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Max-Planck-Institut für Hirnforschung, Abteilung für Tumorforschung und experimentelle Pathologie, und der Neurochirurgischen Universitätsklinik (Direktor: Prof. Dr. W. Tönnis), Köln

BEITRAG ZUR HISTOCHEMIE DES INTRAVASALEN KOLLOIDS IM HYPOPHYSENVORDERLAPPEN DES MENSCHEN

J. Doebel

(Eingegangen am 25. Januar, 1968)

Mit histochemischen Nachweismethoden wird festgestellt, daß das intravasale Kolloid im Hypophysenvorderlappen überwiegend aus Proteinen besteht. Das Vorkommen von Lipoiden wurde nicht untersucht. Das intravasale Kolloid ist bei intrakranieller Drucksteigerung vermehrt. Nach den histochemischen Ergebnissen ist es sehr wahrscheinlich mit dem Kolloid in den Pseudofollikeln identisch. Es wird angenommen, daß vor allen Dingen die Einschmelzung von Alphazellen die Quelle für das Kolloid darstellt.

Nach Untersuchungen von Romeis (1940) sind die intravasalen Kolloide in den Hypophysen höherer Tiere und des Menschen nicht einheitlicher Natur. Es lassen sich vielmehr das amorphe und das geformte intravasale Kolloid, siderophile Körnchen, chromophobe Sekretbläschen und eine »gewisse fadenartige Substanz« unterscheiden.

Das geformte intravasale Kolloid ist als häufiger Befund bei Schwangeren und Pubertierenden, wie überhaupt in Hypophysen mit erhöhter Aktivität bekannt (Rasmussen 1927, Romeis 1940, Graumann 1964). Darüber hinaus war schon Romeis (1940) eine Vermehrung bei intrakranieller Drucksteigerung aufgefallen. Das Auftreten von geformtem, intravasalem Kolloid im Hypophysenvorderlappen (= HVL) konnte von Kuhn (1957) in 76% der Fälle mit intrakranieller Drucksteigerung unterschiedlichster Genese und Lokalisation des raumverdrängenden Prozesses nachgewiesen werden. Die von Kuhn (l. c.) mit färberischen Methoden gewonnenen Ergebnisse sollen durch histochemische Nachweise ergänzt werden.

Material und Technik

Bei 240 durchgesehenen Hypophysen, die von Patienten mit einem klinisch und pathologisch erhöhten intrakraniellen Druck stammten, waren intravasale, geformte Kolloide in 48% vorhanden. Hiervon wurden 10 Hypophysen, bei denen sich die intrakranielle Drucksteigerung auch in einer Blutstauung in den Sinuskapillaren manifestiert hatte, zur histochemischen Analyse ausgewählt (Tab. I). Die Färbe- und histochemischen Methoden gehen aus den folgenden Tabellen hervor. Die Befunde wurden an Paraffinschnitten erhoben. Zum Nachweis von Lipoiden liegt vorläufig noch nicht genügend geeignetes Material vor.

Befunde

Die Proteinnachweise zeigten folgende Ergebnisse (s. Tab. I).

Tabelle I

Kasus

Nr.	Geschlecht	Alter	Ursache der intrakraniellen Drucksteigerung	Dauer der klin. Erscheinungen
6778	männl.	51	Ventrikelmeningiom	1 J.
6779	weibl.	47	Astrocytom, rechts frontal	2 J.
6782	männl.	37	Unklassifizierter Tumor rechts frontal	1 J.
6795	männl.	45	Blutungen, Erweichungen	
6950	weibl.	33	Astrocytom, links in der Fissura Sylvii	
7022	weibl.	53	Glioblastom, links frontal	1 J.
7110	weibl.	19	Spongioblastom im 4. Ventrikel	3 J.
7128	männl.	49	Abszeß, links occipital	4 W.
7174	weibl.	21	Plexuspapillom im rechten Seitenventrikel	4 W.
7332	weibl.	50	Glioblastoma multiforme, rechts parietal	8 M.

J. = Jahre; W. = Wochen; M. = Monate

Tabelle II

Proteine

Aminosäuren und spezifische Gruppen	Nachweisreaktionen	Autoren	Befunde		
			i. v. K.	P. f. k.	Z. l. k.
Amino-Gruppen	o-Diacetylbenzol	Voss (1940)	+++	+++	+++
Amino-Gruppen	Ninhydrin-Reaktion	Fotakis (1960)	++	++	+
Phenol-(Tyrosin)	Millon-Reaktion	Baker (1956)	+++	+++	
Tyrosin u. Tyrosinreste	Diazotierungs-Kupplungs-Reaktion	Glenner u. Lillie (1957)	+++	+++	+++
Indol-(Tryptophan)	p-DMAB-Nitrit	Adams (1957)	+++	+++	+++
Guanidyl-(Arginin)	Sakaguchi-Reaktion	Carver, Brown, Thomas (1953)	++	++	+
SH- u. SS-Gruppen	DDD-Reaktion	Barrnett u. Seligman (1954)	+++	+++	++
Phenyl-, Imidazol-, Indolyl-,Guanidyl-	Gekuppelte Tetrazonium-Reaktion	Danielli (1947), Burstone (1959)	+++	+++	++

i. v. K. = intravasales Kolloid, P. f. k. = Pseudofollikelkolloid, Z. l. k. = Zwischenlappenkolloid

Zusätzlich wurde bei einem Fall die Bestimmung des isoelektrischen Umladebereiches (I. E. U.) nach Pischinger (1926) durch mikrophotometrische Ausmessung von je 25 intravasalen- und 10 Pseudofollikelkolloiden eines jeden der bei verschiedenem pH-Wert gefärbten Schnitte durchgeführt.

Die Kurvenverläufe (Abb. 1) ergeben sich aus den arithmetischen Mitteln der Einzelmessung, sowie den jeweiligen maximalen und minimalen Meßwerten in jedem Präparat. Man erkennt deutlich den mehr im Säurebereich liegenden I. E. U. bei den Pseudofollikelkolloiden.

Die Ergebnisse der Nukleinsäurenachweise finden sich in Tab. III.

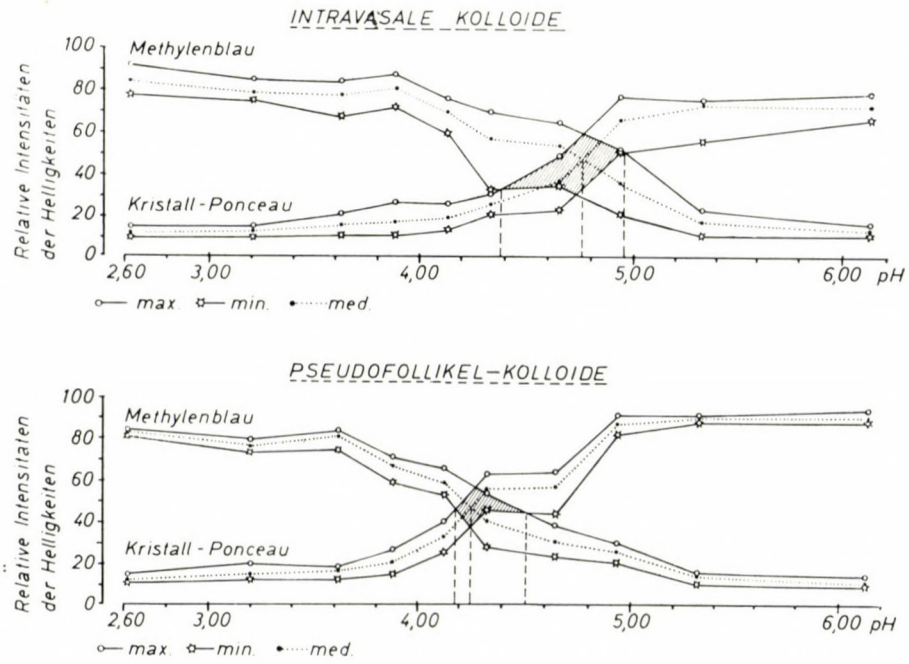

Abb. 1

Tabelle III

Nukleinsäuren

Verbindungen und Gruppen	Nachweisreaktionen	Autoren	i. v. K.	P. f. k.	Z. l. k.
DNS	Feulgens Nukleal-reaktion	FEULGEN u. ROSSENBECK (1924)	—	—	—
DNS und RNS	Gallocyanin-Chromalaun	EINARSON (1951)	—(+)	—	—

i. v. K. = intravasales Kolloid, P. f. k. = Pseudofollikelkolloid, Z. l. k. = Zwischenlappenkolloid

Das histochemische Verhalten der Kolloide bei den Kohlenhydratreaktionen zeigt Tab. IV.

Tabelle IV

Kohlenhydrate

Verbindungen und Gruppen	Nachweisreaktionen	Autoren	i. v. K.	P. f. k.	Z. l. k.
Mukoproteide und Glykoproteide, Polysaccharide, Proteine	Perjodsäure-Leukofuchsin (PAS)	Hotchkiss (1948) Mc Manus (1948)	+++	+++	+++
Saure Mukopolysaccharide	Alcian-Blau 8 GS	Steedman (1950)	–	+(–)	+++
Freie Säuregruppen	Hale's Methode nach G. Müller	Hale (1946) G. Müller (1955)	–	+(–)	+++
Saure Mukopolysaccharide	Metachromasie	Kelly (1958)	alpha	alpha	beta-gamma

i. v. K. = intravasales Kolloid, P. f. k. = Pseudofollikelkolloid, Z. l. k. = Zwischenlappenkolloid

Ergebnisse

1. Intravasales Kolloid

Bei allen Einschränkungen, denen die histochemische Technik unterliegt, ist anzunehmen, daß die intravasalen Kolloide des Hypophysenvorderlappens reich an Proteinen sind und sehr wahrscheinlich Kohlenhydrate enthalten.

Die Proteinkomponente dieser Glyco-(Muco-)-proteide ist ausgesprochen tyrosinreich und enthält Tryptophan und Arginin in geringerer Quantität. Freie Aminogruppen sind reichlich, Sulfhydrylgruppen — wenn überhaupt — nur spärlich vorhanden.

2. Die Kolloide in den Pseudoacini des HVL

Vergleicht man das histochemische Verhalten dieser Kolloide mit dem der intravasalen Kolloide nach Anwendung der Proteinreaktionen, so lassen sich keine Unterschiede feststellen. Dagegen enthalten die interzellulären Kolloide in ihrer äußeren Schale zusätzlich saure Mukopolysaccharide. Es kann angenommen werden, daß die Verschiebung des I. E. U. darauf zurückzuführen ist.

3. Die Kolloide in den Spalten und Zysten der Pars intermedia

Diese Kolloide bestehen trotz färberisch ungleichen Verhaltens aus sauren Mukopolysacchariden. UV-Absorptionsmessungen (Kempe und Meyer-Arendt 1954) lassen auf eine einheitliche Substanz schließen.

Besprechung

Vernachlässigen wir die Zwischenlappenkolloide aus entwicklungsgeschichtlichen Gründen, so ergibt sich eine auffällige Korrespondenz der Befunde an intravasalen- und Pseudofollikelkolloiden. Entschließen wir uns weiterhin zur Deutung des Saumes saurer Mukopolysaccharide in der äußeren Schale der interzellulären Kolloide als nicht zum Kolloidkörper gehöriger zellulärer Reaktion, so sind sie identisch.

Dieser Befund leitet über zur Frage der Entstehung der Kolloide im Hypophysenvorderlappen.

Nach BARGMANN (1962) sind alle Zellen des HVL zu merokriner und holokriner Kolloidsekretion befähigt. Dagegen kommt KUHN (1957) zu dem Schluß, daß die hyperchromatische Alphazelle allein als Quelle des intravasalen Kolloids anzusehen ist. Der Übertritt von kolloidal eingeschmolzenen Alphazellen aus dem interzellulären Raum in eine Sinuskapillare wurde mehrfach festgehalten (ROMEIS 1940 und KUHN 1957). Die vorliegenden Untersuchungen erhärten nun aufgrund des histochemisch gleichen Verhaltens der beiden Kolloidformen die Beobachtung der genannten Autoren, nach der es sich um verschiedene Lokalisationen ein und desselben Degenerationsproduktes handelt.

Darüber hinaus erfährt die Auffassung von KUHN (i. c.), nach der die hyperchromatische Alphazelle allein die Bildungsstätte der Kolloide ist, eine weitere Unterstützung. Es zeigt sich nämlich, daß nach Anwendung der Diazotierungs-Kupplungsreaktion (Tab. II) eine selektive und intensive Färbung von intravasalen Kolloiden, Pseudofollikelkolloiden und Alphazellen eintritt.

Die unter Erhöhung des intrakraniellen Druckes beobachtete Verminderung der Alphazellen (KRAUS 1933) bei gleichzeitiger Vermehrung des intravasalen Kolloids (ROMEIS 1940, KUHN 1957) ist als weiterer wichtiger Hinweis für die Entstehung des Kolloids aus degenerierenden azidophilen Zellen des HVL anzusehen.

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A CONTRIBUTION TO THE HISTOCHEMISTRY OF INTRAVASCULAR COLLOID IN THE ADENOHYPOPHYSIS OF HUMANS

J. DOEBEL

It has been demonstrated by histochemical methods that the intravascular colloid in the anterior lobe of the hypophysis consists mainly of proteins. The possible presence of lipides has not been examined. Higher intracranial tension increases the amount of intravascular colloid which is, according to histochemical results, presumably identical with the colloid contained in the pseudofollicles. The breakdown of alpha cells is supposed to be the principal source of the colloid.

ДАННЫЕ О ГИСТОХИМИИ ВНУТРИСОСУДИСТОГО КОЛЛОИДА В ПЕРЕДНЕЙ ДОЛИ ГИПОФИЗА У ЧЕЛОВЕКА

Й. ДЕБЕЛ

Гистохимическими методами исследования было установлено, что в передней доли гипофиза внутрисосудистый коллоид состоит преимущественно из протеинов. Встречаемость липоидов не была изучена. При повышении внутричерепного давления количество внутрисосудистого коллоида повышается. На основе результатов гистохимических исследований, этот коллоид по всей вероятности идентичен с коллоидом, наблюдаемым в псевдофолликулах. Полагается, что источником коллоида является прежде всего расщепление альфа-клеток.

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THE EFFECT OF THYROTROPIC HORMONE TREATMENT ON THE EPIPHYSEAL CARTILAGE OF THE WHITE RAT

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(Received March 26, 1968)

The effect of the thyrotropic component of Ambinon® on the proximal tibial epiphyseal cartilage has been studied in young white rats. The animals received 0.05 ml (0.5 I.U.) subcutaneously, daily for 4 weeks, while the controls were treated with 0.05 ml of physiological NaCl solution, also subcutaneously. Ambinon, similarly to thyroxine, increased the proliferation of the cellular elements of cartilage and induced a minor osteoblastic activity. In the phase of cartilage breakdown the number of chondroclast giant cells increased. The changes are considered to be due to thyroid hyperfunction, hyperthyroxinaemia, not ruling out the possibility that the results may have been influenced also by the gonadotropic component of the preparation.

The role played by the thyroid gland in growth has been known for long. Gudernatsch [7] in 1912 fed tadpoles with thyroid tissue and found that their metamorphosis and rate of growth were enhanced. These first experimental data were followed by a series of papers on the effect of the thyroid on ossification; these have been surveyed critically by Silberberg and Silberberg in 1943 [20] who attributed the many contradictory data (inhibition or stimulation) to differences in the methods employed, as in the lack of pure hormone preparations at that time, there were differences in the dose of hormone administered, the animals treated were different in age and sex, and there were differences also in the duration of treatment. After some later studies [1—4, 18] Hulth and Nylander [9, 10] as well as Lévai and Zs. Nagy [16] have published evidence indicating that thyroxine increased the rate of ossification. They treated the animals with thyroxine, or inhibited thyroid function by the administration of thiouracil derivatives. Thyroxine treatment allowed to increase the thyroxine level high over the physiological one, so that the changes described must have arisen under aphysiological conditions.

In the present investigations we have undertaken to eliminate thyroxine overdosage so that growing white rats were treated with thyrotropic hormone (Ambinon®, Organon, Netherlands) and the changes taking place in the structure of the epiphyseal cartilage were observed. The results so obtained were thus near to the physiological ones, although Ambinon® has also a gonadotropic component which cannot be ignored in evaluation.

Materials and methods

In the experiments 40 male 35-day rats of 39 g average weight were used. The animals were divided into 4 groups, each containing 7 experimental rats and 3 controls. At the onset of experiment then at weekly intervals we measured body weight, length and rectal temperature. The experimental animals received daily 0.05 ml (0.5 I.U., i.e. 5 Heyl-Laqueur units) of TSH subcutaneously, while the controls were treated with 0.05 ml isotonic NaCl solution. The animals of the different groups were killed 1, 2, 3 and 4 weeks after the onset of treatment. One day before killing them, 1 treated rat and 1 control were given ^{131}I and thyroid I uptake was estimated 2, 8 and 24 hours later. The knee joints were excised and fixed in Susa or in 10% formalin buffered to pH 7. The formalin material was decalcinated in 10% EDTA solution [5]. After embedding in paraffin, 7 μ thick sections were cut and stained with haematoxylin-eosin and Azan.

Results

Especially near the end of the experiments, the treated animals were more agile than the controls. During the last week they weighed 199.4% more than initially, as compared to the 170.1% weight gain in the control group.

Table I

Changes in the average body weight of experimental animals

Duration of treatment	Treated, per cent	Control, per cent
Initial values	100	100
1 week	141.3	121.8
2 weeks	152.9	138.7
3 weeks	242.5	217.8
4 weeks	299.4	270.1

The rectal temperature of the treated animals was about 1 °C higher than that of the controls. The radioiodine studies showed that the thyroid gland of the experimental animals was more active than that of the controls. Histologically, the thyroid exhibited changes indicative of a slight hyperfunction. This tendency decreased toward the end of the experiment.

In describing the histological changes of the epiphyseal cartilage we have used the zone classification of HAM [8], while when discussing the normal course of ossification we have relied upon the work by KROMPECHER [12].

After one week of treatment (Fig. 1a) the epiphyseal cartilage of the treated animals showed a broadening and richness in cells of the proliferation zone. The maturation zone, too, was slightly increased in width. Nuclear staining was diminished in the calcification zone, the lower part of the epiphyseal cartilage disc showed increased capillarisation. The increased breakdown of cartilage was indicated by the increase in capillarisation and the appearance of chondroclasts, giant cells breaking down cartilage (Fig. 4a). In some cases the nuclei stained well even in the lowermost cell layer of the calcification zone.

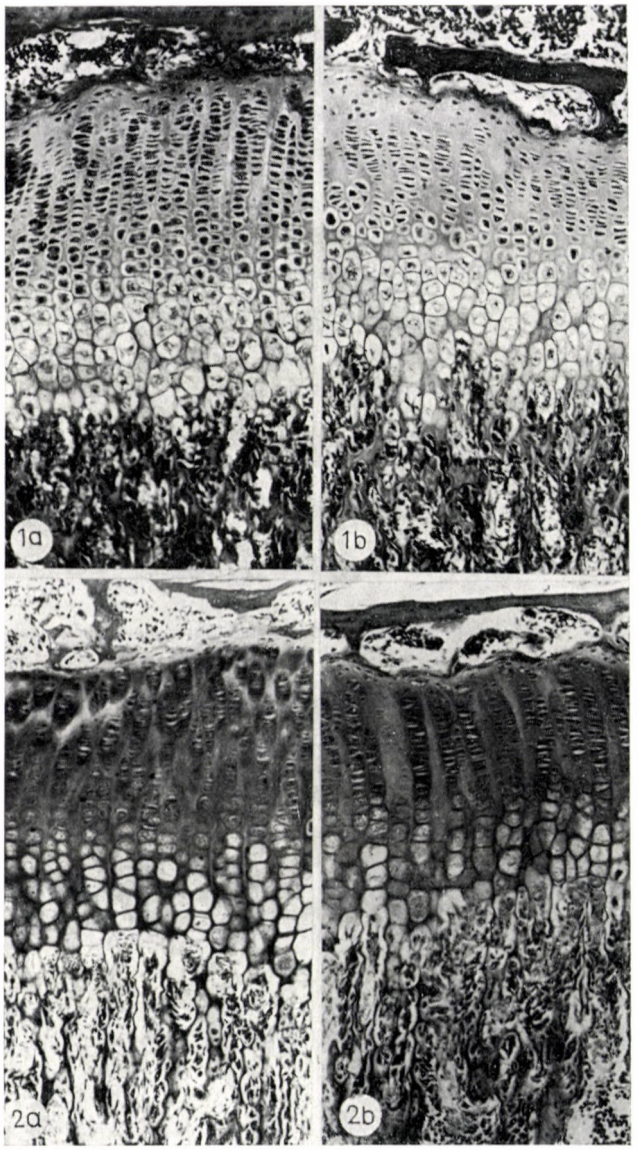

Fig. 1a. Proximal epiphyseal cartilage of the tibia after one week treatment. H. E., ×100; *b.* One-week control. H. E., ×100

Fig. 2a. Proximal epiphyseal cartilage of the tibia after two weeks treatment. H. E., ×100; *b.* Two-week control. H. E., ×100

In the one-week controls (Fig. 1b) the proliferation zone was poorer in cells and the maturation zone less wide. In the vicinity of the broadened calcification zone, capillarisation was not so marked than in the treated rats. Chondroclasts were few or absent.

