необосновано. Подытоживаются диагностические критерии синдрома.

По общераспространенному мнению патологически повышенное количество, и, пожалуй, также патологически измененное качество тромбоцитов вызывают склонность к кровотечениям путем расстройства свертываемости крови. На основании литературных данных и результатов исследования наблюдавшихся ими случаев авторы придерживаются того мнения, что при геморрагической тромбоцитемии патологическим является первичный гемостаз, а именно его вторая фаза, необратимая аггрегация тромбоцитов (вязкий метаморфоз и контракция тромбоцитов), и в силу этого не образуется непроницаемого для крови «platelet plug». Подробный механизм действия еще невыяснен, но полагается, что по какой-то причине не проявляется действия тромбина, необходимого для процесса. На этом основании можно объяснить удлиненное время кровотечения, а также возникновение клинически наблюдаемой склонности к кровотечению. Дефект свертываемости плазмы, имеющей решающее значение во вторичном остановке кровотечения, может вызвать усиление расстройства гемостаза больных тромбоцитемией.

ИЗМЕНЕНИЯ БИОЭЛЕКТРИЧЕСКОЙ АКТИВНОСТИ ГОЛОВНОГО МОЗГА ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ГИПЕРОКСИИ

И. САМ, И. НИКОЛИЧ и Дь. ГОТТЗЕГЕН

На ЭЭГ подопытных крыс, регистрированной при помощи вживленных корковых электродов после применения кислородного давления в 3,7 атм. появлялись грубые, неспецифические изменения амплитуды и частоты, а также спайки. Изменения ЭЭГ появляются раньше, чем нарушения дыхания и работы сердца, и даже раньше самой ранней фазы развития гипероксического отека легких. Кислородная насыщенность артериальной крови при появлении изменения ЭЭГ также еще нормальна. Путем связывания CO_2, выделяемого животными, и предохранением обратного вдыхания CO_2 можно замедлить развитие изменений ЭЭГ и отчасти преодолеть развитие спайков. Нарушение биоэлектрической активности головного мозга представляет собой самый ранний симптом кислородного отравления.

ПОВЕДЕНИЕ КРОВЯНОГО ДАВЛЕНИЯ И ЭКСТЕРОЦЕПТИВНОГО РЕФЛЕКСА КРОВЯНОГО ДАВЛЕНИЯ ПРИ ЛИМФОГЕННОЙ ЭНЦЕФАЛОПАТИИ

М. ФЁЛЬДИ, К. ТУРАНСКИ и Т. Э. ЗОЛТАН

1. После блокады шейного участка лимфотока наблюдаются статистически достоверные колебания кровяного давления.

2. При лимфогенной энцефалопатии после вкапывания в глаза веществ, вызывающих болей (капсаицина) наблюдается — по сравнению с нормой — более значительное повышение кровяного давления и уменьшение времени блефароспазма.

3. Авторы объясняют эти явления нарушением равновесия вегетативных центров и морфологическими изменениями структур центральной нервной системы.

ДЕЙСТВИЕ ТИМЕКТОМИИ, ГИПОФИЗЕКТОМИИ, АДРЕНАЛЕКТОМИИ И ДАЧИ КОРТИКОИДОВ НА СОДЕРЖАНИЕ ГЕПАРИНА В СЫВОРОТКЕ КРОВИ У КРЫС

К. ВАЛЛЕНТ, Й. ФАХЕТ и Э. ШТАРК

Дача формалина и гликокортикоидов вызвала значительное уменьшение количества сывороточного гепарина. Адреналектомия повысила уровень сывороточного гепарина, а тимектомия предотвратила это повышение. Удаление гипофиза в значительной мере повысило концентрацию гепарина в сыворотке крови.

Дача ДОКА повысила содержание гепарина в сыворотке. После тимектомии содержание гепарина в сыворотке уменьшилось в результате дачи гидрокортизона в меньшей степени, а в результате дачи ДОКА оно повысилось в меньшей степени, чем у животных с интактной зобной железой.

Предполагается, что функция коры надпочечников играет значительную роль в регуляции уровня сывороточного гепарина.

ИССЛЕДОВАНИЕ СЫВОРОТОЧНЫХ ЛИПОИДОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ЖЕЛТУХЕ

Т. КРЕММЕР, Э. ПОШ и Э. ФЕРЕНЦИ

Авторы выявили в опытах на животных, что после лигирования общего желчного протока в сыворотке проявляются ненормальные бета-липопротеиды. Изменения обмена липоидов и появление ненормально лабильных липопротеидов объясняются на основании данных химического анализа изолированных липопротеидов.