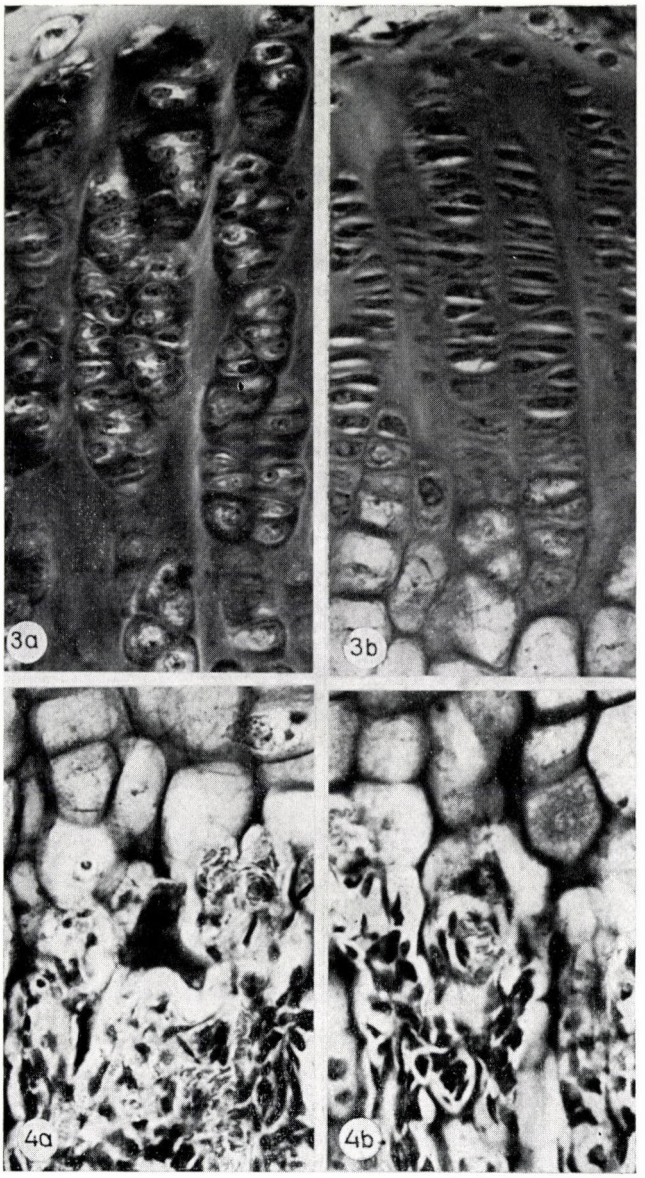

Fig. 3a. Increase in the number of cells in the proliferation zone after two weeks treatment. H. E., ×350; *b.* Two-week control. H. E., ×350

Fig. 4a. Chondroclastic giant cell after one week treatment. H. E., ×350; *b.* Chondroclastic giant cell after two weeks treatment. H. E., ×350

After two weeks treatment (Fig. 2a) the cells of the proliferation zone were markedly enlarged, showing a nestlike, irregular arrangement also in the vicinity of the residual cell zone. There were some mitotic cells (Fig. 3a), the

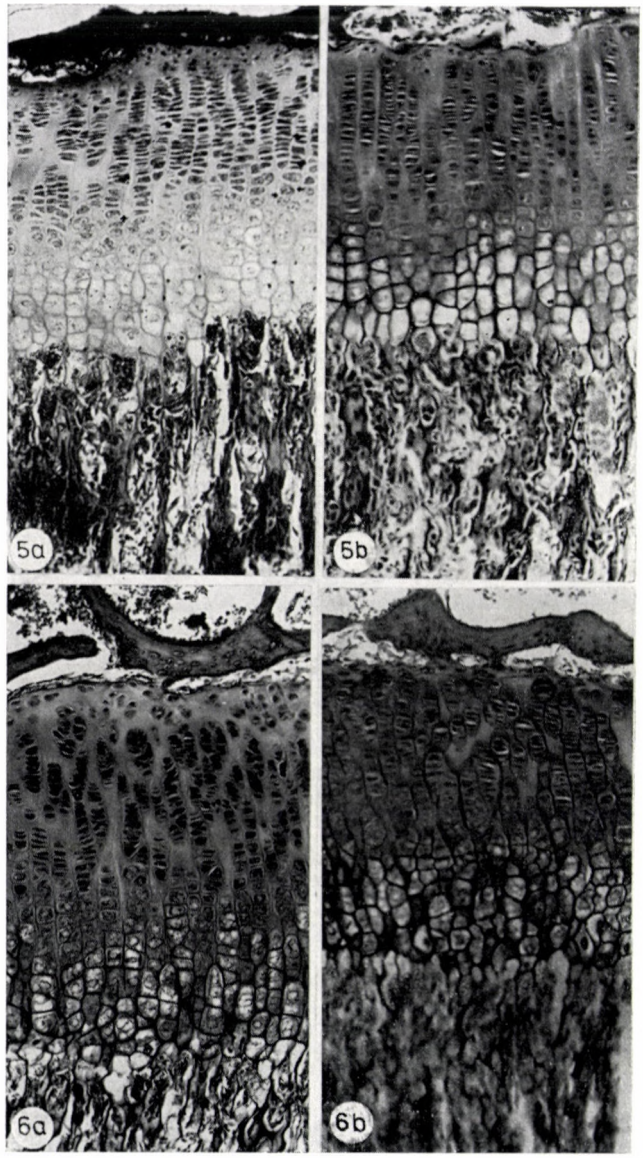

Fig. 5a. Proximal epiphyseal cartilage of the tibia after three weeks treatment. H. E., ×100; *b.* Three-week control. H. E., ×100

Fig. 6a. Proximal epiphyseal cartilage of the tibia after four weeks treatment. H. E., ×100; *b.* Four-week control. H. E., ×100

ground substance was less basophilic, the maturation zone increased in width. Nuclear staining diminished in the calcification zone. As compared to the 1-week findings, capillarisation was less abundant. Giant cells were also seen (Fig. 4b).

The 2-week controls were conspicuously different (Figs 2b and 3b). Here the cells of the proliferation zone were smaller, more regularly arranged, the maturation zone was smaller in diameter and its cells were also smaller. The width of the calcification zone did not reach that of the treated animals, the cells were bigger, the nuclei stained well. There was no marked difference in capillarisation, only in osteoblastic activity in favour of the control group.

After 3 weeks of treatment the proliferation zone and the residual cell zone are still rich in cells. The arrangement of cells in the maturation zone is less regular, it is difficult to find the junction between the proliferation and maturation zones. The calcification zone is composed of 3 to 5 cell rows, in the lower part of the epiphyseal cartilage capillarisation is marked. As compared to the 2-week pattern, osteoblastic activity increased, the bony trabecules increased significantly in number and size (Fig. 5a).

In the 3-week controls the proliferation zone is less rich in cells which are more regularly arranged than in the treated rats. The maturation zone is wider, the junction to the proliferation zone is distinct. There is no difference in the structure of the calcification zone. Osteoblastic activity is weaker than in the 3-week treated specimens (Fig. 5b).

After 4 weeks of treatment the epiphyseal cartilage is wider than in the control group; particularly the proliferation zone is broad and highly cellular. The maturation zone is more conspicuous, while in the calcification zone the nuclei are stained even in the deep layers. Capillarisation is less ample than in the 3-week treated rats or in the 4-week controls (Fig. 6a).

In the 4-week control group the cells of the proliferation zone are larger than in the treated rats and are arranged in irregular nests. The maturation zone is smaller in diameter, less regular in shape, the calcification zone is of about the same size, but its nuclei stain weaker in the treated rats. In the distal part of the epiphyseal cartilage capillarisation is increased, while osteoblastic activity appears to be weaker (Fig. 6b).

Discussion

In the literature of recent years we have been unable to find papers dealing with the correlation between TSH and ossification. The administration of TSH, as indicated by the histological controls and radioiodine studies, induced hyperthyroidism but its severity could be increased to a certain limit only and the increased thyroid activity showed a tendency to diminish with the duration of treatment. This could not be applied without restrictions to the changes in the epiphyseal cartilage. The difference in weight gain (Table 1) has been ascribed to the gonadotropic component of Ambinon®, but an indirect action of the gonadotropic component on the epiphyseal cartilage could not be ruled out.

It is generally accepted that hypothyroidism, as it promotes the persistence of cartilage, reduced the rate of ossification. In cretins, cartilage was found in the clavicle and scapula even after the 5th decade of life [23] and experimental findings allowed similar conclusions [17, 21].

As regards hyperthyroidism, the views are far from being so unequivocal, though all recent papers have reported on an increase in the number of cells in the proliferation zone, a proliferation of the cellular elements of cartilage, an increase in osteoblastic activity, i.e. about an acceleration of ossification in hyperthyroidism. Our experiments lend support to this view. Although a direct or indirect gonadotropic effect could not be ruled out, the changes in the epiphyseal cartilage were the most marked in the most active phase of growth, and almost identical to those observed by us earlier in thyroxine-treated animals.

In the animals treated with TSH the number of cells in the proliferation zone and the increase in its diameter were the highest in the second and third weeks. At that time there was also a difference in favour of the treated rats in the mitotic activity of cartilage cells. The maturation zone was broad at the onset of treatment, then decreased in diameter, to increase again during the fourth week. The rate of cartilage breakdown increased during the second and third weeks, and began to decrease toward the end of the experiment. The increase of osteoblastic activity, the increased rate of ossification followed the changes of the cartilage with a lag of one to one and a half week. The increased vascularisation observed during the first and second weeks, the increase in the number of capillary loops and the appearance of chondroclastic giant cells suggested an increase of cartilage absorption. The rate of this, however, was reduced later, while osteoid formation seemed to increase at an even rate from the second week till the end of the experiment. An increased osteoblastic activity following thyrotropic hormone treatment as described by FORST et al. [6] could not be observed, presumably because the experimental period was too short. Another possibility is that the reaction of the growing animal to hyperthyroxinaemia is different from that of the full-grown one.

KROMPECHER [12—15] stated that in both normal and pathological bone growth it is essential that the proper proportions be maintained between growth and destruction of cartilage and bone, respectively. If the physiological balance is upset, pathological bone formation will result. Analysing our results in the light of these findings, it may be stated that the thyroid hyperfunction in response to TSH treatment increases in the first step the rate of growth and destruction of cartilage, while the subsequent bone-building activity is less intense, but it lasts longer. Our results confirm the data obtained earlier in connection with thyroxine treatment [16] and at the same time dissipate our doubts as to the aphysiological level of the thyroxine dose used then.

For the explanation of the mode of action we may mention the primary tissue metabolism increasing action of thyroxine, the increased Ca and P excretion by the kidneys [11]. The decreased rate of cartilage destruction and the diminished osteoblastic activity at the end of the experiment suggest the possibility that TSH administration increases thyroid activity, i.e. elevates the blood thyroxine level and through a feed back suppresses pituitary TSH production. On the other hand, a decrease in the blood TSH level may slow down thyroid hyperactivity, which may explain the decreasing tendency of thyroid activity.

The chondroclastic activity in response to TSH did not reach the degree observed after thyroxine treatment. In agreement with other authors [13, 16, 22] we regard the appearing giant cells as cells differentiated to destroy cartilage. This type of cell was claimed to play such a role by SCHENK et al. [19] who based their statement on the results of electron microscopic studies.

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DIE WIRKUNG DES THYREOTROPHORMONS AUF DEN EPYPHYSENKNORPEL WEISSER LABORATORIUMSRATTEN

G. LÉVAI, F. MÓRICZ, P. SZERZE, GY. PETRÁNYI jr., und J. LACZKÓ

Die Wirkung der thyreotropen Komponente des Präparats Ambinon® (Organon) auf den Epiphysenknorpel der proximalen Tibia im Wachstum begriffener weißer Laboratoriumsratten wurde untersucht. Über 4 Wochen wurde den Tieren täglich 0,05 ml (0,5 I.E.) subkutan verabreicht, während die Kontrolltiere, ebenfalls subkutan, 0,05 ml physiologische Kochsalzlösung erhielten. Wöchentlich wurden einige der Tiere getötet, bei deren Aufarbeitung sich feststellen ließ, daß das Ambinon, ähnlich dem Thyroxin, zunächst die Proliferation der zellulären Elemente der Knorpel steigert und anschließend eine geringfügige Osteoblastenaktivität bewirkt. In der Knorpelabbau-Phase vermehren sich die chondroclastischen Riesenzellen. Diese Ergebnisse werden auf die Schilddrüsenhyperfunktion, die Hyperthyroxinämie zurückgeführt, wobei aber auch die Möglichkeit erwogen wird, daß die gonadotrope Komponente des Hormonpräparats die Ergebnisse beeinflußt haben könnte.

ДЕЙСТВИЕ ВВЕДЕНИЯ ТИРЕОТРОПНОГО ГОРМОНА НА ГИПОФИЗАРНЫЙ ХРЯЩ У ЛАБОРАТОРНЫХ БЕЛЫХ КРЫС

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Действие тиреотропного компонента препарата Амбинон® (фирмы Органон) на эпифизарный хрящ проксимальной части тибии было изучено у лабораторных белых крыс. Подопытные животные получали на протяжении 4 недель ежедневно 0,05 мл (0,5 ИЕ) препарата подкожно, а животные контрольной группы — 0,05 мл физиологического раствора соли, также подкожно. Каждую неделю убивали часть животных, при обработке которых было установлено, что Амбинон, подобно тироксину, повышает пролиферацию клеточных элементов хряща, и вслед затем вызывает незначительную активность остеобластов. В фазе расщепления хряща размножаются «хондрокластические» гигантские клетки. Полученные результаты авторы приписывают гиперфункции щитовидных желез, гипертироксинемии, причем они учитывают также возможность того, что гонадотропный компонент гормонного препарата также мог повлиять на полученные результаты.

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THE FINE STRUCTURE OF THE AREA POSTREMA OF THE RAT

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The ultrastructure of the area postrema has been studied in the rat. Small nerve cells were found surrounded by astrocyte-like cells and by their processes. The cytoplasm of these glial cells contains many lysosomes and in most of them an acid phosphatase reaction product has been demonstrated. The processes of astrocyte-like cells form lamellar sheaths around the axo-dendritic synapses. In most of these synapses large granulated vesicles were observed. Special nerve fibres containing many small shrunken mitochondria, osmiophilic bodies and granulated vesicles were demonstrated. Axo-somatic synapses were restricted to the peripheral parts of the area postrema. Some flattened ependymal cells contain wide cisternae of endoplasmic reticulum resembling those of secretory cells.

The structure termed area postrema has been described by RETZIUS in 1896. It is situated in the most caudal part of the rhomboid fossa at each side of the apex of the calamus scriptorius. In mammals it is formed of two symmetrical tongue-like formations which run apart in the cranial part and emerge in the area of the passage of the fourth ventricle into the vertebral channel. It is also known in birds and has been investigated by MORATO, TEIXEIRA, TEIXEIRA-PINTO (1958), and recently by DUVERNOY, SCHERRER and KORITKÉ (1966). The shape of the structure shows considerable differences in the various animal species.

The numerous findings concerning the area postrema (KORITKÉ, DUVERNOY, 1962; SHIMIZU, ISHII 1964; RABL 1965) can be summarized as follows.

1. The area postrema possesses a clear-cut vascular organization. The vascular supply is provided by large double, spirally wound sinus-like capillaries and resembles in appearance the vascular organization of other circumventricular organs and also the hypothalamus. This similarity is also obvious in the ultrastructure of the capillary wall (ROHR, 1966; SCHWINK, WETZSTEIN, 1966).

2. The cellular architecture of the area postrema shows some particularities the nature of which is not clear. It was originally presumed that it consists only of glia (promontorium gliosum) but two kinds of cells can be distinguished when stained with methylene blue or one kind consists of gallocyanin, some small neurones, usually apolar or unipolar, with a small perikaryon and a small nucleus and nucleolus. The second type is represented by numerous cells with a round nucleus which in appearance resembles the nucleus of astrocytes, as observed also by SHIMIZU and ISHII (1964).

3. A high activity of some hydrolytic and oxidative enzymes is typical of the cells of the area postrema (WEINDL, 1965; NĚMEČEK, PAŘÍZEK, 1966; ŠPAČEK, PAŘÍZEK, 1967). This chemical activity is different in the various animal species (CAMMERMEYER, 1949). A high catecholamine concentration in the area postrema and hypothalamus has also been shown (VOGT, 1954; FUXE, OWMAN, 1965) and some of the cells have chromaffin qualities (HÖJEBERG, 1962).

4. It is known from neurosurgical practice (HÖJEBERG, 1962) that there is a relationship between changes caused by tumours or operations of the caudal part of the fourth ventricle and the simultaneous appearance of acute ulcers in the upper part of the digestive tract. Some authors (BRIZZEE, BORISON, 1952; see also WANG, 1965) localized in the area postrema the "trigger zone" of the vomiting reflex.

The function of the area postrema is still under discussion; chemoreception, cerebrospinal fluid absorption, metabolic changes between blood and the cerebrospinal fluid and neurosecretion have been attributed to the area. It may be presumed that it performs many functions simultaneously. The close neighbourhood of the vegetative nuclei localized in the floor of the fourth ventricle is the cause of difficulties which arise when an attempt is made to isolate the area for purposes of study.

Only a few studies dealing with the detailed ultrastructure of the area postrema have been published: on the rabbit by SHIMIZU and ISHII (1964), on the cat by RIVERA-POMAR (1966) and on the rabbit by LEONHARDT (1967).

The present study deals with the electron microscopic description of the structure in the rat.

Material and methods

The area postrema has been studied in eleven healthy mature rats of both sexes weighing from 180 to 260 g. The material was obtained and fixed by two methods.

1. Under ether anaesthesia the skull was opened, the floor of the fourth ventricle was made accessible and prefixed *in situ* in 2% glutaraldehyde in 0.05 M Na-cacodylate buffer pH 7.2. The area postrema was excised under a dissecting microscope and fixed by immersion in the same fixative for one hour at 4°C. This was followed by postfixation in Caulfield's fluid.

2. 200 ml of a solution of 2% formaldehyde and 2.5% glutaraldehyde in 0.07 M Na-cacodylate buffer pH 7.2 (TÖRŐ jr., JOÓ, 1966; PAŘÍZEK, ŠPAČEK, 1967) with the addition of 0.135% $CaCl_2$ was perfused for 20 minutes through the left heart chamber under ether anaesthesia. Frontal sections of the region of the lower part of the fourth ventricle, containing the area postrema, were immersed for 40 minutes in the same fixative. The area postrema was isolated under the dissecting microscope and was fixed again in Caulfield's fluid.

The block was in both cases dehydrated and embedded in Vestopal W.

Ultra-thin sections were cut by the Reichert ultramicrotome, contrasted with uranyl acetate and lead citrate (PEASE, 1964) and examined and photographed under the Tesla BS 242 B electron microscope. An Orwo Elektronen Platte was used as negative material for photography.

Observations

The presence of small nerve cells in addition to a large number of glial elements, which we term astrocyte-like cells after SHIMIZU and ISHII (1964), was confirmed under the optical microscope in preparations stained with methylene blue (after PISCHINGER), gallocyanin (after EINARSON) and in Vestopal-embedded sections stained with paraphenylendiamine.

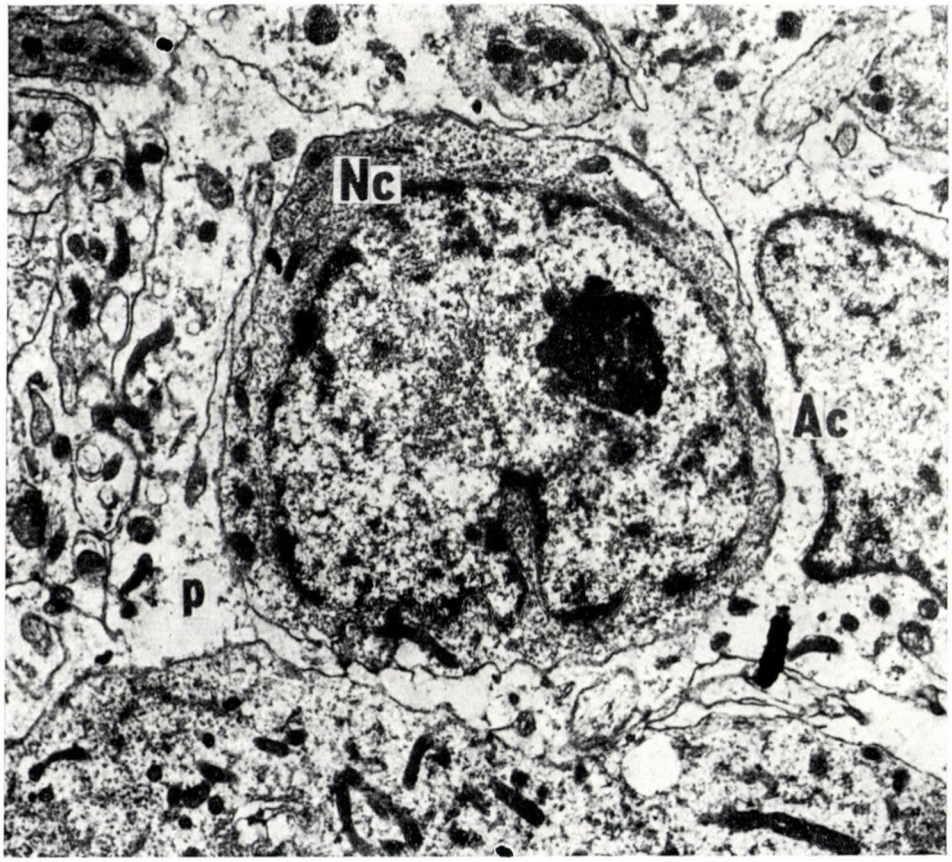

Fig. 1. Nerve cell from the area postrema of the rat (Nc). Nucleus shows an indentation. Part of astrocyte-like cell (Ac) and processes of astrocyte-like cells (p). ×7900

The electron microscopical picture showed that the cytoplasm of the nerve cells has on the whole the same ultrastructure as the rest of the nerve cells belonging to the central nervous system (Fig. 1). The majority of nuclei of the cells showed indentations. The chromatin of the nucleus was uniformly dispersed following fixation by perfusion; on the other hand, when fixation was performed by immersion of the preparation, the chromatin formed agglo-

merations mainly on the nuclear membrane. A thin granular endoplasmic reticulum formed only a few areas of Nissl's substance. A great number of free ribosomes, a well developed Golgi's complex, often multivesicular corpuscles and numerous lysosomes of different shape and content, the majority of which is delimited by a simple membrane, were discovered in the fundamental cytoplasm. The content of the lysosomes is either homogenous or granular, in some cases they contain vacuoles or agglomerations of myelin-like lamellae.

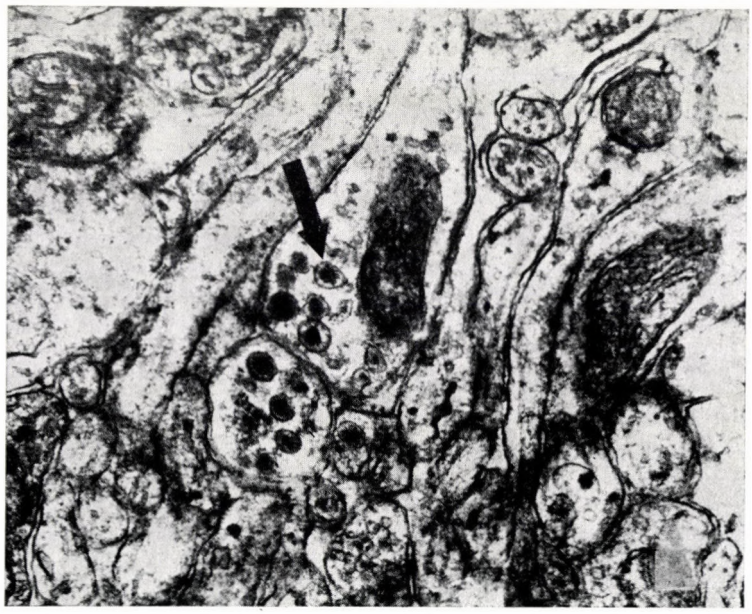

Fig. 2. Enlarged terminal part of the axon containing numerous granular vesicles (arrow). ×32 800

A small number of granulated vesicles, resembling those described in the sympathetic nerves, and considered to be catecholamine carriers (LENN, 1965), were regularly found in the cytoplasm of the perikaryon. The surface of the perikaryon was almost entirely covered by glial processes.

Granular vesicles grouped in rows between the neurotubules were discovered while studying the neuropil. Large numbers of them were situated in the distended parts of the axons (Fig. 2) and in the presynaptic sacs between the synaptic vesicles (Fig. 7).

Myelinized nerve fibres were present in small numbers and often contained formations resembling the degenerative bodies (Fig. 3). We discovered also what were probably terminations of the nerve fibres, with neurofilaments and neurotubules, numerous small shrunken mitochondria, variously formed

osmiophilic bodies, multivesicular bodies, lamellae with middle-line and typical vesicules with a dense centre (Fig. 4). These terminations were similar to the formations described by ROHR (1966) in SFO. This author found them also

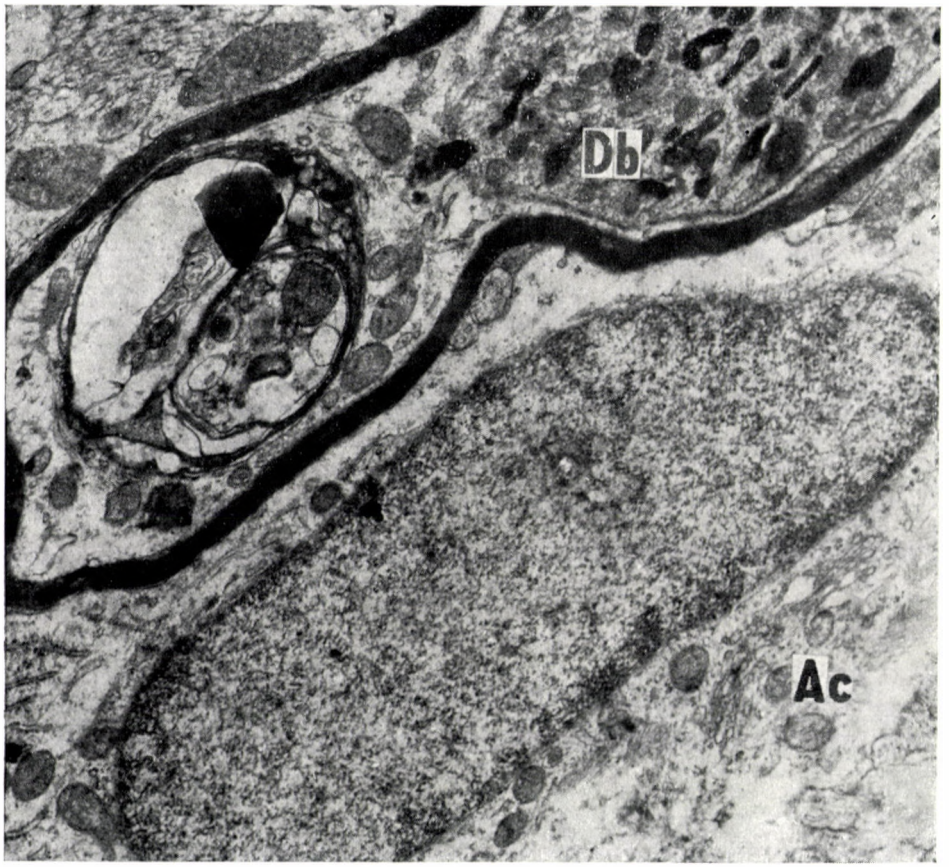

Fig. 3. Myelinized nerve fibre with formations resembling degenerative bodies (Db). Astrocyte-like cell (Ac). ×17 800

in the perivascular space and supposed that they were Hering's bodies with neurosecretory material.

The astrocyte-like cells were similar to the plasmatic astrocytes of the cerebral cortex (Fig. 5). The appearance of the nucleus was influenced by the method of fixation; the content of the nucleus formed dense agglomerations when fixation had been performed by immersion. When it had been accomplished by perfusion the content of the nucleus was finely dispersed and the

nuclei resembled those of ependymal cells. Astrocyte-like cells which contained a light thin cytoplasm, as well as cells with a dense cytoplasm, were found. We supposed that they were two different poles of the same cell. The cisterns of the endoplasmic reticulum were smooth or granular. The fundamental

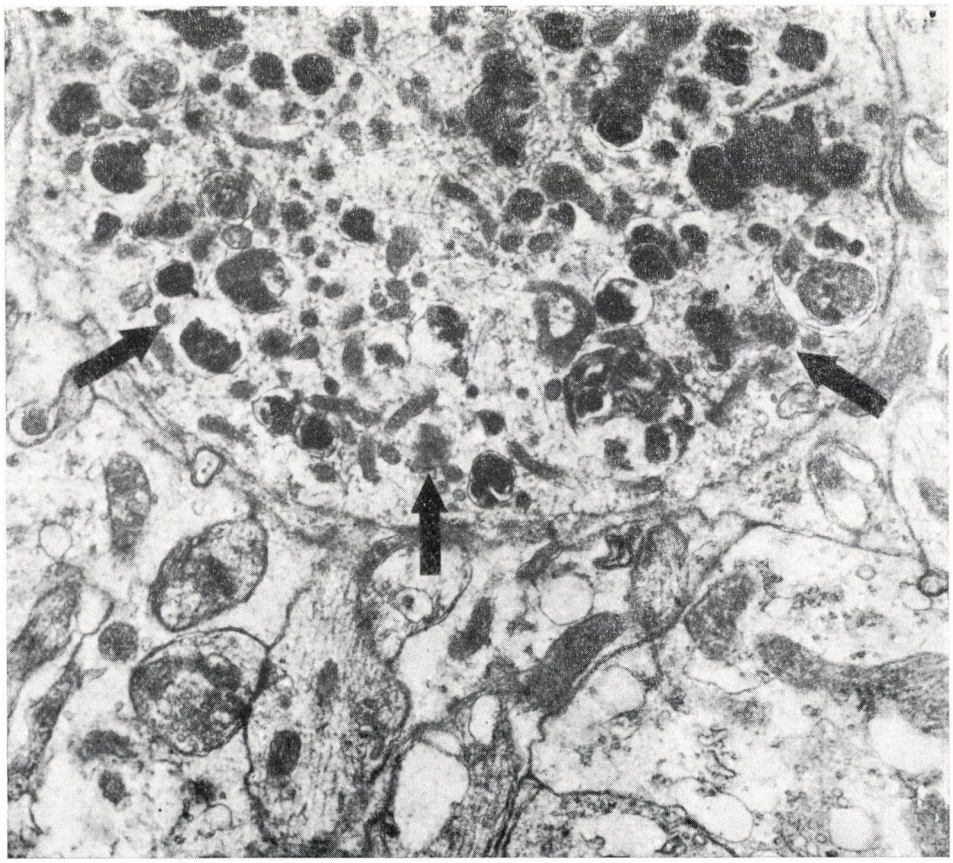

Fig. 4. Structure assumed to represent the terminal part of a special nerve fibre (arrows). ×16 300

cytoplasm contained a small number of free ribosomes and fine tubules. Some cells contained more and others less gliofilaments and practically always many lipofuscin bodies were present without membrane or other lysosomal bodies demarcated by a single membrane (Fig. 6). In most of these bodies we demonstrated acid phosphatase. The content of lysosomes are thus the source of the high acid phosphatase activity of the area postrema (ŠPAČEK, PAŘÍZEK, 1966).

In some cases we found in the processes of astrocyte-like cells formations consisting of smooth membranes coating a centrum of thickened cytoplasm. Similar formations were described as "fingerprints" in the SCO of sheep by BARLOW et al. (1967). Axodendritic synapses were often surrounded by flattened lamelliform processes of astrocyte-like cells (Figs 7 and 14).

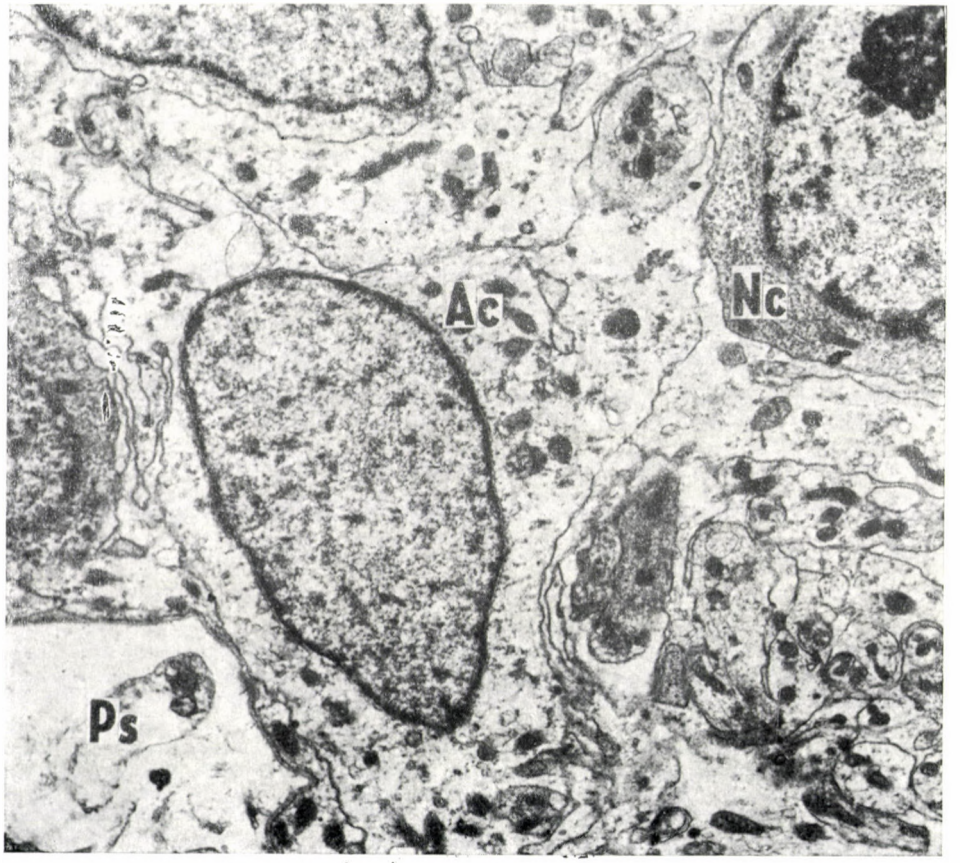

Fig. 5. Astrocyte-like cell (Ac) and part of a nerve cell (Nc). Perivascular space (Ps). ×7900

Oligodendroglial cells with a dark nucleus having a typical chromatin arrangement and with a dense cytoplasm containing many free ribosomes were present (Fig. 8). No microglial cells were discovered in our sections.

The surface of the area postrema facing the fourth ventricle was lined by the ependyma which here changed from typical cylindrical cells into flat cells and choroid plexus. Cylindrical cells had on their free surface microvilli, polypoidal protrusions, kinocilia and terminal bars. The nuclei resembled those of astrocyte-like cells, the mitochondria were long and often ramifying.

Flat ependymal cells had no kinocilia on their free surface, microvilli were rare and the nucleus was elongated parallel with the long axis of the cell. We found between these cells also elements with wide cisterns of endoplasmic reticulum with a content of low density (Fig. 9). These ependymal cells reminded of secretory cells.

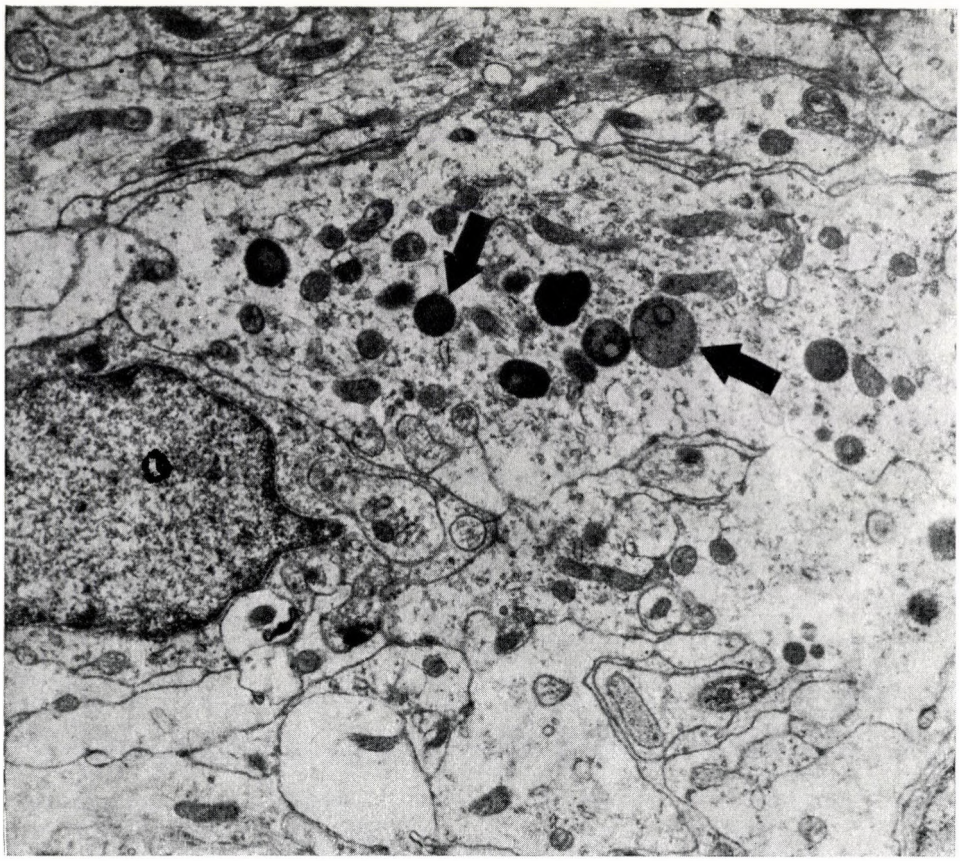

Fig. 6. Lysosomal bodies in the cytoplasm of astrocyte-like cell (arrows). × 11 200

The vascular system in the area postrema showed some similarity to the vascular pattern of some "specialized areas" connected with neurosecretion. According to our observations the area postrema is supplied by two types of vessel. The most typical and most frequent ones are blood capillaries of a sinusoidal character with a wide perivascular space (Fig. 10). The endothelium became thinner in some areas forming pores with a thin diaphragma (Fig. 11), and pseudopodia-like protrusions into the lumen in the neighbourhood of their terminal bars. A perivascular space was marked off by the inner

basement membrane under the endothelium and by the outer basement membrane of the parenchyma proper. In this space we found pericytes, fibrocytes, macrophages, or undifferentiated peri-capillary cells and bundles of collagenous fibrils (Fig. 12). Endothelial cells and pericytes contained a large number of pinocytotic vesicles. On the outer basement membrane only glial

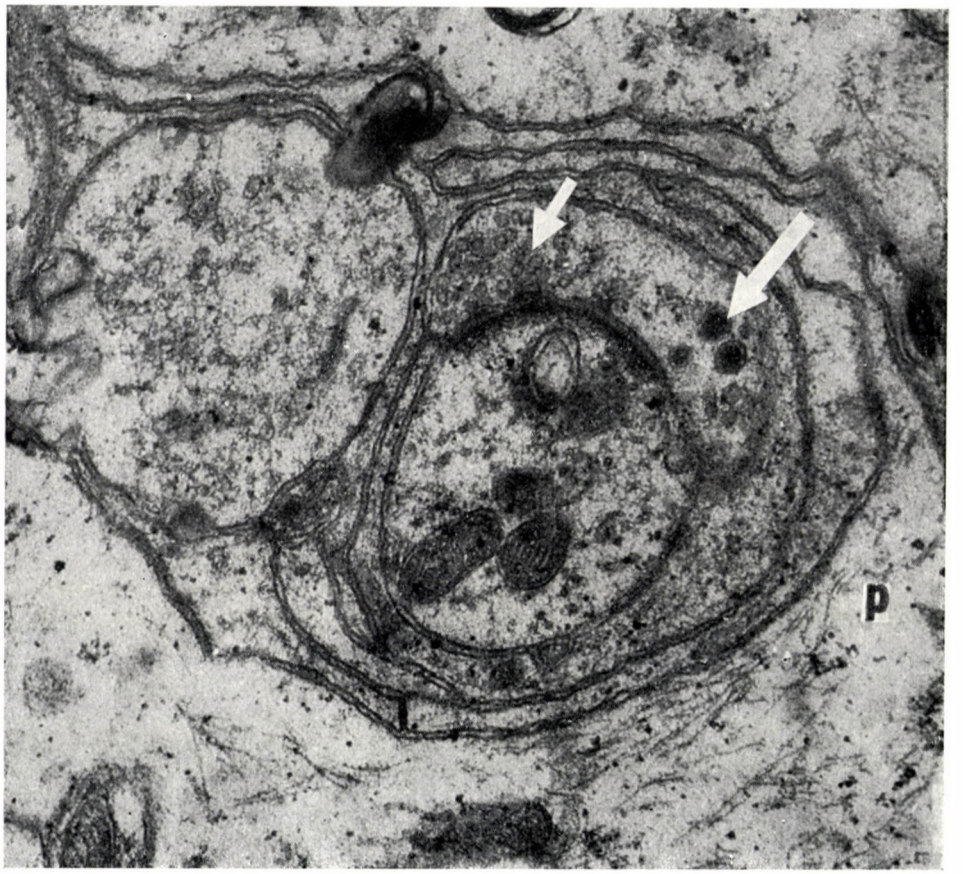

Fig. 7. Flattened lamelliform processes of astrocyte-like cell surrounding the axo-dendritic synapse (p). Small synaptic vesicles (the small arrow) and large ones with a dense centre (the large arrow). ×50 700

processes were located which often penetrated into the perivascular space and formed there a labyrinth (Fig. 13). The cytoplasm was thickened in these penetrating processes (Fig. 14). In the periphery of the area postrema the glial cells and their processes were reduced in number, the tissue resembled other areas of the central nervous system. There were wide sinusoidal capillaries and their thick basement membrane expanded in some cases into the paren-

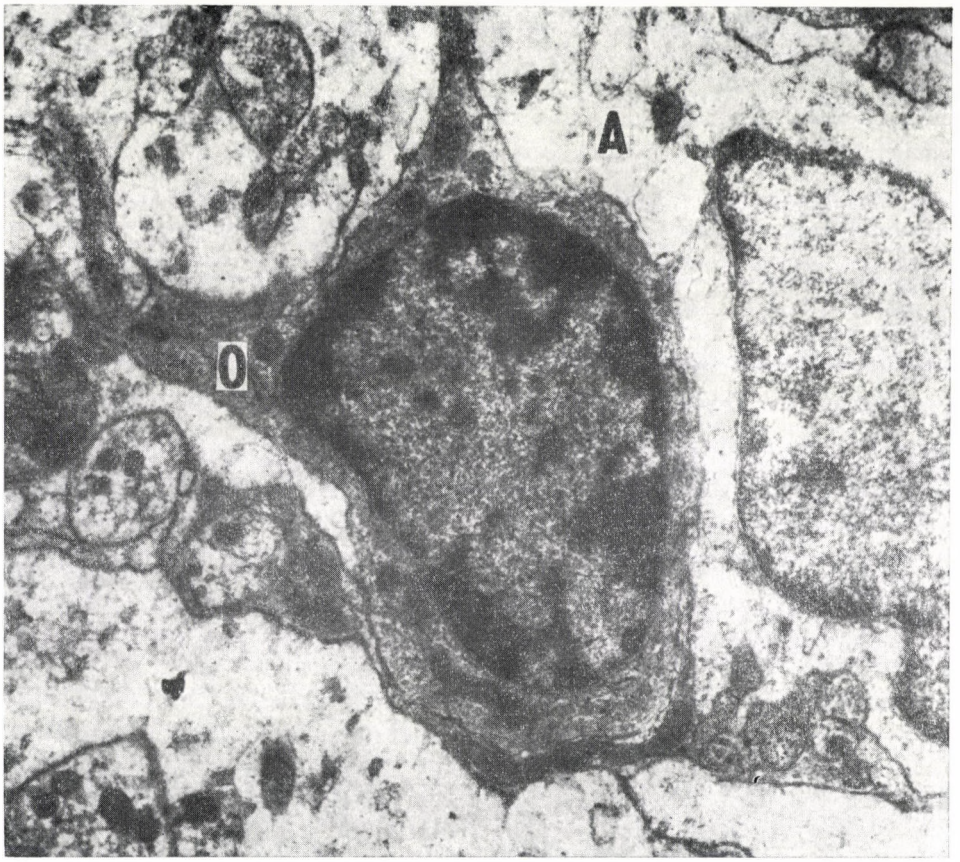

Fig. 8. Oligodendroglial cell (O) and part of astrocyte-like cell (A). ×16 300

chyma. We saw no perivascular space and no pores in their endothelium. Their basement membrane was surrounded by glial processes between which we sometimes found close contacts.

On the nerve cells bodies, axo-somatic synapses were discovered; their presynaptic bags reached a considerable size. Presynaptic bags of numerous axo-dendritic synapses (Fig. 15) were filled in this peripheral area with small synaptic vesicles and large vesicles with a dense centre (granulated vesicles).

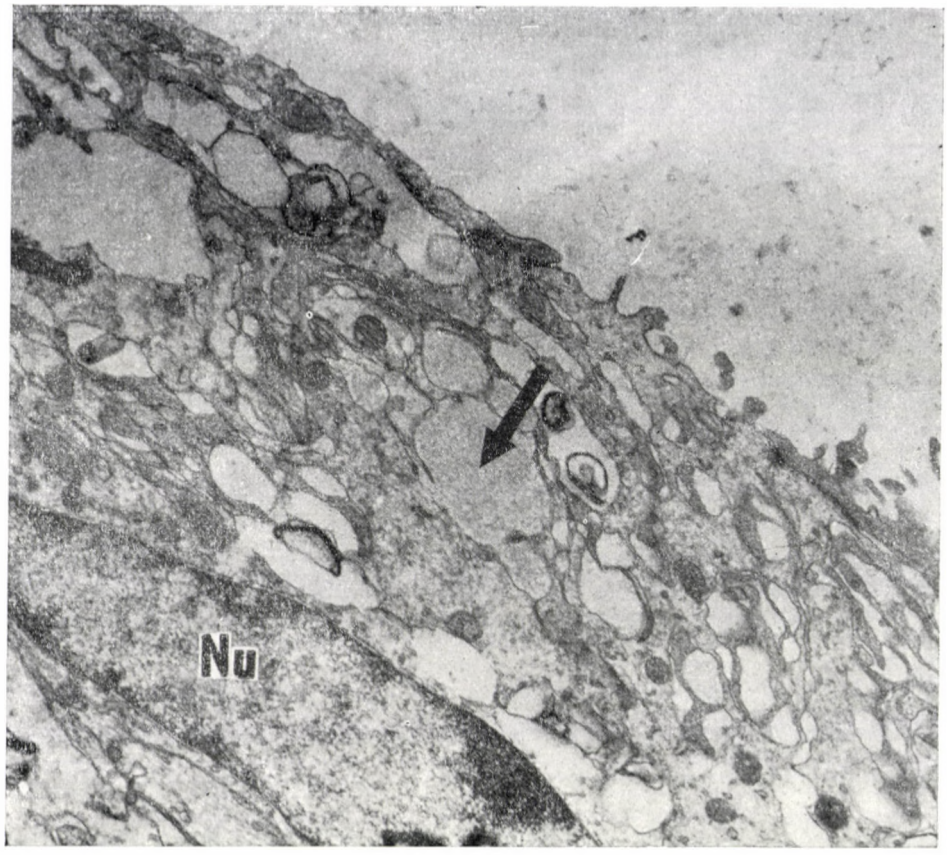

Fig. 9. Part of a flat ependymal cell with a wide cistern of endoplasmic reticulum (arrow) Nucleus (Nu). ×12 200

Discussion

The selective excision of such minute structures as the rat's area postrema represents a problem; still, its appearance, shape and position provided the possibility of obtaining samples with the exclusion of areas lying below it.

In all the cases when the area postrema has been isolated from the medulla oblongata and fixed by immersion, practically all nerve cells were shrunken. This shrinkage could be avoided by fixation by perfusion. This shows the high sensitivity of such cells to influence of external factors. Other nerve cells, for example the pyramid cells of the cerebral cortex, never showed such considerable shrinkage when fixed by immersion.