ДИСПАНСЕРИЗАЦИЯ ПОЧЕЧНЫХ БОЛЬНЫХ

(400 случаев)

А. ХАМОРИ, Ю. ЦИРНЕР, З. БИБОР и Л. ГОФМАН

В диспансеризации нуждаются а) больные с хроническими болезнями почек, б) больные с затяжной формой острого нефрита, у которых излечение можно ожидать лишь по истечении двух лет. К критериям выздоровления относится также нормализация фильтрационной фракции.

Медленное прогрессирование хронического гломерулонефрита, предположительно, обуславливается автоиммунизацией. Наилучшим объяснением краткого скрытого периода внезапного обострения болезни является классическая теория о сенсибилизации стрептококками («оседлые» противотела). Предрасполагающими факторами являются: 1. почечный камень, 2. аномалии развития в мочеполовом аппарате и 3. беременность.

Признаком активности хронического нефрита является микрогематурия, определяемая по методу Эддиса, и/или низкое содержание комплемента. Динамизм хрони-

ческого пиелонефрита можно оценить на основании числа микробов + числа лейкоцитов (Эддис).

Прогрессирование хронических состояний можно замедлить 1. предотвращением агрессивных факторов, 2. при гломерулонефрите преодолением активности болезни продолжительной дачей стероидов, а при пиелонефрите продолжительной антибиотической химиотерапией. После излечения очагов непременно следует произвести хирургическое лечение урологических изменений. При амилоидном перерождении почек стероидная терапия противопоказана.

В скрытой фазе хронических болезней почек попытка реабилитации требует исключительно тщательного контроля.

Диспансеризацию почечных больных приходится продолжать на протяжении всей жизни пациентов. В центр диспансеризации следует поставить нефрологическую амбулаторию.

ГИСТОЛОГИЧЕСКАЯ ОСНОВА НЕКОТОРЫХ ОЛИГУРО-АНУРИЧЕСКИХ ПАТОЛОГИЧЕСКИХ ПРОЦЕССОВ

Ф. РЕНЬИ-ВАМОШ, М. ЗОМБОРИ и Х. ЕЛЛИНЕК

Дается описание некоторых болезней, при которых патогенез олиго-анурии in vivo нельзя было доказать с достоверностью. Однако вопросы, очень важные для соответствующей терапии и для прогноза, частично или полностью были выяснены гистологическим исследованием.

ОСТРАЯ ОЛИГО-АНУРИЯ, НАБЛЮДАЕМАЯ ПРИ ХРОНИЧЕСКИХ БОЛЕЗНЯХ ПОЧЕК

Ф. РЕНЬИ-ВАМОШ, Ш. ЧАТА, М. ЧЕЛЛАР и Дь. ХАМВАШИ

В отделении по применению диализирующего аппарата Будапештской урологической клиники у 96 из 413 больных основной болезнью была хроническая болезнь почек. Внезапное обострение болезни привело к олиго-анурии. 44 больных выжило, 52 чел. умерло (54,1%). В 61 случае основной болезнью был хронический пиелонефрит, в 20 случаях — хронический гломерулонефрит, в 9 случаях — подострый гломерулонефрит, в 3 случаях амилоидоз, в 2 случаях — нефросклероз и в одном случае — периартериит. Дается описание гистологических изменений почек, а также причин, вызвавших обострение. Подчеркивается, что результаты лечения были бы лучшими, если олиго-анурические больные поступали бы в отделение раньше и в менее тяжелом состоянии.

АНЕМИЯ ПОСЛЕ ОЖОГА

II. Обмен железа

Й. БЕРНАТ, Г. ДОЖАН, Й. НОВАК и Ш. ЕЛЕК

Статья указывает на то, что после ожога развивается тяжелое расстройство обмена железа, для которого характерно, между прочим, одновременное значительное снижение содержания железа в сыворотке и уменьшение железосвязывающей способности сыворотки.

У 20 больных с ожогами проводились исследования при пероральной нагрузке железом. Результаты показали, что при нагрузке железом кривая начинается с низкой величины и в течение 7 часов остается очень плоской.

У 10 больных с ожогами проводились исследования при внутривенной нагрузке железом. Результаты показали, что введенное в кровообращение железо быстро — в течение 3—5 часов — исчезает из сосудистой системы. Из этого факта можно заключить об исключительно интенсивном обмене железа.

У 40 крыс-альбиносов проводились гистохимические исследования. На основании результатов было установлено, что железо, быстро исчезающее из кровообращения

поглощается или присоединяется прежде всего клетками ретикуло-гистиоцитарной системы.