KING (1937) did not discover nerve cells in the area postrema of the cat and considered all cells to be spongioblasts and astroblast-like cells. CAMMER-

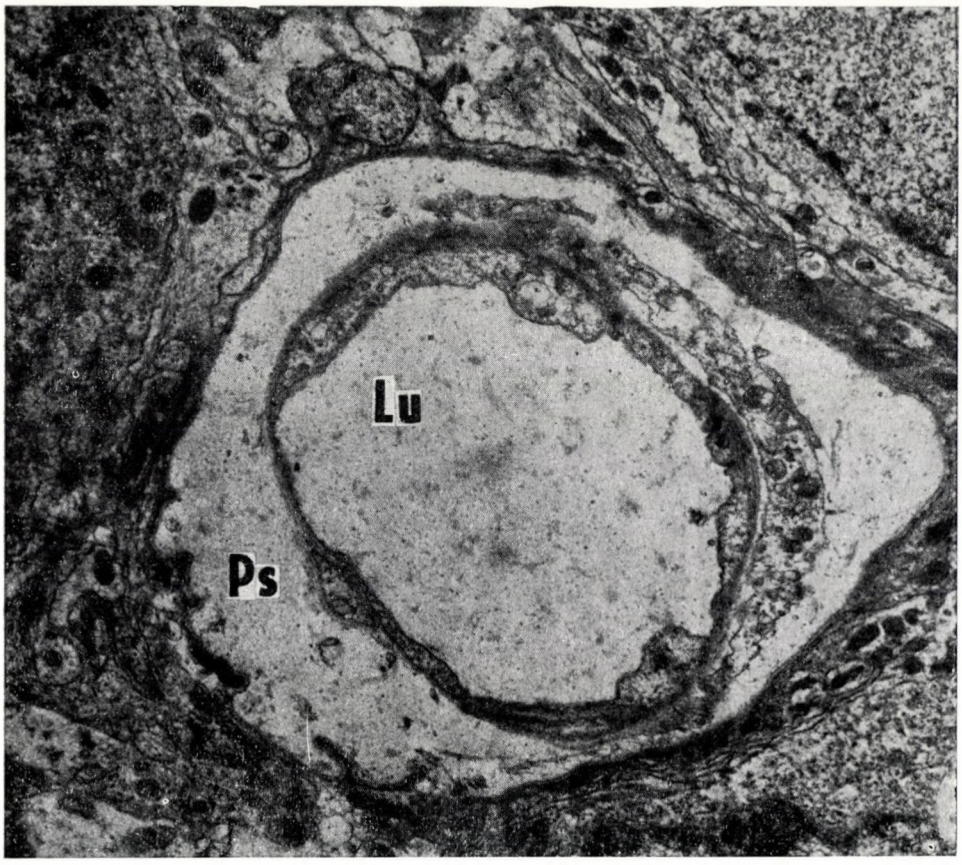

Fig. 10. Blood capillary with wide perivascular space. Capillary lumen (Lu), perivascular space (Ps). ×8400

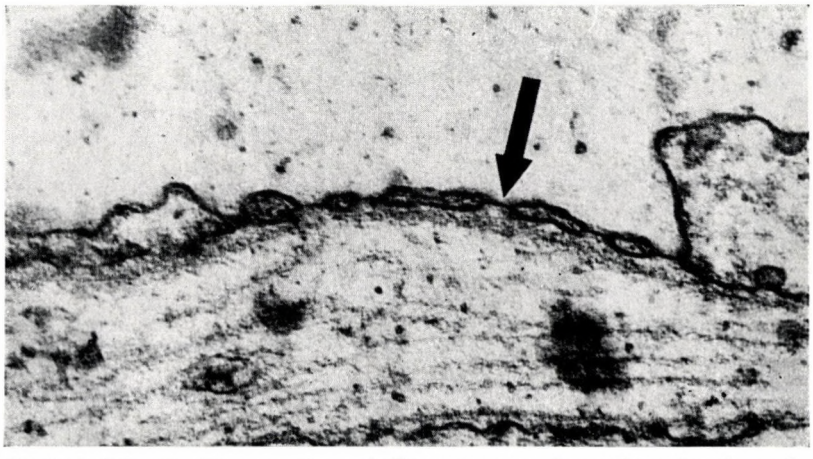

Fig. 11. Endothelial cell with pores (arrow). Basement membrane is under the endothelium. ×62 400

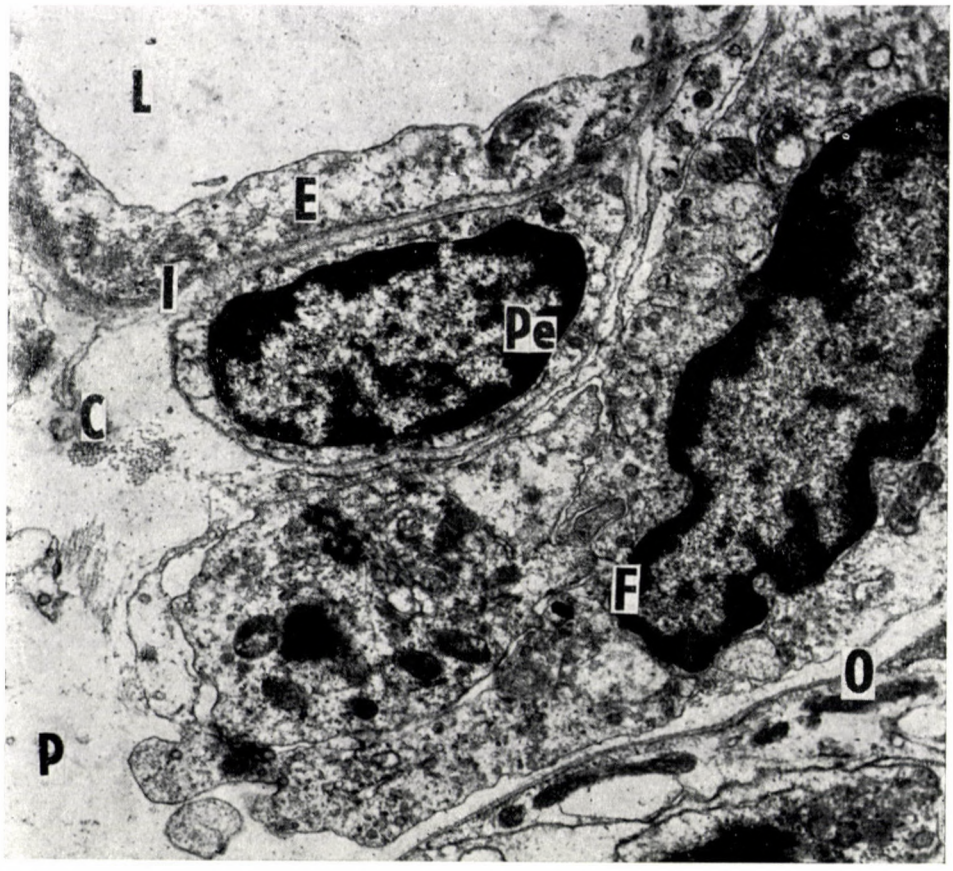

Fig. 12. A perivascular space. Capillary lumen (L), endothelial cell (E), inner basement membrane (I), perivascular space (P), collagenous fibrils (C), pericyte (Pe), other pericapillary cell without basement membrane on its surface (F), outer basement membrane (O). ×11 500

MEYER (1947) described nerve cells in the area postrema of mice and rabbits. WISLOCKI and LEDUC (1952) found nerve cells in man; their presence in animals is not certain according to these authors. BRIZZEE and NEAL (1954) found nerve cells and glial cells, called glia-like cells, in cats. IIJIMA et al. (1963) considered all cells found in the area postrema to be neuroglial cells; SHIMIZU and ISHII (1964) found nerve cells by optical and electron microscopy. These authors classified the glial cells, which other authors retarded as immature elements, as mature and differentiated cells because, as was shown by SMART (1961), they do not incorporate ^{3}H thymidine, and termed them astrocyte-like cells.

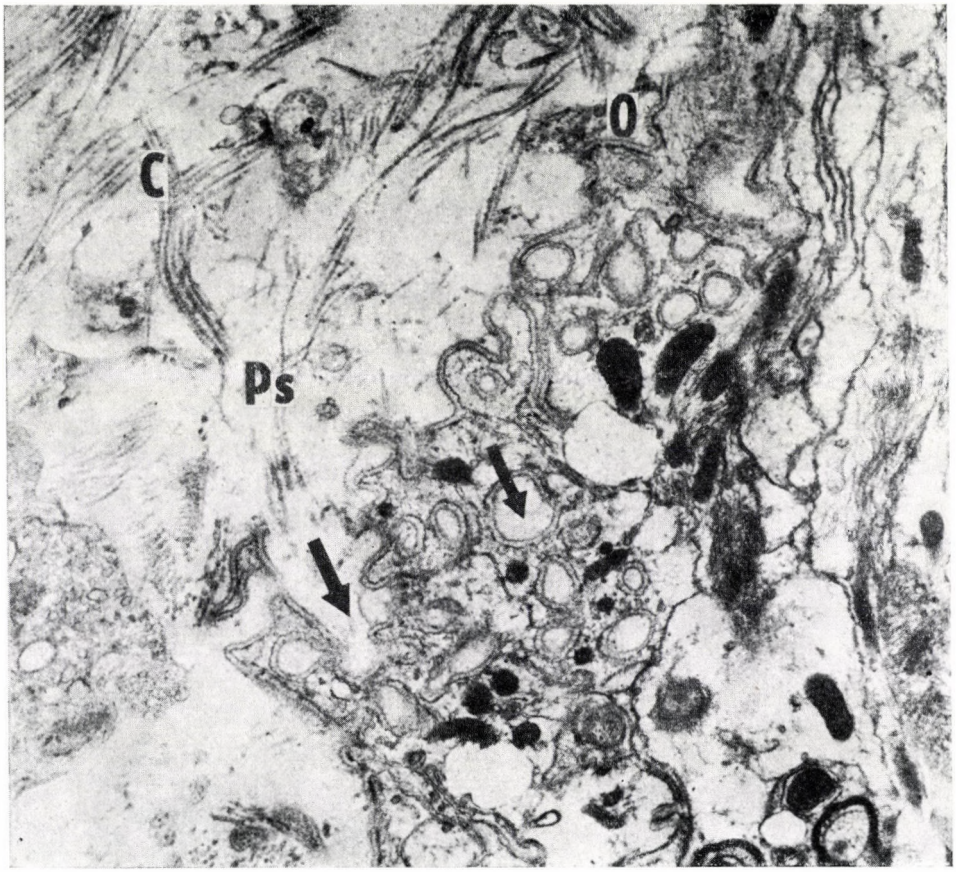

Fig. 13. Labyrinth of a perivascular space. Perivascular space (Ps), collagenous fibrils (C), outer basement membrane (O), labyrinth (arrows). ×12 200

We could demonstrate nerve cells in agreement with the findings of SHIMIZU and ISHII (1964). Granular vesicles were found in a small number in their perikaryon and in large numbers in the axons. There were granular vesicles in the presynaptic sacs. Axo-somatic synapses on the body of nerve cells of the cat were described by RIVERA-POMAR (1966); we confirmed this only in the peripheral parts but could not be quite certain whether or not these belonged to the area postrema because in our sections we could not find any distinct morphological border.

WISLOCKI and PUTNAM (1924) reported on the intravital staining by acid dyes of the area postrema and VAN BREEMEN and CLEMENTE (1955) and DEM-

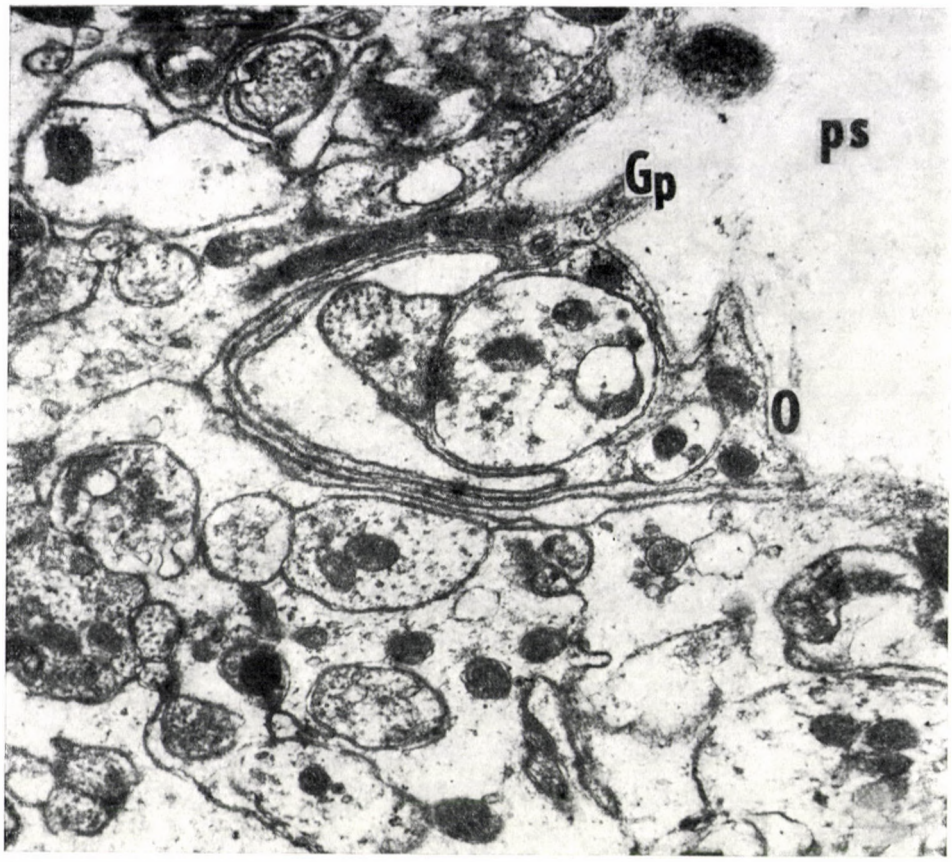

Fig. 14. Glial processes penetrating into the perivascular space (Gp). Perivascular space (ps), outer basement membrane (O). Axo-dendritic synapse surrounded by lamelliform glial processes next to perivascular space. ×26 200

PSEY and WISLOCKI (1955) found silver deposits in the neighbourhood of vessels after the administration of $AgNO_3$. This proves their changed vascular permeability. MORATO and FERREIRA (1957) described a broad perivascular space in the area postrema of the rabbit; SHIMIZU and ISHII (1964) and LEONHARDT (1967) found a wide perivascular space around the capillaries. This space contained collagenous fibres, pericytes and also terminal axons supplied with a basement membrane. We did not succeed in showing the terminal axons in the perivascular spaces, nor could RIVERA-POMAR (1966) find them in the cat. The pores in the endothelial cells of the capillaries in the area postrema were found also by RIVERA-POMAR (1966) in the cat and by LEONHARDT (1967) in the

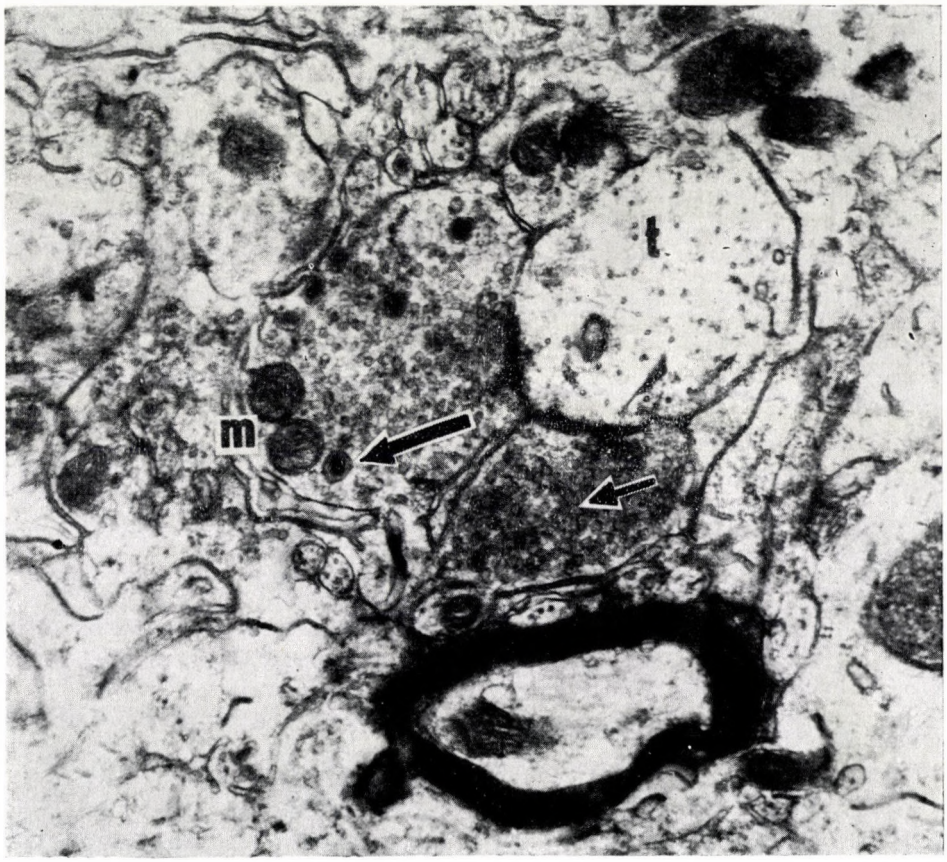

Fig. 15. Axo-dendritic synapses at the periphery of the area postrema. Dendrite with cross sectioned neurotubules (t), two large presynaptic bags with mitochondria (m), small synaptic vesicles (small arrow) and large ones with a dense centre (large arrow). ×27 000

rabbit. Similar capillaries with perivascular spaces or pores were described by ROHR (1966) in the subfornical organ and by SCHWINK and WETZSTEIN (1966) in the subcommissural organ.

The lamellar glial sheaths surrounding the axo-dendritic synapses observed by us have not yet been described in area postrema, nor have the special ependymal cells and special nerve fibres been mentioned in the literature.

Consequently, it will be necessary to study the area postrema in a topographical relationship to the nervous nuclei localized below it. It will be also necessary to take into consideration the shape differences, the differences connected with the presence of enzymes, the age and sex of the investigated

individuals, and also the influence of some biological rhythms and the close relation to the ependyma of the fourth ventricle.

*

The authors are indebted to Professor J. HROMADA and Professor V. VRTIŠ for valuable advice, to Mrs. E. MATĚJKOVÁ for technical assistance, and to Dr. Z. CHARVÁT for kind supervision of the English text.

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DIE ULTRASTRUKTUR DER AREA POSTREMA DER RATTE

J. ŠPAČEK und J. PAŘÍZEK

Die Ultrastruktur der Area postrema der Ratte wurde untersucht. Es wurde festgestellt, daß die kleinen Nervenzellen von Astrozytoidzellen und deren Fortsätze umgeben sind. Das Cytoplasma der Gliazellen enthält viele Lysosomen, von denen die meisten ein Reaktionsprodukt der Gomori-Bleimethode für den elektronenmikroskopischen Nachweis der sauren Phosphatase aufweisen. Die Gliafortsätze bilden lamelläre Scheiden um die axodendritischen Synapsen. In den meisten Synapsen werden große granuläre Vesikel beobachtet. Die speziellen Nervenfasern enthalten kleine geschrumpfte Mitochondrien, osmiophile Körperchen und granuläre Vesikel. Axosomatische Synapsen wurden nur an den peripheren Rändern der Area postrema festgestellt. Einige flache Ependymzellen mit breiten endoplasmatischen Retikulumzisternen erinnern an die sekretorischen Zellen.

УЛЬТРАСТРУКТУРА AREA POSTREMA У КРЫСЫ

Й. ШПАЦЕК и ПАРИЗЕК

При изучении ультраструктуры area postrema крысы было установлено, что небольшие нервные клетки окружены астроцитоидными клетками и их отростками. Цитоплазма клеток невроглии содержит множество лизосом, большинство которых дает продукт реакции со свинцовым методом Гомори для электронномикроскопического выявления кислой фосфатазы. Отростки глии образуют пластинчатые влагалища вокруг аксодендритных синапсов. В большинстве синапсов наблюдаются крупные зернистые пузырьки. Специфические нервные волокна содержат небольшие, сморщенные митохондрии, осмиофильные тельца и зернистые пузырьки. Аксосоматические синапсы были обнаружены лишь в периферических частьях area postrema. Некоторые плоские эпендимальные клетки с широкими эндоплазматическими ретикулярными цистернами напоминают секреторные клетки.

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EFFECT OF A SINGLE NEONATAL DOSE OF ACTH, TSH, STH, THYROXINE AND ALDOSTERONE ON SERUM HEPARIN AND TISSUE MUCOPOLYSACCHARIDES

K. VALLENT and M. PALKOVITS

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A single neonatal dose of ACTH decreased, and one of TSH increased, the serum heparin level. A single neonatal dose of aldosterone induced a significant increase in the acid mucopolysaccharide contents of cartilage. PAS-positivity was enhanced by ACTH. TSH and thyroxine slightly increased the amount of mucopolysaccharides in cartilage. A single neonatal dose of ACTH and thyroxine intensified the PAS reaction in the thymus whereas TSH and STH had no such effect.

The endocrine balance in neonatal age may play a role in determining the amount of mucopolysaccharides contained in blood and tissues.

Neonatal thymectomy (MILLER 1961, GOOD et al. 1962; FACHET et al. 1965) as well as neonatal treatment with glucocorticoids (SCHLESINGER and MARK 1964; FACHET et al. 1966) are known to cause permanent inhibition of growth and involution of the lymphatic organs. It was demonstrated earlier (VALLENT et al. 1968) that a single neonatal dose of cortisol produced a chronic decrease in the serum and tissue mucopolysaccharide contents. It was also shown (FACHET et al. 1966) that a single injection of glucocorticoid into newborn rats delayed the rejection of homologous skin grafts. Several authors have proved that immune reactions are accompanied by an elevation of the mast cell count (WINQVIST 1960; KELLER 1966) and of the blood and tissue mucopolysaccharide level (SONNET 1955; FEHÉR et al. 1966). It has been suggested that the equilibrium between the pituitary-adrenal and the thymo-lymphatic systems is of a biological importance in the neonatal period (FACHET et al. 1967).

The present experiments were designed to study in the rat the effect of ACTH, TSH, STH, thyroxine and aldosterone on the serum and tissue mucopolysaccharide level.

Material and methods

Sixty-one newborn Wistar rats, with body weights of 8 ± 2 g, were divided into six groups and subjected on the first day of life to the following intraperitoneal treatments:

1. ACTH 0.2 I.U. (Organon OSS)
2. TSH, lyophilised 0.2 I.U. (Calbiochem, Los Angeles, Calif.)
3. STH, lyophilised 1.0 mg (Calbiochem, Los Angeles, Calif.)
4. Aldosterone 20 μg (CIBA, Basel)

5. Thyroxine 2.0 μg (Hoffmann-La Roche, Basel)
6. Control animals 0.9% physiological saline solution, 0.1 ml.

The rats were decapitated on the 28th day of life. The blood heparin level was determined in 0.1 ml of fresh serum on the evidence of the thrombin-inactivation time (Gerendás 1946) by a method described earlier (Vallent et al. 1966). The thymus, spleen and suprarenal gland were weighed on a torsion balance. For histological examination the thymus, spleen, liver, skin, and xyphoid process were fixed in neutral formalin for two days, embedded in paraffin and 6 μ sections were stained with haematoxylin-eosin and combined PAS-alcian blue. Alcian blue staining for 30 min. was followed by periodic acid treatment for 5 min., then PAS reaction for 10 to 20 min., treatment with metabisulphide and 3 min. contrasting with haemalaun. Blood smears were stained according to May–Grünwald–Giemsa.

Results

A single neonatal dose of ACTH reduced ($p < 0.01$), while TSH elevated ($p < 0.01$) the blood heparin level. STH and thyroxine had no effect on serum heparin, while aldosterone slightly raised its level (Table I).

Table I

Effect of a single neonatal dose of ACTH, TSH, STH, thyroxine and aldosterone on the serum heparin level, 28 days after treatment

Groups	Number of animals	Serum heparin, μg/ml	Significance
I. NaCl, 0.9%	15	15.3±0.4	
II. ACTH, 0.5 I.U.	10	13.7±0.3	$p < 0.01$
III. TSH, 0.2 I.U.	10	17.1±0.2	$p < 0.01$ (I—III)
IV. STH, 1.0 mg	8	16.4±0.3	
V. Thyroxine 2 μg	10	15.0±0.4	
VI. Aldosterone 20 μg	8	16.2±0.3	$p > 0.10$ (I—VI)

A single neonatal injection of aldosterone increased the amount of acid mucopolysaccharides in the cartilaginous tissue. ACTH intensified the PAS reaction, a phenomenon indicative of increased neutral mucopolysaccharide contents. TSH and thyroxine increased the positivity of the PAS and alcian blue reactions (Fig. 1).

The thymus became slightly more PAS positive after ACTH and thyroxine administration. Thyroxine produced a loosening of the thymic medulla and a relative reduction in the number of thymocytes (Fig. 2).

Examination at 28 days revealed no effect of STH on the mucopolysaccharide contents of cartilage. Under the given experimental conditions neither STH nor TSH had any effect on the mucopolysaccharides of the thymus. No significant changes were registered in spleen, thymus and adrenal weight, nor in the qualitative blood picture.

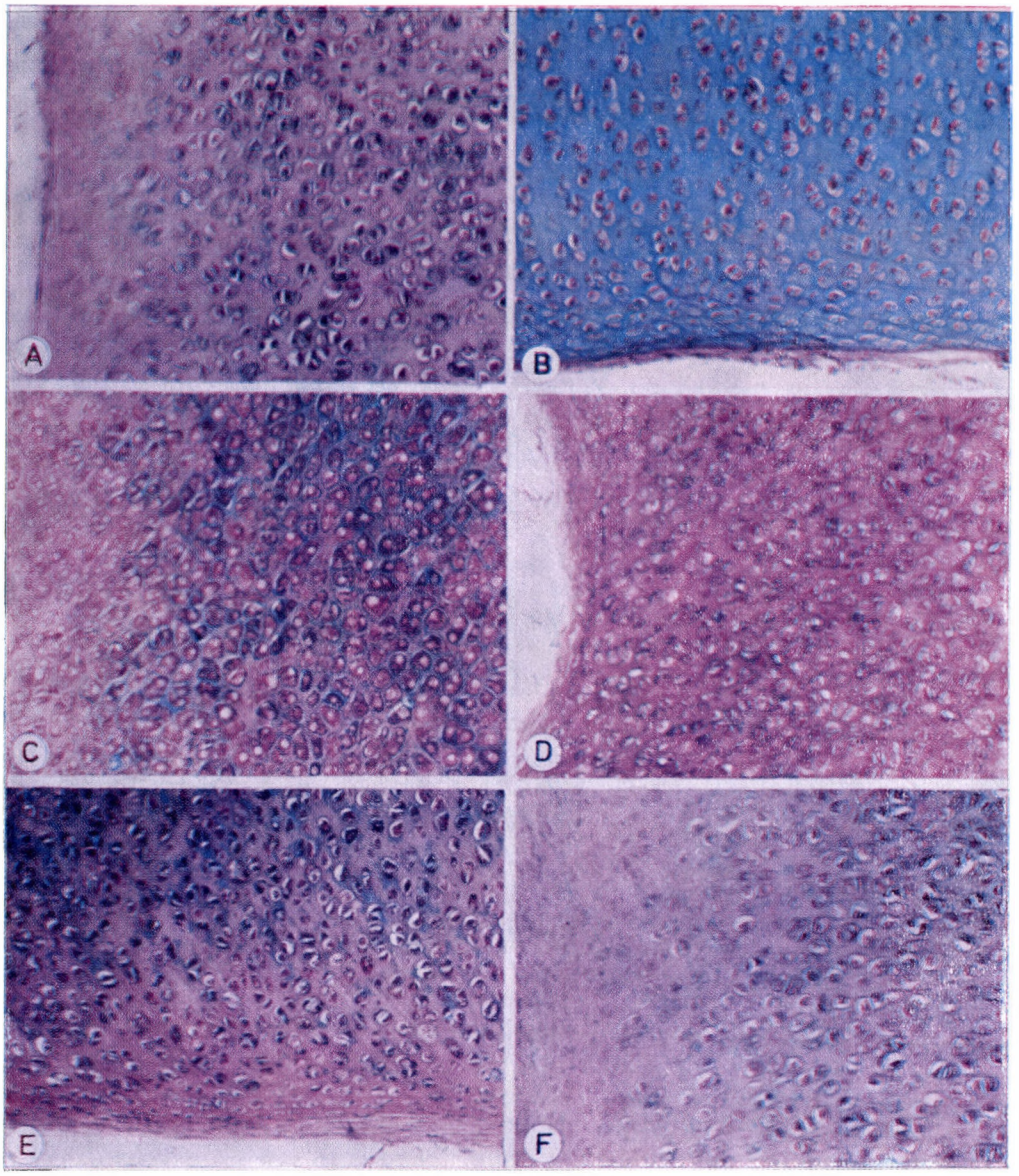

Fig. 1. Effect of a single neonatal dose of aldosterone, TSH, ACTH, thyroxine and STH on the mucopolysaccharide content of cartilage. (Combined PAS–alcian blue staining, ×340.)
A = NaCl 0.9%; B = aldosterone; C = TSH; D = ACTH; E = thyroxine; F = STH

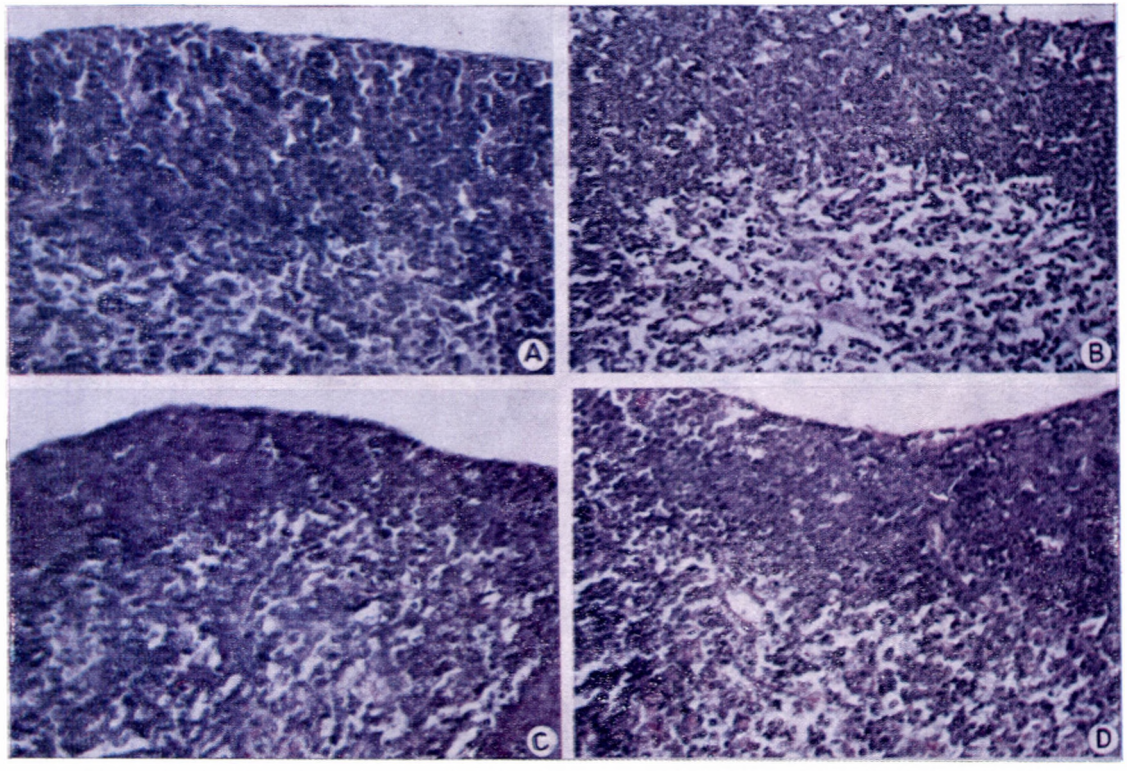

Fig. 2. Effect of a single neonatal dose of TSH, thyroxine and ACTH on the mucopolysaccharide content of thymus. (Combined PAS-alcian blue staining, ×340.) A = NaCl 0.9%; B = TSH; C = thyroxine; D = ACTH

Discussion

A single neonatal dose of cortisol was shown by SCHLESINGER and MARK (1964) to induce fatal cachexia in mice and by Fachet et al. (1966) to cause permanent involution of the thymo-lymphatic apparatus in the rat. Numerous symptoms of these conditions resemble those seen in the "wasting-syndrome" following neonatal thymectomy. SCHAPIRO (1965) observed similar phenomena after repeated doses of glucocorticoid given in the early days of life, while in adult animals glucocorticoid was found to induce but a temporary (1 to 5 days) involution of the lymphatic organs (DOUGHERTY and WHITE 1943).

The questions of mucopolysaccharide metabolism and regulation are being debated but it stands clear from KROMPECHER's investigations (1960) that hypoxia has a significant role in their regulation, while several authors (MONKHOUSE 1956; DAVIDSON 1964; LORENZEN and ZACHARIAE 1966; VALLENT et al. 1966) attribute significance in this respect also to endocrine functions. Still other authors emphasize the importance of fibroblast in this connection (BERNSON and DALFERESE 1960; KENNEDY 1960).

In contrast to glucocorticoids, the other hormones employed in the present experiments failed to induce any significant change in the weight of the animals and the examined organs, whereas changes in the blood and tissue mucopolysaccharide contents induced by ACTH, TSH, and aldosterone were still demonstrable after 28 days. Several authors observed thymo-lymphatic hyperplasia and an elevation of the blood and tissue acid mucopolysaccharide level (GYLLENSTEN 1953; FACHET et al. 1964; JÓZSA et al. 1964; VALLENT et al. 1966) following treatment with TSH and thyroxine. It was shown that the thymus contained an excessive number of mast cells (FREEMAN et al. 1956; ARNESEN-KRISTEN 1958), much PAS positive matter (ARNESEN-KRISTEN 1958) and heparin (CHARLES and SCOTT 1933).

A single neonatal dose of thyroxine increased the PAS positive contents of the thymus in the present experiments. It is noteworthy that this phenomenon occurred also after the administration of ACTH. The question thus arises why ACTH did not have an effect like that of the glucocorticoids although it is known to mobilize the glucocorticoids. It is likewise known that the suprarenal gland of newborn animals responds vigorously to ACTH (MITRO and PALKOVITS 1967). The presumable explanation is that the amount of glucocorticoids mobilized by ACTH is less than that employed by FACHET et al. (1966) and SCHLESINGER and MARK (1964) or else endogenous and exogenous glucocorticoids may act in a different way on the thymo-lymphatic system and the mucopolysaccharides in blood and tissues.

The biological significance of the neonatal period is confirmed by the fact that in adult animals the same compounds fail to induce similar changes (VALLENT 1967). The present observations justify the conclusion that a well-

balanced functioning of the endocrine glands during the neonatal period may play a role in determining the amount of mucopolysaccharide in blood and tissues.

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DIE WIRKUNG EINMALIGER VERABREICHUNG VON ACTH, TSH, STH, THYROXIN ODER ALDOSTERON IM NEUGEBORENENALTER AUF DEN SERUMHEPARINSPIEGEL UND DEN MUKOPOLYSACCHARIDENGEHALT IN DEN GEWEBEN

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Einmalige ACTH-Injektion im Neugeborenenalter bewirkt die Herabsetzung, TSH-Injektion jedoch den Anstieg des Serumheparinspiegels. Aldosteron-, STH- oder Thyroxingabe hatte keinen signifikanten Einfluß auf den Serumheparinspiegel (28 Tage nach der Injektion).

In der Grundsubstanz des Knorpelgewebes führt im Neugeborenenalter einmalige Aldosteronadministration einen erheblichen Anstieg des sauren Mukopolysaccharidengehalts herbei. Nach ACTH-Zufuhr erhöhte sich die PAS-Positivität, die auf einen neutralen Mukopolysaccharidengehalt hinweist. TSH-oder Thyroxinzufuhr bewirkte einen mäßigen Anstieg im Mukopolysaccharidengehalt des Knorpelgewebes.

Im Neugeborenenalter bewirkt einmalige ACTH- oder Thyroxin-Injektion die Erhöhung der PAS-Positivität der Thymusdrüse, während TSH oder STH — 28 Tage nach der Administration — keinen Einfluß auf den Mukopolysaccharidengehalt ausübten.

Im Neugeborenenalter dürfte der ausgeglichenen Funktion der endokrinen Drüsen eine Rolle in der Gestaltung des Mukopolysaccharidengehaltes der Gewebe und des Blutes zukommen.

ДЕЙСТВИЕ ОДНОКРАТНОГО ВВЕДЕНИЯ АКТГ, ТТГ, СТГ, ТИРОКСИНА ИЛИ АЛЬДОСТЕРОНА В ПЕРИОД НОВОРОЖДЕННОСТИ НА СОДЕРЖАНИЕ ГЕПАРИНА В СЫВОРОТКЕ И МУКОПОЛИСАХАРИДОВ В ТКАНЯХ

К. ВАЛЛЕНТ и М. ПАЛКОВИЧ

Однократная инъекция АКТГ, введенная в период новорожденности, понижает содержание гепарина в сыворотке, а инъекция ТТГ повышает его. Через 28 дней после введения альдостерона, СТГ или тироксина не наблюдается достоверного изменения содержания сывороточного гепарина.

Однократная инъекция альдостерона в период новорожденности в существенной мере повышает содержание кислых мукополисахаридов в основном веществе хрящевой ткани. После введения АКТГ повышается ПАСК-положительность, указывающая на нейтральное содержание мукополисахаридов. Введение ТТГ или тироксина вызывает умеренное повышение содержания мукополисахаридов в хрящевой ткани.

Однократное введение АКТГ или тироксина новорожденным повышает ПАСК-положительность зобной железы, в то время как через 28 дней после введения ТТГ или СТГ нельзя выявить изменения содержания мукополисахаридов.

В период новорожденности уравновешенная функция внутрисекреторных желез, по-видимому, играет роль в отношении содержания мукополисахаридов в крови и в тканях.

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ADENOSINE TRIPHOSPHATASE ACTIVITY IN THE INITIAL PHASE OF FRACTURE HEALING

A HISTOCHEMICAL STUDY ON RATS*

J. RAEKALLIO and M. KOVÁCS

(Received May 14, 1968)

Adenosine triphosphatase (ATPase) activity was investigated histochemically in the healing experimental fractures of rats. The animals were sacrificed 1, 2, 4, 8, 10, 12 and 16 hours, and 1, 2, and 3 days after fracturing the right tibia. In a peripheral zone situated at a distance of 200—500 μ and farther away from the fracture line, ATPase activity began to increase 10 hours after injury in the osteoblasts and osteogenic cells of the periosteum. Proliferation of these cells after 16 hours further enhanced ATPase activity which then persisted throughout the experimental period. Similar phenomena were noticed in the endosteum.

Since the description by GOMORI of the histochemical demonstration of alkaline phosphatase considerable work has been reported in reference to the activity of this enzyme in later phases of fracture repair [3]. There are, however, few reports on the appearance of adenosine triphosphatase (ATPase) in healing fractures. Further, the view still prevails that an inert latent period occurs up to the second or third day after fracturing [2, 5]. This seems to be due to lack of investigations into the earliest phase of healing. On the other hand, the activity of several enzymes, including ATPase, has been histochemically demonstrated to increase during the very first postoperative hours in healing skin wounds [7, 8, 9, 10, 11]. To study the appearance of ATPase in the initial phase of fracture healing, an experimental investigation was made, demonstrating the enzyme histochemically.

Material and methods

4-month-old albino rats of both sexes were used. In ether anaesthesia the right tibia of each animal was fractured in the mid-diaphyseal region by digital pressure. Groups of three rats were killed 1, 2, 4, 8, 10, 12 and 16 hours, and 1, 2 and 3 days after fracturing.

The fractured tibias were removed immediately after sacrificing, and most of the soft tissues were dissected away from the bones. The unfixed tibias were demineralized for 48 hours in 10% disodium ethylenediaminetetraacetate (EDTA) in 0.1 M phosphate buffer pH 7.2 [15]. The EDTA solution with the fractured bone was kept at +4 °C, slowly circulated by a magnetic stirrer and changed every 24 hours.

The demineralized tibias were frozen on a block of dry ice, attached to a chuck and cut at 16 μ in a cryostat. The frozen sections were placed on albuminized slides, kept at room temperature, oriented, thawed, dried in a cool air stream, and fixed for 10 minutes in cold

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(—2 °C to —3 °C) 10% formalin, buffered with sodium acetate and adjusted to pH 7.2. The sections were washed and incubated for 30 minutes at +37 °C in the lead—ATP medium of WACHSTEIN and MEISEL [6]. After washing, the sections were treated with 1% yellow ammonium sulphide for three minutes, rewashed, and mounted in glycerine jelly. Control sections were incubated without the substrate. In addition, alternate sections were stained by the van Gieson technique for histologic study.

Results

The sites of ATPase activity stained brownish-black. In the uninjured bone (far from the fracture end) the cytoplasm of the osteoblasts and osteoclasts demonstrated various degrees of intracellular activity. The vessel walls stained intensively. The osteocytes showed no activity.

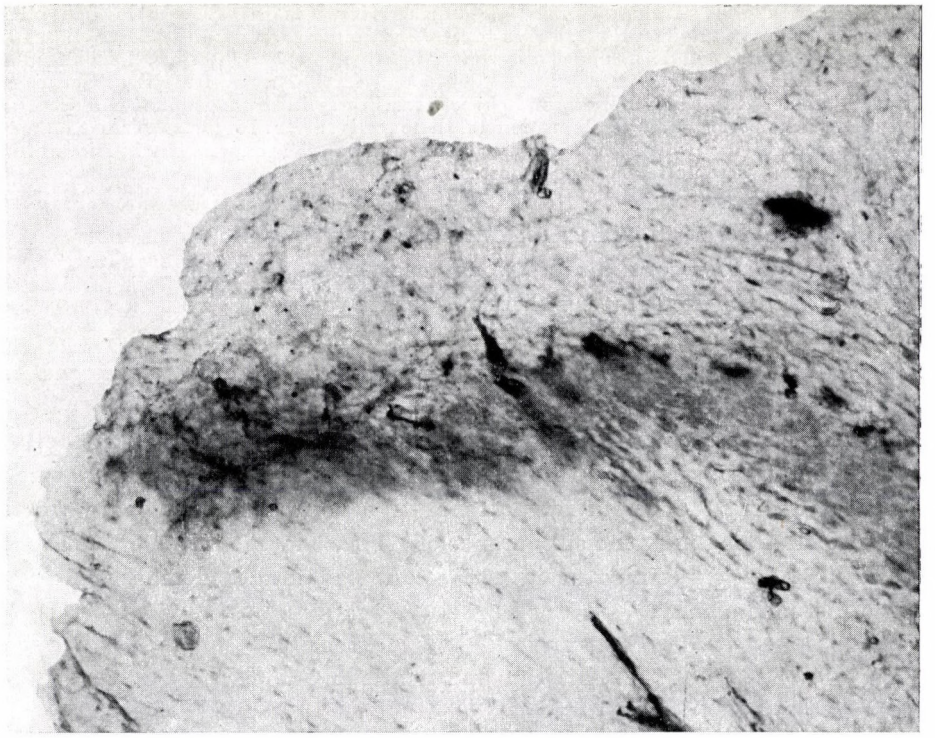

Fig. 1. ATPase activity in a 10-hour fracture. The first signs of increase in enzyme activity are noticed in the periosteal cells (×150)

At one to eight hours after the injury the fracture defect was filled and surrounded by extravasated blood and inflammatory exudate. Numerous polymorphonuclear leucocytes with ATPase active cytoplasm appeared in the exudate after four hours.

At ten hours the first signs of increase in ATPase activity were noticed in the osteoblasts and undifferentiated osteogenic cells of the periosteum (Fig. 1).