Упомянутые изменения развиваются очень быстро (в течение 24—72 часов) и они сохраняются в течение всей болезни. В период реконвалесценции это расстройство обмена веществ самопроизвольно нормализуется.

ДЕЙСТВИЕ ПОВРЕЖДЕНИЯ ЛАТЕРАЛЬНЫХ ОБЛАСТЕЙ ГИПОТАЛАМУСА НА БЕРЕМЕННЫЕ КРЫСЫ И НА СМЕРТНОСТЬ ПЛОДОВ

З. АВАР и Э. МОНОШ

Авторы исследовали у крыс действие электрического повреждения латеральных областей гипоталамуса (**regio tuberalis dorsalis, regio infundibularis ventralis**), проводившегося на 16—18-ый день беременности, на смертность плодов, на суточный прием пищи и воды беременными крысами и на изменения их веса и температуры.

Установлено, что повреждения указанных областей вызывают значительно большее повышение доли мертворождений и ранней смертности детенышей, чем ложная операция или повреждение других областей гипоталамуса. Вес детенышей при рождении и их число в пересчете на одну крысу-самку в группе животных с повреждением латеральных частей гипоталамуса и в контрольной группе не показали существенных отклонений. Вес детенышей повысился лишь в контрольной группе. Повреждение латеральных областей гипоталамуса привело к значительному временному уменьшению потребления пищи и воды и, в соответствии с этим, вес тела самок до наступления родов показал убывающую тенденцию. В то же время в контрольной группе после ложной операции отмечалось 10—11%-ное повышение веса тела. Относительно температуры тела ни в подопытной, ни в контрольной группе не наблюдались значительных изменений.

ДАННЫЕ К ПАТОМЕХАНИЗМУ ШОКОВОЙ ПОЧКИ IV

Регенерация почки после повреждения вследствие временной аноксии

Л. ТАКАЧИ-НАДЬ и И. ЮХАС

Авторы исследовали у крыс регенерацию функции почек по истечении 4, 7 и 14 дней после лигирования на протяжении различного времени почечных артерии и вены одной стороны. Исследования проводились при помощи Ивенсевой синьки. Было установлено, что между продолжительностью аноксии и необходимым временем регенерации существует связь и, что возникающие повреждения почек в пределах 14 дней по большей части восстанавливаются. Степень аноксического повреждения эпителия канальцев оказывает влияние на восстановление функции почек.

ДЕЙСТВИЕ ЭКСИКОЗА НА МИНУТНЫЙ ОБЪЕМ И НА КРОВООБРАЩЕНИЕ ОРГАНОВ У НАРКОТИЗИРОВАННЫХ И БОДРСТВУЮЩИХ КРЫС

П. БЕНЧАТ и Л. ТАКАЧ

Авторы исследовали действие эксикоза на наркотизированных, на бодрствующих и фиксированных а также, на бодрствующих и не фиксированных крысах. Установлено, что у наркотизированных животных изменения подобны перераспределению кровообращения, наблюдаемому при различных состояниях застойной гипоксии. В бодрствующем состоянии уже возбуждение, вызванное вмешательством, имеет следствием тяжелые изменения кровообращения. Под влиянием эксикоза повышается коронарная фракция минутного объема, уменьшается кожная фракция, в то время как почечная фракция остается неизменной.

КРИЗИСЫ ПРИ ТЯЖЕЛОЙ МИАСТЕНИИ, I

Встречаемость и патомеханизм

А. СОБОР

Формирование понятия о миастеническом кризисе, обсуждение его теоретического и практического значения на основании литературных данных. — Описание характера кризисов, обсуждение возможного патологоанатомического и патофизиологического механизма. — Обсуждение теоретических вопросов механизма кризисов. — Частота встречаемости кризисов. — Характеристики семиотики кризисов, наблюдаемых при миастении. — Возможность дифференциации отдельных типов кризисов.

КРИЗИСЫ ПРИ ТЯЖЕЛОЙ МИАСТЕНИИ, II

Причины, характер, лечение

А. СОБОР

Формирование, теоретическое и практическое значение понятия о миастеническом кризисе. — Описание характера кризисов, обсуждение возможного патологоанатомического и патофизиологического механизмов. Обсуждение теоретических вопросов механизма кризисов. — Частота встречаемости кризисов. — Характеристика семиотики кризисов, встречаемых при миастении. — Возможность дифференциации отдельных типов кризисов. — Казуистика собственных случаев автора: из 80 больных миастенией, наблюдавшихся автором в течение 14 лет, дается описание истории болезни 14 больных, имевших кризисы. — Подробное изложение более часто встречаемых и более важных причин кризисов, целесообразная категоризация и анализ посттимектомических кризисов. — Современные принципы и практика лечения кризисов; профилактика кризисов.