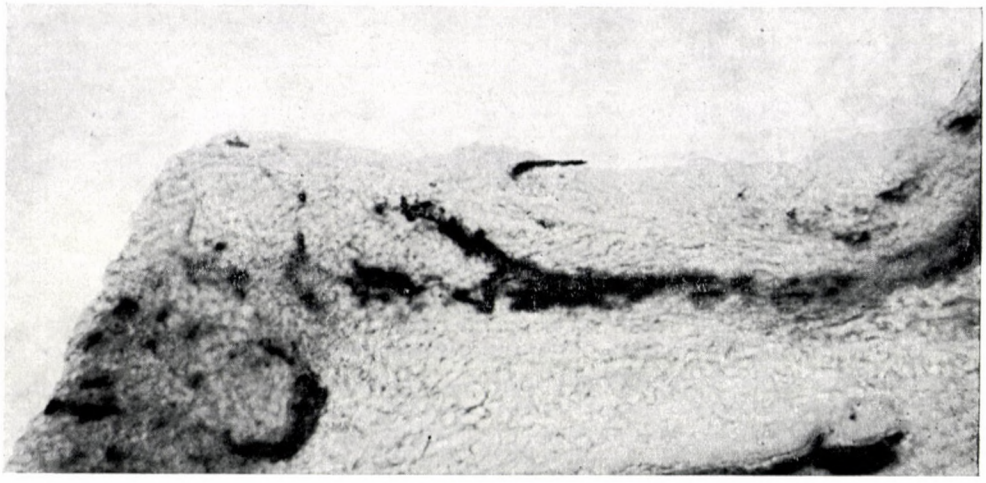

Fig. 2. Intensified ATPase activity of periosteal cells in a 12-hour fracture (×150)

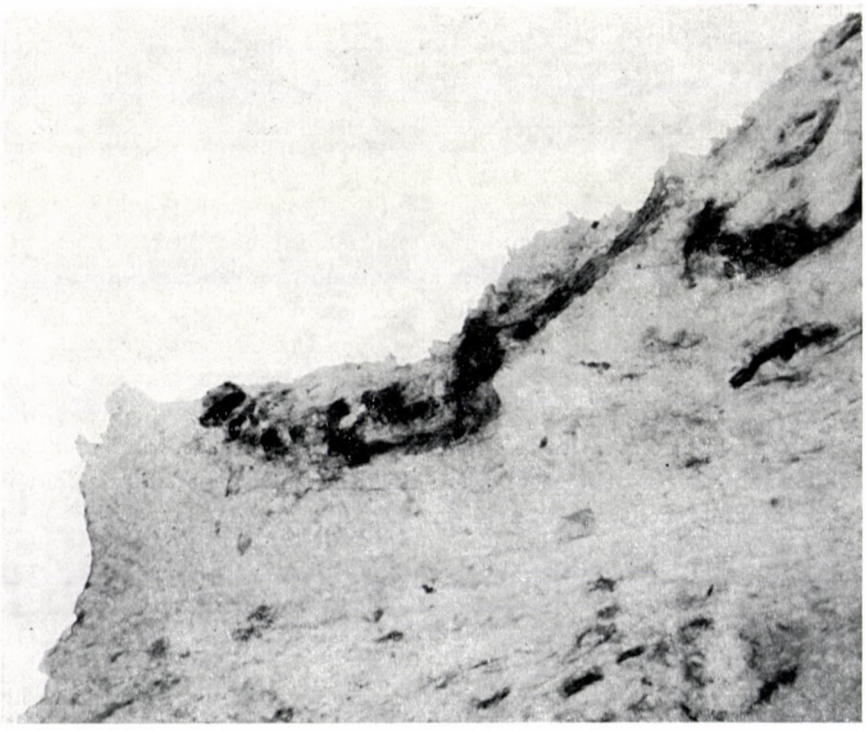

Fig. 3. ATPase activity in a 16-hour fracture. Initial proliferative response contributes to the intensification of enzyme activity (×170)

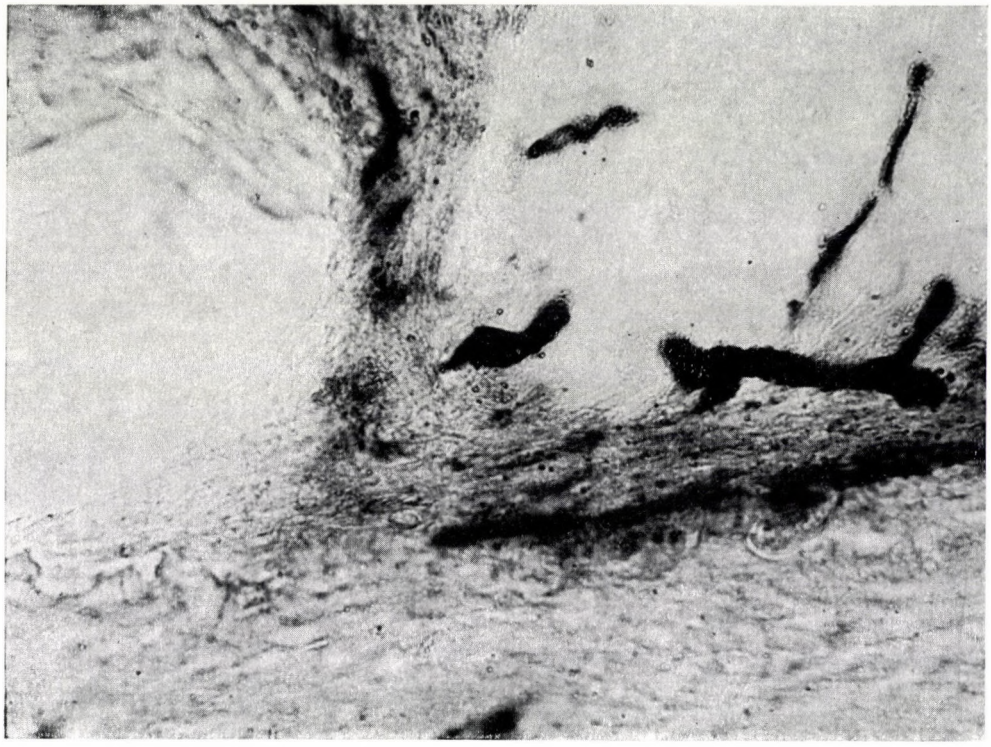

Fig. 4. Intense ATPase activity in proliferating periosteal cells and in vessel walls. 24-hour fracture (×360)

ATPase activity of these cells and of osteoclasts increased first in a peripheral zone situated 200—500 μ and farther away from the fracture line. Between 10 and 12 hours the ATPase activity of the mentioned periosteal cells became more intensive (Fig. 2), but no cell proliferation could then be noticed. At 16 hours (Fig. 3) and subsequently an initial proliferative response was seen in the periosteum. This occurred in the same peripheral zone which had showed the first increase in enzyme activity at ten hours. The proliferation became more evident at 24 hours and during the second and third day after the trauma. The proliferating periosteal cells showed an intense ATPase activity (Figs 4 and 5). Similar phenomena were noticed also in the endosteum.

Discussion

Far from being inert, the earliest days and even hours of fracture healing are characterized by increasing ATPase activity, located in the peripheral zone of periosteum and endosteum. The initial increase in enzyme activity occurs in

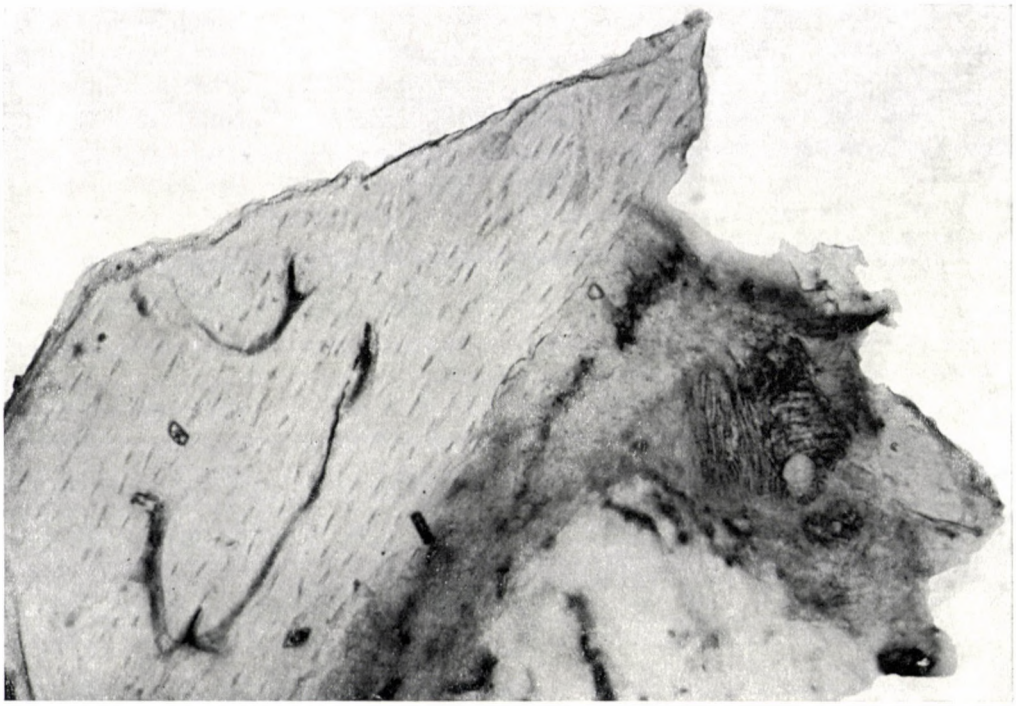

Fig. 5. Proliferating periosteum with intense ATPase activity in a 48-hour fracture (×150)

the osteoblasts and osteogenic cells at ten hours. Proliferation of these cells after 16 hours further increases the ATPase activity observed there. By using tritiated thymidine, the initial proliferative response to fracture has been seen at the same time [16].

The first reaction to fracture is essentially a sterile inflammation which is the consequence of trauma and haemorrhage. According to SENDA [14] both the mobility and the phagocytic function of leucocytes are related to ATPase dependent biochemical reactions. This may explain the intense ATPase activity of the invading cells.

Also in the local periosteal cells there is a very early enzymatic response to injury, demonstrable after 10 hours by using the histochemical method for ATPase. This enzyme may play a role in converting the preosseous matrix to calcifiable matrix, providing a source of energy [3]. In addition, ATPase activity has been found at the sites of vigorous fibre formation [4]. The high amounts of adenosine triphosphate and ATPase are related to the calcifying mechanism, by providing readily renewable organic phosphate [1]. ATPase and its substrate may further play an important role also in the membrane phenomena in rapidly growing tissues, being responsible for movement of phosphate ions across cellular membranes against concentration gradients [17]. Whatever

the most decisive function of ATPase *in vivo*, the increase in the activity of ATPase and other hydrolases [12, 13] probably represents one of the first steps in the repair process, starting early in the so-called latent period of fracture healing.

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ADENOSINTRIPHOSPHATASEAKTIVITÄT IN DER INITIALPHASE DER BRUCHHEILUNG BEI RATTEN

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Ratten wurden 1, 2, 4, 8, 10, 12 und 16 Stunden sowie 1, 2 und 3 Tage nach Frakturierung der rechten Tibia getötet und die ATPase in den Schnitten wurde histochemisch untersucht. In einer peripheren Zone, 200—500 μ und mehr entfernt von der Frakturlinie, setzte 10 Stunden nach der Frakturierung der Anstieg der ATPase-Aktivität in den Osteoblasten und osteogenetischen Zellen der Knochenhaut ein. Nach 16 Stunden rief die Proliferation dieser Zellen einen weiteren Anstieg der ATPase-Aktivität hervor, die dann während der gesamten Beobachtungszeit intensiv blieb. Ähnliche Erscheinungen wurden auch am Endosteum beobachtet.

АКТИВНОСТЬ АДЕНОЗИНТРИФОСФАТАЗЫ В НАЧАЛЬНОЙ ФАЗЕ ЗАЖИВЛЕНИЯ ПЕРЕЛОМОВ КОСТЕЙ. — ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ У КРЫС

Й. РАЕКАЛЛИО и М. КОВАЧ

Активность аденозинтрифосфатазы (АТФазы) была изучена гистохимическим методом исследования в связи с заживлением экспериментально вызванных костных переломов у крыс. Животных убивали через 1, 2, 4, 8, 10, 12 и 16 часов и 1, 2, и 3 дня после создания перелома правой большеберцовой кости. В периферической зоне, находящейся на расстоянии 200—500 μ и еще дальше от линии перелома, активность АТФазы начинает повышаться через 10 часов после травмирования, в остеобластях и костеобразовательных клетках надкостницы. По истечении 16 часов пролиферация этих клеток еще более повышает активность АТФазы, которая в течение всего экспериментального срока оказывается очень интенсивной. Подобные явления отмечаются и во внутреннем покрове костномозговых полостей (эндостеуме).

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SPECIFIC STAINING AND EXACT QUANTITATIVE EVALUATION OF THE GRANULATION IN THE JUXTAGLOMERULAR CELLS

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(Received May 27, 1968)

A combined trichrome staining method for the demonstration of the juxtaglomerular cells is described. The method is a combination of Mallory's phosphotungstic acid haematoxylin and Heidenhain's azan staining. The combined trichrome method gives good results also on the arteriolar granulated cells of the kidneys of different kinds of fish after a periodic and chromic acid pretreatment. Besides, it is excellent also for general histological purposes. The elective staining of the JGC granules is caused by the phosphotungstic acid haematoxylin; the mechanism of this effect is discussed. Authors give detailed description of the precise determination of the juxtaglomerular index.

A few years ago we developed a new combined trichrome stain for demonstrating the hyalin and fibrinoid deposits in the arteriolar walls (ENDES 1954). The procedure, a combination of Mallory's phosphotungstic acid haematoxylin (PTAH) and Heidenhain's azan methods, proved to be excellently suitable for the elective staining of the granules of epitheloid smooth-muscle cells in the preglomerular arterioles.

For demonstrating the granules, BOWIE's staining (BOWIE 1925) is usually applied, but its specificity has been doubted (BIAVA and MICHEL 1966) because it stains also the non-specific lipofuscin granules of the arteriolar smooth-muscle cells. This has made us to describe our trichrome staining for the demonstration of juxtaglomerular cell (JGC) granulation, the procedure being more specific than Bowie's method and devoid of the disadvantage of the latter. In addition we describe the method for estimating the JGC index because we feel a want of its detailed and exact survey in the literature.

Method and results

Combined trichrome staining. 1. Fixation in 10% formol, embedding in paraffin, thin sections.

2. Extraction of paraffin in xylol, alcohol, distilled water.

3. Deparaffinated sections remain overnight in 1% HCl, in 80% ethanol solution, then are washed thoroughly in tap water, then in a

4. 0.1% solution of aniline oil in 96% alcohol, for 2—3 minutes.

5. Rinse in 96% alcohol and subsequently twice in distilled water.

6. Stain with Azocarmine for 2—3 minutes. (The solution is prepared by dissolving 0.1 g of Azocarmine G in 100 ml distilled water, bringing to the boil, cooling, filtering and adding 1 ml of concentrated acetic acid.)

7. Rinse in distilled water.
8. Differentiate in the 0.1% aniline oil solution in alcohol.
9. Rinse in 1% acetic acid solution in alcohol for 1 minute.
10. Rinse twice in distilled water.
11. Stain in a solution of crystalline phosphotungstic acid, 20 g; haematoxylin, 1 g, distilled water, 1000 ml; for 3—6 hours. (For ripening of the phosphotungstic acid haematoxylin, add 0.177 mg $KMNO_4$.) It is important that the PTAH solution be at least 3 months old and not older than 9 months.
12. Rinse in 96% alcohol.
13. Stain in aniline blue orange G solution for 10—15 minutes. (Aniline blue 0.5 g; orange G, 2.0 g; glacial acetic acid, 8 ml; distilled water, 100 ml. The solution is boiled, cooled. Before use it is filtered and diluted 1 : 7 with distilled water.)
14. Rinse and differentiate for 3 minutes in 96% alcohol, changed twice.
15. Acetone, xylol, mounting in Canada balsam.

(The staining times mentioned apply to work with dyes used by us; they might need modification with the use of dyes from other sources.)

The newly deposited, lipid-rich hyalin material in the vascular wall gives a reddish, while the old, collagenous hyalin gives a blue and the fibrinoid a dark blue-violet staining. The heavily granulated cells of the preglomerular arterioles catch one's eye, but the cells containing only 1—2 granules are also recognized by their localization in the media of the arterioles, their approximately identical size, their spheroidal shape and their violet colour. The granules of mast cells have a similar tint but are easily differentiated on the basis of their localization and morphological characteristics.

BIAWA and MICHEL (1966) have called attention to the non-specific lipofuscin-like granules which after treatment with Bowie's method stain similarly as the JGC granules. With our trichrome staining the non-specific granules display a very faint grey colour, which can be differentiated with absolute certainty from the violet specific granules. The non-specific granules vary in size, have irregular borders and the best method for their light-microscopical visualization is Movat's silver-methenamine staining. The trichrome staining allows to avoid any false high JG index, because it eliminates the possibility of estimating the non-specific granulation. This is rare in the kidneys of rats and mice, but frequent in human kidneys, especially in older patients with hypertension. Performing the three granule-staining methods simultaneously on consecutive sections offers a good comparison of their different staining effects.

Our trichrome method ensured good results on kidneys of many species including human, rat, mouse, dog, cat, pig, cattle, horse, bat, and even guinea pig kidneys although using Bowie's method the guinea pig kidney was reported to be devoid of JG granulation (Figs 1 to 3).

Results with the trichrome stain were, however, not satisfactory in the kidneys of sea- and fresh-water fishes. BOHLE and WALVIG (1964) succeeded in demonstrating granulated cells in the kidneys of sea-fishes with a PAS—alcian blue method and in the kidneys of teleosts with Movat's silver-methenamine (MOVAT 1961). According to LONGLEY and BURTNER (1962) oxidative pretreatment enhances the staining capacity of PTAH. Therefore our trichrome method was supplemented by pretreatment with periodic acid and chromic acid. In this way we succeeded in staining electively the granulated cells in the kidneys of sea- and fresh-water fishes. The procedure is as follows.

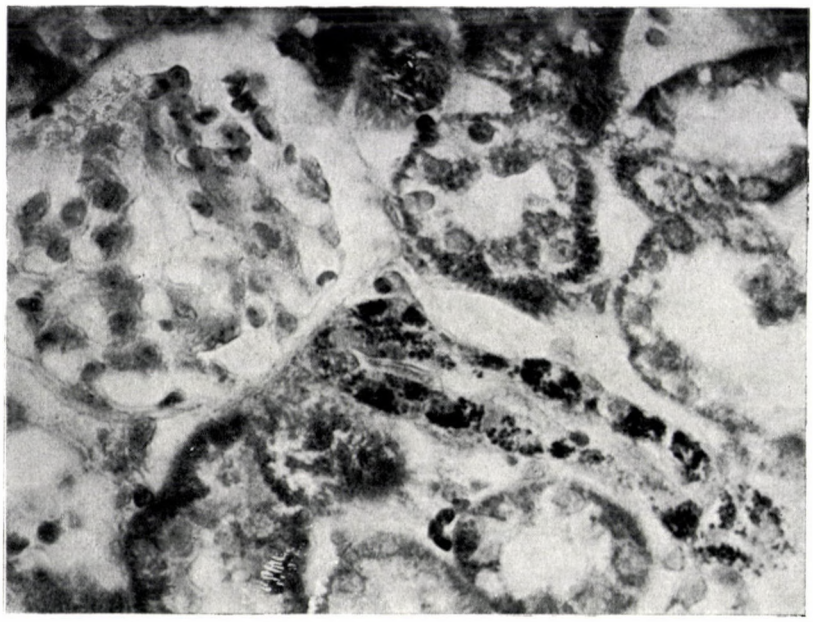


Fig. 1. Mous kidney. Glomerulus with afferent arteriole, containing many heavily granulated JGC in the media. ×600

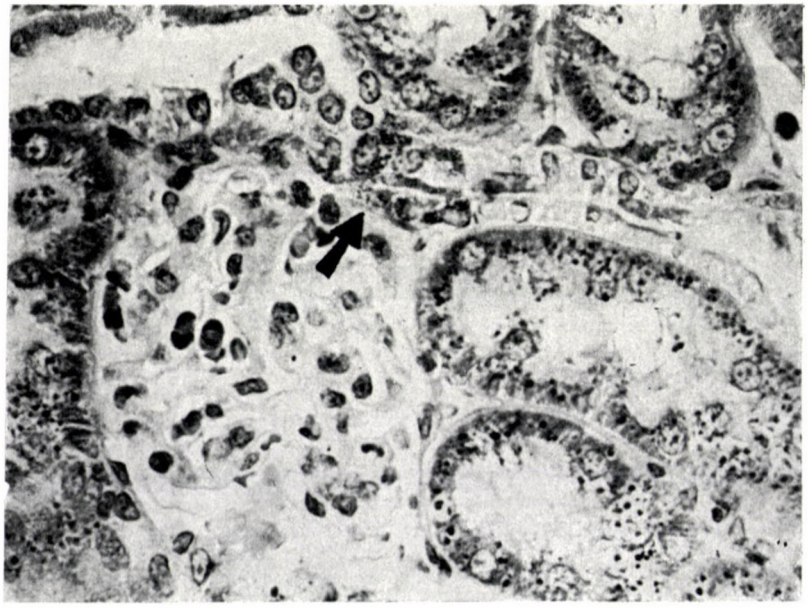


Fig. 2. Rat kidney. Glomerulus with afferent arteriole, in the vascular pole the arteriole contains five granulated cells (arrow). ×600

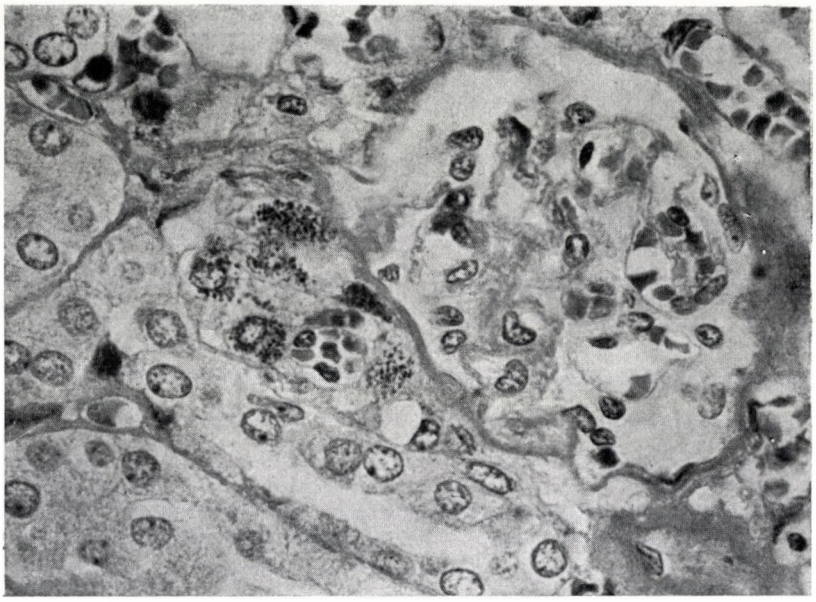


Fig. 3. Human kidney. In the vascular pole afferent arteriole in cross section. The media contains many enlarged, richly granulated cells, partly localized eccentrically, forming a s.c. Polkissen. ×600

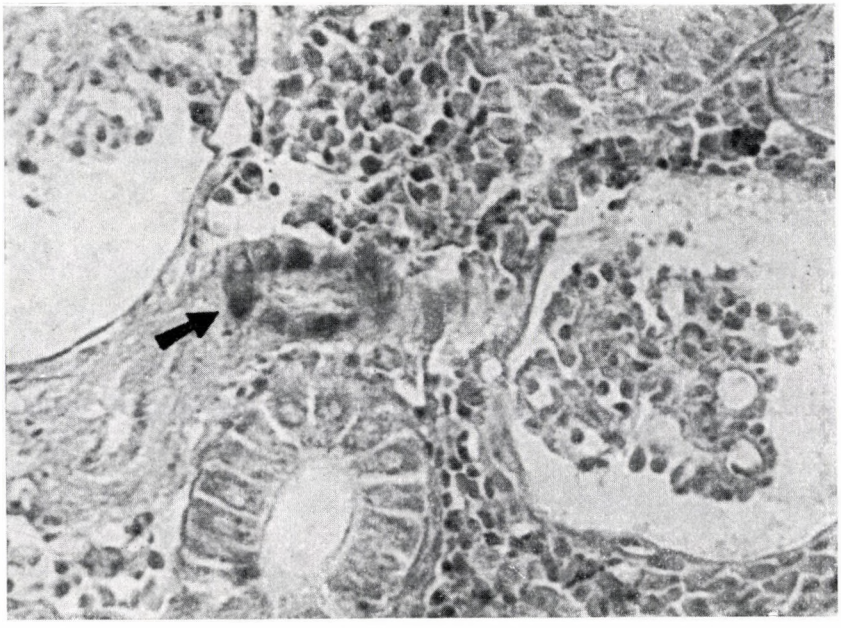


Fig. 4. Kidney of the sea-water fish Pleuronectes flesus. Two glomeruli, between them afferent arteriole in cross section. Some of the medial cells are filled with fine dust-like granulation (arrow). ×600

1. Oxidize 10 minutes in 1% aqueous periodic acid solution.
2. Wash 5 minutes in running water.
3. Oxidize 20 minutes in 5% aqueous chromic acid solution.
4. Wash 5 minutes in running water.
5. Wash 2 minutes in distilled water.
6. Combined trichrome staining described above.

The fact that the demonstration of the granulated cells of the fish kidney is so complicated might have been the reason for considering it devoid of granulated cells (Fig. 4).

In addition, our trichrome staining ensures as good results as any other trichrome method for general purposes in any kind of tissues.

Recognition of the JGC is rendered difficult by the heavily congested vessels as it happens in laboratory animals sacrificed in deep anaesthesia. Therefore it is recommended to kill them by bleeding in superficial ether anaesthesia, which induces renal ischaemia which is favourable for the examination of the JGC.

We have ample experience with rat and mouse kidneys of which we cut 1 or 2 horizontal slices without any pressure, with a sharp blade.

Estimation of juxtaglomerular index (JGI)

The original method of HARTROFT and HARTROFT (1953) is applied by us for estimating the frequency of granulated cell-groups and the degree of granulation. Various modifications have been recommended by different investigators. We have developed a standard method; according to our experience it is more exact than the others.

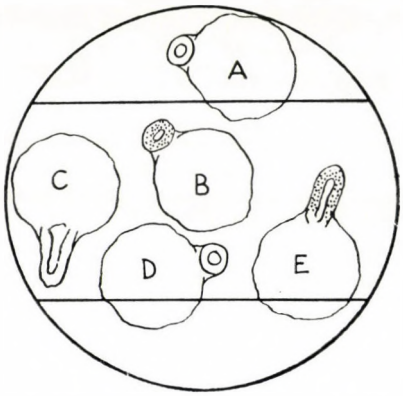
If we estimate 2 slices from each of the kidneys, we obtain the index of an animal (rat) on the basis of counting 900 glomeruli on the average. According to our experience, estimation of 1 slice each of the kidneys, 450 glomeruli on the average, gives a similar result. We consider it important to estimate the total in horizontal slices, so the whole cortex, the superficial and the deep glomeruli are counted.

Counting is done at a magnification of 600 with the use of a frame. The latter is a thin metal ring applied on the diaphragm of one of the eye-pieces. The ring holds two parallel 0.2 mm wires which enclose a 6 mm wide stripe in the centre of the field. The glomeruli and JGA are counted in this zone, which comprises about 1/3 or 1/4 of the field. The narrower the stripe the more exact and the more time-consuming the counting. In a 6 mm wide stripe a horizontally cut rat kidney slice may be looked through in 50—55 stripes, and this will take 45 to 60 minutes. Before counting the examiner should so rotate the eye-piece that the stripe lies horizontally.

It is best to begin on the surface of the cortex, and counting stripe for stripe moving the preparation horizontally. Glomeruli part of which overlaps the lower margin of the stripe should be counted in the next stripe where they overlap the upper margin of the next stripe. Granulated JGA should be counted even if their glomerulus overlaps the lower margin, but in such cases one must subtract one from the number of "negative" (devoid of granulated cells) glomeruli because this glomerulus will again appear in the next zone as an overlapping "negative" one on the upper margin and will be counted as such. (Graph 1.)

Granulated cells should naturally be estimated even if they are seemingly independent of a glomerulus. About 10% of the glomeruli appear to be atypical, because their Bowman's

capsules are cut tangentially, or the glomerulus had dropped out of its capsule. In the latter case the empty Bowman's capsule should be distinguished from thin-walled venules or from empty tubular basal membrane rings. Proper estimation of such elements is important to avoid false high index values. When the whole surface of the kidney section had been counted,



Graph 1. Scheme of the counting-frame. The negative glomeruli (A, C) and the positive glomerulus (B) should be counted in this stripe. The negative glomerulus (D) will be counted in the next stripe. The other positive one (E) should be counted here, but because of its reappearing in the next stripe in a negative form, one should be subtracted from the number of negatives

the number of "negative" glomeruli should be added to the number of granulated complexes to obtain the total glomerulus-count. The so-called JG index is computed according to the formula $\frac{GV \cdot 100}{TGC}$, where TGC = total glomerulus count; GV = granulation value, i.e. the sum of the estimated classifications according to the degree of granulation (HARTROFT and HARTROFT, 1953).

Discussion

In our method, JG granules are stained by PTAH. The other constituents of the trichrome staining give a background to ensure prominence to the granules. Recently ERTL (1966) has published a simplified modification of our method, applying PTAH alone. TERNER et al. (1964) dealt with the mechanism of PTAH staining and found that PTAH differs from the group of alum and iron haematoxylin dyes by its anionic mordant and the two colours produced by PTAH was considered a true polychromasy. PTAH acts as an acid dye, having an affinity to basic protein-rich tissue components, and its substrate is very likely an amino or some other basic group. According to our histochemical investigations (GOMBA, SOLTÉSZ, ENDES, 1963) the granules are rich in tyrosine and tryptophan and this might explain their PTAH binding capacity.

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KOMBINIERTE TRICHROM-FÄRBUNG FÜR DEN NACHWEIS DER GRANULIERTEN ZELLEN DES JG-APPARATES UND EINE GENAUE METHODE FÜR DIE BESTIMMUNG DES JG-INDEXES

P. ENDES, SZ. GOMBA und I. DÉVÉNYI

Die kombinierte Trichrom-Färbung für den Nachweis der juxtaglomerulären granulierten Zellen, eine Kombination der Malloryschen PTAH-Färbung und der Heidenhainschen Azan-Färbung wird beschrieben. Nach Vorbehandlung mit Perjodsäure und Chromsäure lassen sich mit der beschriebenen Methode sogar die arteriolären granulierten Zellen der Fischniere färben. Das Verfahren ist für histologische Untersuchungen hervorragend geeignet. Die spezielle Färbung der Granulen wird durch PTAH herbeigeführt. Die Eigenart dieses Effekts wird analysiert. Ein genaues und zuverlässiges Verfahren in Form eines Abzählrahmens wird für das präzise Zählen der granulierten Zellen des juxtaglomerulären Apparates mitgeteilt.

КОМБИНИРОВАННЫЙ МЕТОД ОКРАШИВАНИЯ ТРИХРОМОМ ДЛЯ ВЫЯВЛЕНИЯ ГРАНУЛИРОВАННЫХ КЛЕТОК ЮКСТАГЛОМЕРУЛЯРНОГО АППАРАТА И ТОЧНЫЙ МЕТОД ДЛЯ УСТАНОВЛЕНИЯ ЮКСТАГЛОМЕРУЛЯРНОГО ПОКАЗАТЕЛЯ

П. ЭНДЕШ, С. ГОМБА и И. ДЕВЕНЬИ

Приводится комбинированный метод окрашивания трихромом для выявления гранулированных клеток юкстагломерулярного аппарата. Предложенный метод является комбинацией методов окраски PTAH по Маллори и окраски азаном по Хейденхейну. После подготовки метайодной кислотой и хромовой кислотой, комбинированным методом авторов окрашиваются также гранулированные клетки артериол почек рыб. Метод весьма пригоден и для общих гистологических исследований. Специальное окрашивание зернышек обусловлено PTAH. Анализуется природа этого эффекта. Сообщается точный и надежный метод в виде так наз. счетной рамки для точного подсчета гранулированных клеток юкстагломерулярного аппарата на препаратах почек.

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RECENT DATA ON THE FINE STRUCTURE OF CHONDROCYTES

PRELIMINARY REPORT

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(Received September 3, 1968)

Since GODMAN and PORTER's fundamental work [2] the methodology of ultrastructural research has undergone a considerable evolution. SABATINI, BENSCH and BARRNETT [7, 8] introduced aldehyde fixatives and the use of Millonig's phosphate buffer [4], formerly disapproved of, has become general. Good results were reported by KARNOVSKY [3] with a mixture of fixatives containing aldehydes. Numerous authors found this mixture to ensure good preservation of the fine structure of the cells of various tissues and these observations have been confirmed by our study on cartilage tissue.

Apart from the study of PALFREY and DAVIES [5] on chondrocytes fixed with g'utaraldehyde, no data have been found concerning the use of aldehyde fixatives.

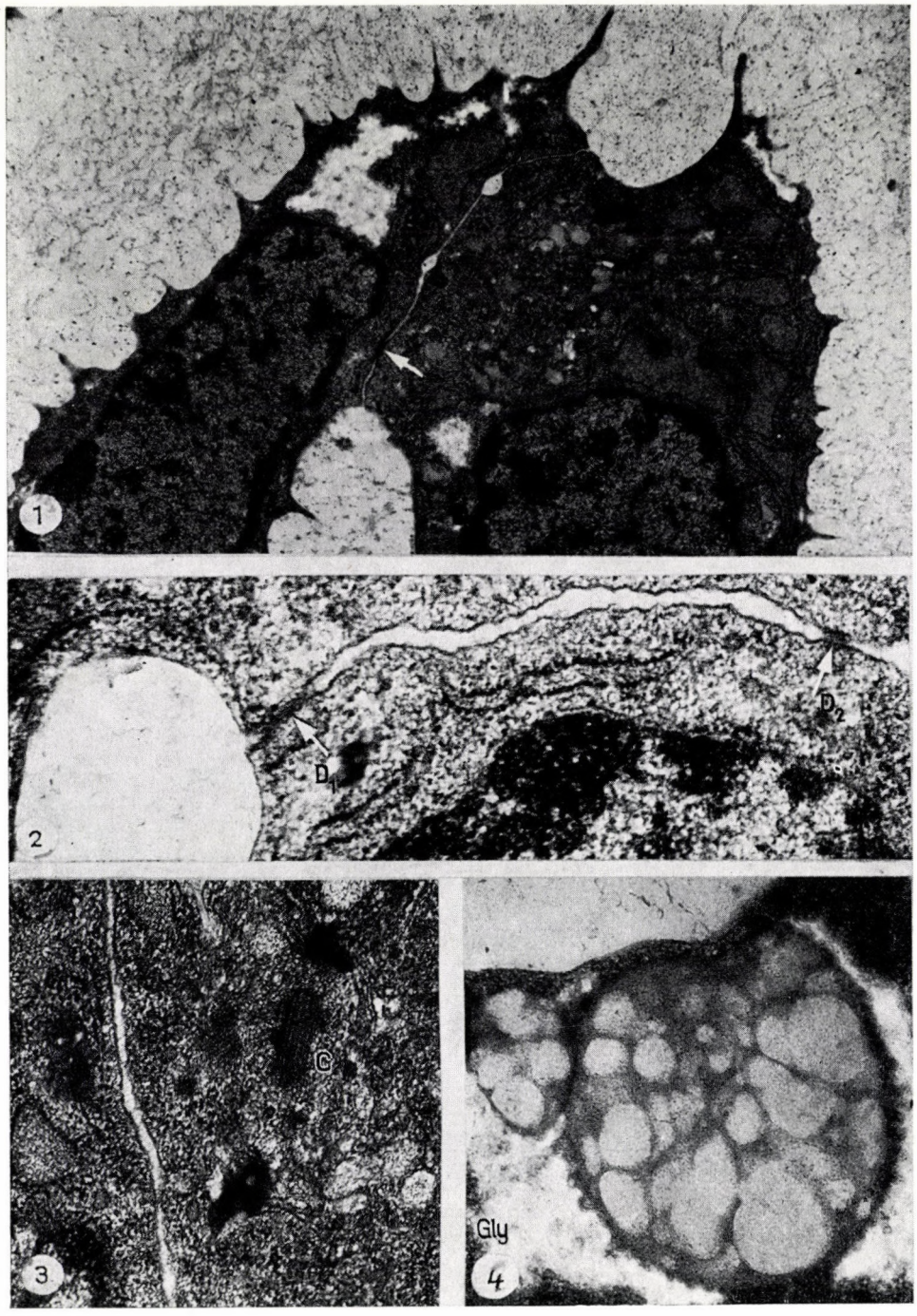
In the present study we have described some appearances noted in cartilage cells which to our best knowledge have not been described or reported.

Material and methods

The cartilage used was obtained from the proximal tibial epiphyseal cartilage of newborn mice. The specimens were fixed in Karnovsky's mixture at room temperature for 30 min., then quickly washed in 0.1 M phosphate buffer and fixed in Millonig's solution for 90 min. After dehydration in an acetone series the specimens were embedded in Vestopal W (Jaeger), sectioned by a LKB ultrotome and stained with uranyl acetate in alcohol or with lead citrate according to REYNOLDS [6]. Electron micrographs were made with Elmiskop IA (Siemens), electron microscope (60 kV accelerating voltage).

Results

The details observed in the chondrocytes were as follows. Figures 1 and 2 show desmosomes (arrow) and zonulae occludentes, while Fig. 3 the typical structure of a cytocentre, in twin cartilage cells separated by a gap not more than 4—600 Å in width. On Fig. 4 a larger delimited structure measuring 1.146×1.097 μ, varying in electron density and in its proximity a similar though smaller structure (0.512×0.341 μ) were situated in a glycogen pool, near the cell membrane.


1
2
D1
D2
3
C
Gly
4

The desmosomes and zonula occludens seen in Figures 1, 2 could not be identified with those described by FARQUHAR and PALADE [1]. Such structures between two cartilage cells have been identified as desmosomes also by PALFREY and DAVIES [5], and their opinion seems to be acceptable in that these cells lying in close apposition to one another have probably undergone mitosis. The centriole visible in Fig. 3 has not so far been described in cartilage cells.

The structure seen in Fig. 4 has been observed on one sole occasion; it has not been analysed as to its origin and function, in the lack of histochemical and biochemical data. There might be chemical difference between its parts of different density, as the denser area displayed a fine granular structure.

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Fig. 1. Narrow intercellular gap between two cartilage cells (arrow). Increased density in both cells in the narrowed area. Desmosome. ×12 000

Fig. 2. Narrowed intercellular gaps (marked by arrows). Part of the cytomembranes is parallel. D_1 seems to be a zonula occludens, while D_2 resembles a desmosome. ×45 000

Fig. 3. Centriole (C) with tubules. ×36 000

Fig. 4. Structures of unknown function, localized in a glycogen pool (Gly). In the denser areas fine granules are visible. ×41 000

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COMBINED EFFECTS OF CHOLESTEROL FEEDING AND METHYLCELLULOSE ADMINISTRATION ON THE HISTOLOGICAL STRUCTURE OF INTERNAL ORGANS AND BLOOD VESSELS

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(Received October 3, 1968)

The histological structure of the internal organs and blood vessels of rabbits treated with methylcellulose, cholesterol, and their combination, has been studied. On combined treatment the total lipid contents of the aorta and liver were lower and the ensuring atheromatosis was less severe than after treatment with cholesterol alone. This has been attributed to an increased metabolisation of fats by the methylcellulose activated RES.

1. Introduction

Methylcellulose, a polymeric carbohydrate macromolecule of 84,000 molecular weight by chronic intravenous or intraperitoneal administration is known to induce in the experimental animal thesaurotic RES hyperplasia, proliferative arteriitis with intimal proliferation in the small arteries, mainly in the lungs, thesaurotic and degenerative renal glomerular vascular changes similar in many respects to the Kimmestiel-Wilson intercapillary glomerulosclerosis, and marked splenomegaly (Horváth et al., 1957, 1958; Benkő, 1958a, b; Benkő et al., 1959, 1962a, b, c; 1966a, b). With the storage of methylcellulose the alpha and beta globulins increase, lipoprotein fractions of triple distribution appear and the glycoprotein components increase in blood plasma (Fröhlich et al., 1957, 1959; Balázs et al., 1960). The methylcellulose-induced splenomegaly is accompanied by haemolysis, caused partly by the hypersplenism and partly by the damage to the erythrocytic membrane caused by the increase of $beta_{2M}$-globulin (macroglobulin) (Andrássy et al., 1958).

The changes induced by cholesterol administration are somewhat similar in nature and location to those caused by methylcellulose. Chronic cholesterol feeding is followed by lipid thesaurosis and atherosclerotic vascular changes associated with hyperlipaemia and hypercholesterolaemia (Antonini et al., 1960). Exogenous cholesterol is stored in the RES, mainly in Kupffer's cells and the macrophages of the spleen (Byers et al., 1957; Berliner and Dougherty, 1960), leading to haemolytic anaemia (Pintér et al., 1961; Rowley et al., 1962; Silver et al., 1964). Stehbens and Silver (1966) found a similarity between the methylcellulose-induced vascular changes and the cholesterol-induced atherosclerosis. These data from the literature and our own previous

experiments (BENKŐ et al., 1960, 1961; SZARVAS et al., 1963) called for a study of the combined actions of methylcellulose and cholesterol. Such experiments seemed to be suitable also for studying the correlation between RES and atherosclerosis, a problem in the foreground of recent interest. Of the RES-stimulators, oestrogens and zymosan have been investigated and both were shown to lessen the cholesterol-induced coronary sclerosis by some unclarified mechanism (PICK et al., 1952, 1957, 1962, 1966; PATEK and BERNICK, 1960).

From the study of the combined actions of methylcellulose and cholesterol we wished to draw conclusions to the following problems. How does the storage of methylcellulose in the RES influence the lipid thesaurosis induced by cholesterol feeding in the viscera, mainly in the liver? Does cholesterol storage modify the deposition of methylcellulose in the RES? Do the methylcellulose-induced RES hyperplasia and RES activity modify, decrease or increase the atherosclerosis induced by cholesterol feeding? Finally, does methylcellulose in the vascular wall modify the cholesterol-induced atheromas and does the cholesterol deposition in the vascular wall modify the methylcellulose-induced vascular changes?

To find the answers to these questions, rabbits have been treated with methylcellulose intraperitoneally and have been fed cholesterol, trying to approach the problems by these experiments.

2. Materials and methods

A total of 34 male rabbits of 2500 to 3000 g body weight were maintained on a mixed diet composed of grits, carrots and green fodder. The animals were divided into four groups. Eight rabbits received 2.0 ml/kg body weight of physiological sodium chloride solution intraperitoneally twice weekly for 18 weeks (Group I, controls). Nine rabbits were treated with 2.0 ml/kg of a 2.5% aqueous methylcellulose solution twice weekly for 18 weeks (Group II). Eight rabbits were fed cholesterol, admixing 1 g of cholesterol to the grits ball every day over the 18-week experimental period (Group III). Nine rabbits were fed cholesterol and were given intraperitoneally the above doses of methylcellulose (Group IV).

Methylcellulose of 84,000 molecular weight was dissolved in sterile distilled water under gentle shaking, stored at +4 °C under aseptic conditions and used within 48 hours.

At the end of the experiments the animals were anaesthetised by intravenous thialbarbital and exsanguinated through a cannula inserted into the abdominal aorta. The internal organs were inspected, the aorta was cut up and the severity of atheromatosis estimated from the number and extent of plaques. The severity of changes was graded from 0 to 5 according to FODOR and ZEMPLÉNYI (1958).

Specimens from aorta, liver, spleen, kidney, lung, heart and adrenal gland were examined histologically. The specimens were fixed in 4% formalin or 96% alcohol, embedded in paraffin and the sections were stained with haematoxylin-eosin, PAS, Oil-Red O and alcian blue, the aortic sections also with elastica and van Gieson staining.

Liver and aorta were tested for total lipids and total cholesterol, by the methods of SWAHN (1952) and of ZLATKIS et al. (1953), respectively.

3. Results

The gross changes were as follows. In Group I the liver, spleen, peritoneum and aorta appeared to be intact, except in one animal, in which a slight fatty degeneration of the liver was found. In Group II (methylcellulose

treatment) the capsule of the liver and the peritoneum showed spotty thickening of variable extent. These were believed to constitute proliferative chronic inflammatory changes secondary to the injection of methylcellulose. The spleen was moderately enlarged. In Group III (cholesterol feeding) marked enlargement of the light yellowish-brown fatty liver and splenomegaly were found in every animal. In Group IV (combined treatment) peritoneal changes similar to those described in Group II and marked enlargement of the spleen occurred, but only 3 animals showed marked fatty degeneration of the liver. In the rest the liver was apparently normal in colour, cut surface, structure and lobular structure.

Table I

Grade of atheromatosis in the aorta of rabbits, as estimated by gross examination in the different groups

Group No.	Treatment	Grade of severity in the members of the different groups										Mean
	Animal No.	1	2	3	4	5	6	7	8	9	10	
(control) I	Physiological NaCl	—	—	—	—	—	—	—	—	—	—	—
II	2.5% methylcellulose	—	—	—	—	—	—	—	—	—	—	—
III	Cholesterol	4	5	4	5	4	5	3	5	—	—	4.375
IV	2.5% methylcellulose + + cholesterol	4	4	3	4	4	4	4	4	3		3.777

Probability: III/IV : $p > 0.05$

Atheromatic changes of the aorta is shown in Table I. On inspection the inner surface of the aorta was intact in the control (I) and methylcellulose treated (II) rabbits. In 4 of the 8 cholesterol-fed animals (III) grade 5 changes appeared in the form of big confluent plaques. In 3 animals these were restricted to the aortic arc, while distally there were only solitary plaques, mainly near the orifices of the side branches of the aorta (grade 4). In 1 animal there were only solitary plaques (grade 3). Of the 9 animals subjected to combined treatment (IV) 7 showed grade 4, 2 grade 3 atheromatosis, and none of them exhibited grade 5 changes. The pattern was obviously less severe than in Group III, but the difference between the two groups was not significant statistically ($p > 0.05$).

Histologically, in Group I the viscera were normal.

In Group II (methylcellulose treatment) the liver was intact. The Kupffer's cells stored moderate amounts of methylcellulose. Storage was substantial in the spleen, the storing histiocytes accumulated in foci in the red

pulpa. In some cases eosinophilic cells were also found (Fig. 1). The heart, adrenal gland and aorta were normal except for 2 animals which displayed proliferative arteriitis in the pulmonary arterioles. In the kidneys of 3 animals the basement membrane was moderately thickened. No methylcellulose storage could be demonstrated in the glomerules.

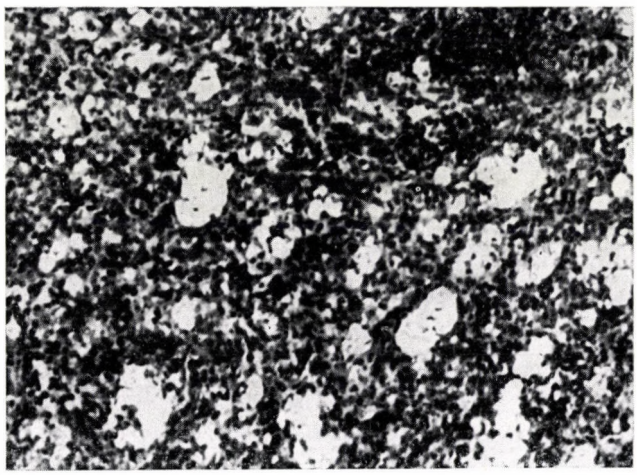

Fig. 1. Rabbit spleen following treatment with methylcellulose. Focal accumulation of histiocytes showing signs of storage. H. E., ×176

Table II

Total lipid content of the liver following methylcellulose treatment and cholesterol feeding, in percentage of wet organ weight

Group No.	Treatment	Number of cases	Total lipid, per cent
I (control)	Physiological NaCl	4	3.56±1.47*
II	Methylcellulose	3	3.95±1.16
III	Cholesterol	4	9.47±0.26
IV	Methylcellulose + cholesterol	4	4.07±0.92
		I/II	$p>0.05$
		I/III	$p<0.001$
	Probability	I/IV	$p<0.05$
		II/III	$p<0.01$
		II/IV	$p>0.05$
		III/IV	$p<0.01$

* Mean error

In Group III (cholesterol feeding) the liver showed diffuse and severe fatty degeneration in 5 animals out of 8. In three cases the fatty degeneration was acinocentral, very marked. In the spleen of four animals the macrophage cells stored lipids. The adrenocortical cells, mainly in the zona fasciculata and reticularis, were swollen and vacuolated and contained much lipid in every animal. In the small and medium-sized arteries of the heart, lung, spleen and liver atheromatosis was visible. This was most marked in the coronaries, but the pulmonary, hepatic and splenic arterioles were also seriously

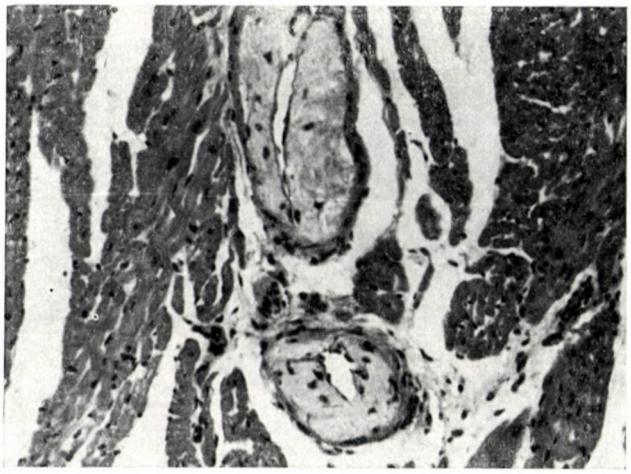

Fig. 2. Cross section of a medium-sized coronary artery from a cholesterol-fed rabbit. Note subintimal accumulation of lipids, causing narrowing of the lumen. H. E., ×176

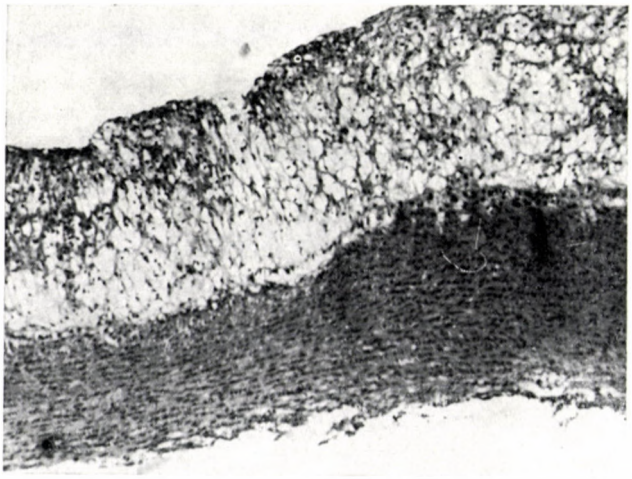

Fig. 3. Atheromatosis of aorta in a cholesterol-fed rabbit. The confluent subintimal plaques do not affect the elastic fibres of the media. H. E., ×176

affected. Usually, there was a circular subintimal accumulation of lipids, causing a marked narrowing of the lumen (Fig. 2). The small renal vessels were normal.