ЭПИЛЕПТИФОРМНЫЕ АНОМАЛИИ ЭЭГ В СЛУЧАЕ СИНДРОМА ПЕРИОДИЧЕСКИХ БОЛЕЙ В ЖИВОТЕ

Ф. КАЙТОР и Т. КАСАШ

Авторы анализировали ЭЭГ 38 детей и подростков, страдающих интенсивными приступами болей в животе. ЭЭГ были записаны в бодрствующем состоянии и под эвипановым наркозом.

В 56,7% ЭЭГ, сделанных в бодрствующем состоянии, удалось выявить спонтанный нефизиологический медленный ритм, а в 13,5% — эпилептиформные изменения. В 83,3% ЭЭГ, сделанных под наркозом, наблюдались типичные спайки. В 64% эпицентры спайков локализовались в Роландовой борозде и на верхушке над одним полушарием или над обоими полушариями. Остальные спайки появлялись в лобной области. В одном случае удалось локализовать передний базотемпоральный спайк, а в другом случае была провоцирована генерализированная активность спайковых волн в 3 ц/сек. Многоочаговая эпилептиформная активность не была редким явлением.

Возможность эпилептогенного повреждения мозга была выявлена у 31% больных; эти больные рассматриваются авторами как эпилептики. Остальные 69% авторы причисляют к «пограничной области эпилепсии», предполагая доброкачественную аномалию мозга, связанную с возрастом (незрелость).

Ссылаясь на результаты физиологических исследований по выяснению желудочно-кишечной регуляции и на наблюдения, сделанные у эпилептиков, авторы придерживаются того мнения, что спайки в Роландовой борозде и в верхушке указывают на конвульсоидную повышенную раздражимость неспецифических висцеромоторных элементов сенсо-моторных центров коры головного мозга. Следовательно, в случае синдрома возвратных болей в животе показано проведение противосудорожного лечения.

ОСТРОЕ ДЕЙСТВИЕ НИКОТИНА НА КРОВООБРАЩЕНИЕ В КОНЕЧНОСТЯХ

Ф. ШОЛЬТИ, И. КРАСНАИ, Ю. РЕВ и Ю. НАДЬ

Острое действие никотина на кровообращение изучалось у 21 кардиально компенсированного и не страдающего сужением периферических сосудов больного, после внутривенного введения 1,0 мг тартарата никотина. Периферическое кровообращение измерялось в 12 случаях при помощи закиси азота, а в 9 случаях методом венозной изотопной дилюции, разработанным авторами и пригодным для измерения полного периферического кровообращения. Помимо этого проводились также измерения кожной и мышечной температуры и исследование радиоактивного «мышечного клиренса». Под острым влиянием никотина минутный объем несколько повысился, температура кожи значительно уменьшилась, в то время как температура мышцы и время исчезновения J^{131} из мышцы не изменились. Периферическое кровообращение и сопротивление периферических сосудов, в сущности, не изменились. При действии никотина фракция минутного объема периферического кровообращения также не показала оцениваемых изменений.

После дачи никотина поразительными и прочными изменениями были выраженное снижение потребления O_2 тканями конечностей и повышение венозного давления в нижних конечностях.

Обсуждается патомеханизм действия никотина на кровообращение в конечностях.

РОЛЬ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ В ПАТОМЕХАНИЗМЕ ОТЕКА ЛЕГКИХ, ВЫЗВАННОГО АДРЕНАЛИНОМ

М. КЕЛЛНЕР, Э. МАКЛАРИ, А. Г. Б. КОВАЧ и Д. ГОТТЗЕГЕН

Исследовалась роль нейрогенных факторов в патомеханизме отека легких, вызванного адреналином. В 41 эксперименте по перекрестному кровообращению авторы вводили адреналин в кровообращение головы, а также в кровообращение туловища. Введение адреналина в кровообращение головы вызывало более тяжелые отеки легких и в большем проценте случаев, чем введение его в кровообращение туловища. По мнению авторов это действие адреналина осуществляется нейрогемодинамическим путем, так как в ходе экспериментов в малом круге кровообращения величины кровяного давления сильно повысились, а коллоидосмотическое давление незначительно понизилось. В проницаемости капилляров было выявлено незначительное повышение.

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