Histologically, the aorta showed atheromatosis corresponding in severity to what is usual after cholesterol feeding for four and a half months (Fig. 3). The plaques were confluent, broad, composed mostly of foamy cells, containing ample amounts of lipid. The process was subintimal and did not affect the elastic fibres of the media. Calcification, ulceration of the plaque surfaces were not observed. From the intimal side the plaques were covered by a thin lipid-free crust, considered to be an early manifestation of a capsule.

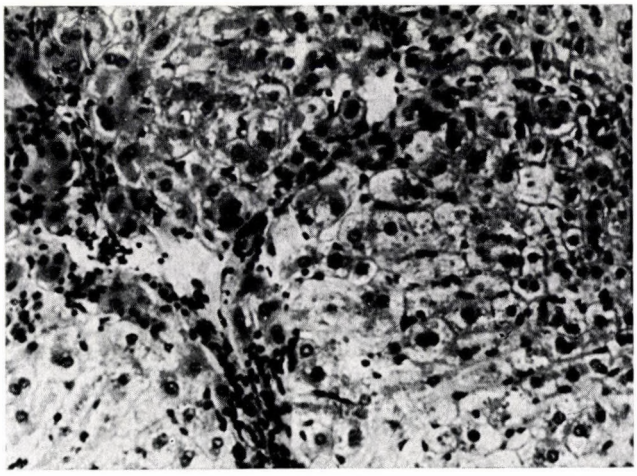

Fig. 4. Periportal mesenchymal reaction, with initial transformation in the liver of a rabbit treated with cholesterol and methylcellulose. H. E., ×176

In Group IV (combined treatment) vacuolisation of the liver parenchymal cells was visible. Fatty degeneration was less severe than in the cholesterol group. This observation was supported by the biochemical results. In Table II are presented the liver total lipid values after methylcellulose treatment and chronic cholesterol feeding. In the cholesterol-fed Group III the total lipid content of the liver was significantly increased. No similar increase was noted in Group IV (combined treatment). Methylcellulose alone did not increase the total fat content of the liver (Group II). Of the 9 animals only 2 showed diffuse fatty degeneration, in 2 others there was marked acinocentral fatty degeneration, and in 5 very slight acinocentral fat deposition. In 3 animals a moderate mesenchymal reaction was visible in the periportal region. In a fourth animal this was so severe that it corresponded to early cirrhosis (Fig. 4). In every animal hepatic and splenic methylcellulose storage of slight extent was demonstrated.

Lipid storage by the adrenals and spleen was similar to that observed in response to cholesterol feeding. The methylcellulose-induced vascular changes in the lungs and kidneys were slight and occurred only at sites. Atheromatosis of the small and medium blood vessels of the heart, lung, liver and spleen was similar to that seen after cholesterol feeding. These changes were not inflammatory or granulomatous in character, but corresponded to subintimal cholesterol plaques.

As regards structure and histochemical reactions (alcian blue, PAS and fat staining) the aorta of the animals subjected to combined treatment was not more severe and did not differ from that of the animals treated with cholesterol alone. The plaques were less confluent and somewhat narrower, though their lipid content was marked. Histological changes suggesting methylcellulose storage, inflammation or granulomatous reaction were not visible in these plaques.

Table III

Total lipid content of the aorta following methylcellulose treatment and cholesterol feeding (absolute values)

Group No.	Treatment	Total lipid, mg/g wet weight
I (control)	Physiological NaCl	138.88
II	Methylcellulose	150.0
III	Cholesterol	443.0
IV	Methylcellulose + cholesterol	312.5

The results for the total lipid content of the aortas in the different groups are presented in Table III. It was the highest in the cholesterol group (Group III); and significantly lower in Group IV. These results were in agreement with the gross (Table 1) and the histological findings.

4. Discussion

After 18 weeks of intraperitoneal methylcellulose treatment storage in the RES, most markedly in the spleen, could be demonstrated in the rabbits. Enlargement of the spleen did not reach the grade found earlier in methylcellulose-treated rats (Horváth et al., 1957; Benkő, 1958). It seems that the absorption of methylcellulose from the rabbit peritoneum differs from that of the rat peritoneum. Proliferative arteriitis and granulomas similar to those found in the lungs of dogs treated with methylcellulose intravenously (Benkő et al., 1959, 1962) did not develop. This might have been due to the fact that on intravenous administration the macromolecular aggregates reach

in larger numbers the pulmonary arterioles and therefore the granulomatous vascular changes are more severe (LAUTSCH et al., 1958). If administered intraperitoneally, methylcellulose enters the circulation slower and in greater dilution and there may be differences also in dispersion. These may account for the lesser severity of pulmonary changes. Such arteriosclerotic changes as had been described by HUEPER (1942, 1945) and later by STEHBENS and SILVER (1966) did not occur in our material.

The glomerular changes were slight, a circumscribed thickening of the basement membrane occurred in the kidney of a few animals only. Spherical masses were not found in the glomerular loops (HORVÁTH et al., 1957, 1958; HALL, 1962; BENKŐ et al., 1966). Thus, the morphological signs of RES activation were in the foreground and the vascular changes were less severe in our experiments.

After 18 weeks of cholesterol feeding the liver and the adrenals showed marked fatty degeneration, without any major mesenchymal reaction. The splenic macrophage cells also stored fat.

Cholesterol feeding induced atherosclerosis in the small and medium-sized arteries of the heart, lungs, spleen and liver, as well as in the aorta. The changes varied greatly in severity and were as usual after 18-week cholesterol feeding.

In the animals treated with methylcellulose and cholesterol there were two kinds of changes in the small and medium-sized arteries. The majority did not differ from what was found after cholesterol treatment alone and the granulomatous changes were neither more frequent nor more severe than in the animals treated with methylcellulose alone.

Under the present experimental conditions no histological and histochemical changes suggesting an interference of methylcellulose and cholesterol were found. Methylcellulose diminished the vascular changes by acting on the fat metabolism and not by a local action.

In the rabbits treated with methylcellulose and cholesterol, methylcellulose was stored in the same measure as after methylcellulose treatment alone. In the same rabbits, fatty degeneration of the liver was less severe than in the animals treated with cholesterol alone. Another difference from the cholesterol-fed group was the intensive mesenchymal reaction in the liver, attributed to an increase of RES activity following methylcellulose administration.

In the aorta the atheromatous changes following combined treatment did not differ from those following treatment with cholesterol alone, except that they were less severe. The total lipids in the liver and aorta decreased significantly also in the group treated with methylcellulose and cholesterol, although the daily cholesterol intake was the same. The total serum lipid value was also less in these animals than in the animals treated with cholesterol only. The fact that in spite of the identical doses of cholesterol less lipid was stored

in the liver and aorta and the plasma lipid level was also lower may be explained first of all by an increased metabolisation of fats, which in turn might have been due to the increase of RES activity induced by methylcellulose. This effect is believed to be similar to that of other agents acting through RES activation, for example to the influence on lipid metabolism and to the inhibition of atheromatosis by zymosan and oestrogens (PICK et al., 1952, 1962, 1966; HALPERN et al., 1957; DI LUZIO et al., 1961).

It is remarkable that methylcellulose stored in the RES should enhance the proliferation of the histiocytic system and at the same time damage the blood vessels and one would have expected an increase in the severity of atheromatosis under the combined effect of methylcellulose and cholesterol. It seems that under the given experimental conditions methylcellulose reduced the atheromatosis changes by activating the RES and diminishing lipid storage. This allows to conclude to a fundamental role of the RES in the pathogenesis of atheromatosis.

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DIE WIRKUNG DER GLEICHZEITIGEN VERABREICHUNG VON CHOLESTERIN UND METHYLZELLULOSE AUF DIE HISTOLOGISCHE STRUKTUR DER INNENORGANE UND DER GEFÄßE

S. BENKŐ, F. BILICZKI, und F. SZARVAS

Die histologische Struktur der Innenorgane und der Gefäße wurde an Kaninchen nach Verabreichung von Methylzellulose und von Cholesterin, ferner nach gleichzeitiger Verabreichung beider Stoffe untersucht. Der Gesamt-Lipoidgehalt der Aorta und der Leber war nach gleichzeitiger Cholesterin- und Methylzellulose-Zufuhr erniedrigt und bei den Tieren dieser Gruppe war auch der Schweregrad der Atheromatose mäßiger. Dieses Ergebnis wird dem im methylzelluloseaktivierten RES zustandekommenden gesteigerten Fettmetabolismus zugeschrieben.

ДЕЙСТВИЕ ОДНОВРЕМЕННОГО ВВЕДЕНИЯ ХОЛЕСТЕРИНА И МЕТИЛЦЕЛЛЮЛОЗЫ НА ГИСТОЛОГИЧЕСКУЮ СТРУКТУРУ СОСУДОВ И ВНУТРЕННИХ ОРГАНОВ

Ш. БЕНКЕ, Ф. БИЛИЦКИ и Ф. САРВАШ

У кроликов была изучена гистологическая структура сосудов и внутренних органов после дачи метилцеллюлозы, холестерина, а также после одновременного введения обоих препаратов. В группе животных, получивших оба средства совместно, содержание общих липоидов в аорте и печени оказалось пониженным. У этих же животных степень тяжести атероматоза была более умеренной. Авторы приписывают этот эффект повышенному метаболизму жира, возникающему в ретикулоэндотелиальной системе, активированной под влиянием метилцеллюлозы.

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COMPARATIVE MORPHOLOGY OF SMALL VESSEL LESIONS IN RHEUMATOID ARTHRITIS AND PERIARTERITIS NODOSA*

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Comparative histological study of the lesions of small arteries observed in rheumatoid arthritis and periarteritis nodosa revealed that the peripheral arteritis common to both conditions differs only in a quantitative respect.

In the acute and subacute stages, more distinctive proliferation of the connective tissue elements of the vessel wall was found in periarteritis nodosa than in rheumatoid vasculitis. This phenomenon may explain the finding that corticosteroid treatment may have a different effect on the vascular lesion in the two diseases.

In the chronic stage, fibrosis of the vessel wall, in particular of the intima, leading to obliteration of the vascular lumen, was more pronounced in periarteritis nodosa than in rheumatoid arthritis; in the genesis of this phenomenon hypertension is supposed to play a role.

Although none of the various forms of arteritis associated with rheumatoid vasculitis and periarteritis nodosa can be considered specific, their clinical, pathological and histological picture permits to distinguish between the two vascular processes with great probability. The cases where the morphologic characteristics of both conditions are demonstrable, correspond to the clinical definition "malignant rheumatoid arthritis".

It has been known for more than a decade that patients with rheumatoid arthritis develop inflammatory vascular lesions in the skeletal muscles [38] and peripheral nerves [34, 35]. In arterioles less than 200 μ in diameter, acute necrosis or subacute proliferation occurs in one third, while chronic fibrous arteritis in one half, of the cases. After the first relevant observations the question has been raised whether arteritis is a specific lesion typical for rheumatoid arthritis, or it is a form of periarteritis nodosa developing in association with rheumatoid arthritis. As both types of vascular lesion are relatively rare and of disseminated focal character, clarification of the problem required a detailed parallel study of necropsy material from both conditions.

Vascular lesions of muscles and nerves associated with periarteritis nodosa have been examined by various authors [1, 9] in contrast to similar vascular changes occurring in other collagen diseases. It is long known that periarteritis nodosa often manifests itself by nervous signs, and peripheral neuropathy may be one of its characteristic symptom complexes. As to the lesions of peripheral nerves due to periarteritis nodosa, investigations by Hungarian pathologists

* This work is dedicated to Professor Joseph Baló on the occasion of having completed five decades in the service of science.

have been of pioneer importance [6, 5, 20, 27]. Their studies were mostly centred on the histology of the large arteries; since the lesions due to rheumatoid arthritis can reasonably be compared only with lesions of small arteries, in the present study the lesions of small muscular and nervous arteries were examined.

The definition of periarteritis nodosa and the differentiation of its individual forms has been based on the studies by ZEEK et al. [40, 41], although the five varieties of necrotizing arteritis described by them (classical form of periarteritis nodosa, allergic angitis, angitic granulomatosis, angitis associated with various collagen diseases, temporal arteritis) cannot be sharply distinguished [3]. Differentiation of the above varieties on the basis of inflammatory lesions of the small arteries is still more uncertain, as for instance the classical form of periarteritis nodosa is characterized by an affection of medium arteries.

Prior to the recognition of the association of generalized arteritis with rheumatoid arthritis, several observations have already indicated that a coexistence between "rheumatism" and periarteritis nodosa seems more frequent as expected [17, 39]. KLINGE et al. [24, 25] have shown that rheumatism affected the entire vascular system and the associated vessel lesions differed hardly from the microscopic appearance of periarteritis nodosa. A comparative study of vascular lesions is preconditioned by a more precise knowledge of rheumatoid vasculitis and the correct definition of the forms of periarteritis nodosa.

Part of the earlier observers held the view that the vascular lesions associated with rheumatoid arthritis and periarteritis nodosa were distinguishable by morphological features. CRUICKSHANK [13] stated that rheumatoid arteritis differed from periarteritis nodosa in being localized to the small muscular arteries and not involving aneurysm and thrombosis. According to BALL [4], SOKOLOFF and BUNIM [38], rheumatoid arteritis was distinguishable from periarteritis nodosa by the absence of a massive destruction of the vessel wall. Later authors have advocated the morphological identity of the two vascular lesions and inferred that only one of them is independent; some of them regarded rheumatoid arthritis the primary condition and periarteritis nodosa its complication, while others believed that rheumatoid arthritis is one of the many varieties of periarteritis nodosa.

The primary nature of periarteritis nodosa is supported chiefly by clinical data [7, 36]. It is known that in one third of the cases the condition is accompanied by arthritic symptoms and often manifests itself clinically in the form of rheumatic muscle pain or peripheral neuropathy; the rheumatoid factor may be positive in 30% of the cases of periarteritis nodosa. Inconsistently with this supposition, the occurrence of periarteritis nodosa is three times more common among males than among females, while rheumatoid arthritis is chiefly a disease of women. Also, the above hypothesis hardly accounts for the paradoxical effect of steroid treatment which is unequivocally favourable in periarteritis nodosa,

whereas in patients with rheumatoid arthritis it seemed to elicit sometimes generalized arteritis.

A more generally accepted view has been to regard periarteritis nodosa as a complication, or occasionally an accompanying condition of rheumatoid arthritis [14, 26]. This opinion has been advocated particularly by the investigators who have doubted the independence of periarteritis nodosa, regarding it merely as a symptom or syndrome whose one aetiologic factor may be rheumatoid arthritis [16, 30, 31].

The majority of authors accepting the independence of both the classical rheumatoid arthritis and the classical periarteritis nodosa, distinguish a third form designated "malignant rheumatoid arthritis" which bears the morphological and clinical characteristics of both conditions and may be regarded as an intermediate condition [2, 8, 18]. This "malignant" pattern differs from classical rheumatoid arthritis and periarteritis nodosa not only in the type of vascular lesions, but also in certain typical laboratory findings such as a positive Rh factor and LE cell phenomenon, as well as in clinical signs such as the association of neuropathy and the more frequent presence of subcutaneous nodules [26, 28, 33, 37].

Interestingly, the above data have been derived from relatively few morphological examinations and still scarcer have been the comparative histological studies of lesions associated with rheumatoid arthritis and periarteritis nodosa [12, 29, 30].

Investigations

We carried out comparative histological examinations of vascular lesions in muscle and peripheral nerve tissue from necropsy material in 30 cases of rheumatoid arthritis, 7 cases of classical periarteritis nodosa, 1 case of hypersensitive angitis and 2 cases of necrotic polyangitis and granulomatosis.

Out of the 30 rheumatoid arthritis cases 26 were females and 4 were males, with an average age of 65 and an average duration of the disease for 17 years. In every case, characteristic lesions of the hand joints were encountered, and in 11 cases chronic arthritis deformans involved also other joints. Histologically, acute necrotic, subacute proliferative and chronic fibrous arteritis of small muscular and nerve vessels was found in 9 cases, chronic fibrous vascular lesion alone in 5 cases, while inflammatory lesions of small arteries were absent in 16 cases. In the 7 cases of classical periarteritis nodosa (6 men, 1 woman), average age was 47; in 4 cases acute necrotic, subacute proliferative and chronic fibrous arteritis occurred uniformly in the muscular and nervous arteries less than 200 μ in diameter, while in 3 cases solely chronic arteritis was found.

Comparison of acute and subacute inflammation of small muscular and nerve arteries in rheumatoid arthritis and periarteritis nodosa revealed a nearly

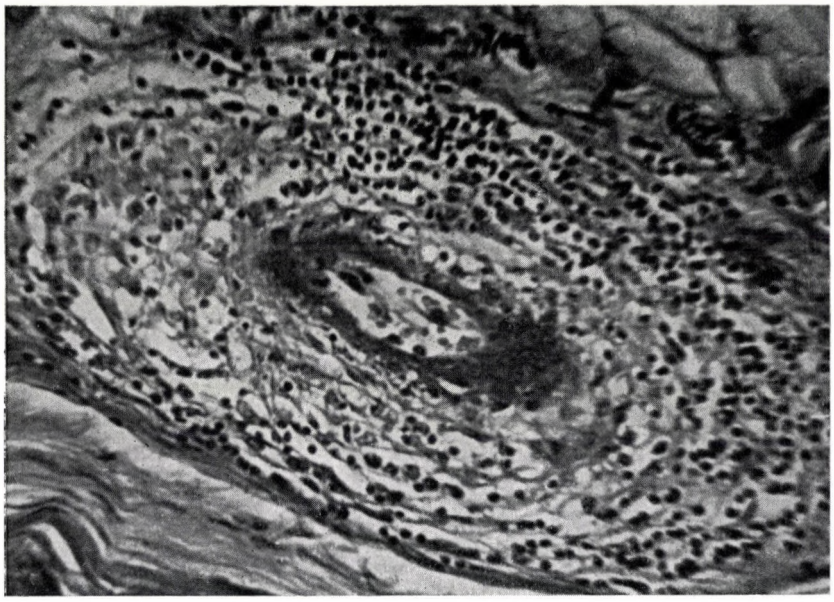

Fig. 1. Rheumatoid arthritis. Female, age 62, femoral nerve. Subacute arteritis. Haematoxylin-eosin. ×360

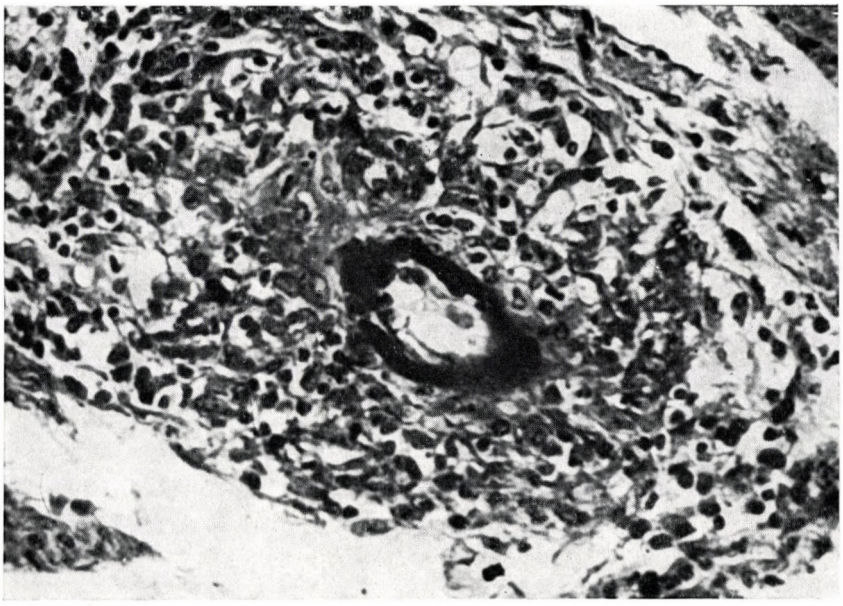

Fig. 2. Periarteritis nodosa. Male, age 30, median nerve. Subacute arteritis. Haematoxylin-eosin. ×360

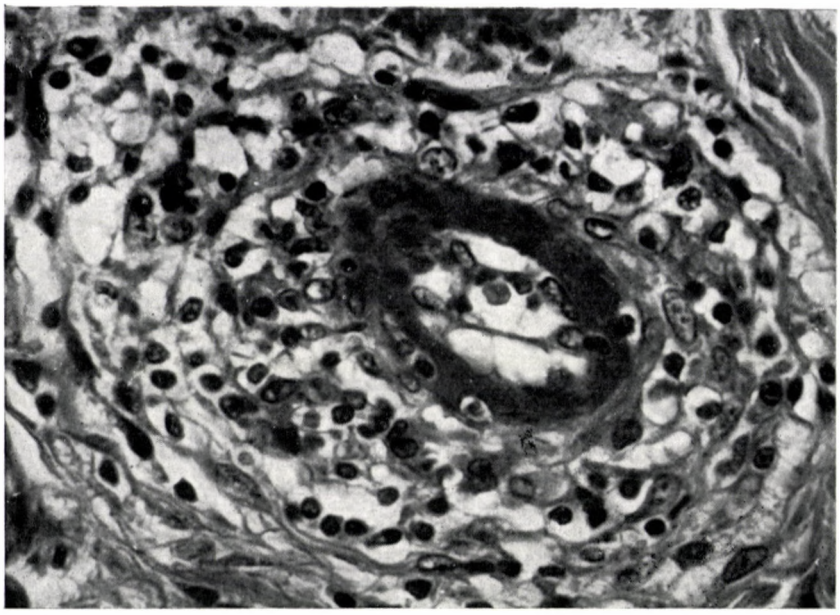

Fig. 3. Periarteritis nodosa. Male, age 30, median nerve. Subacute arteritis. Haematoxylin-eosin. ×480

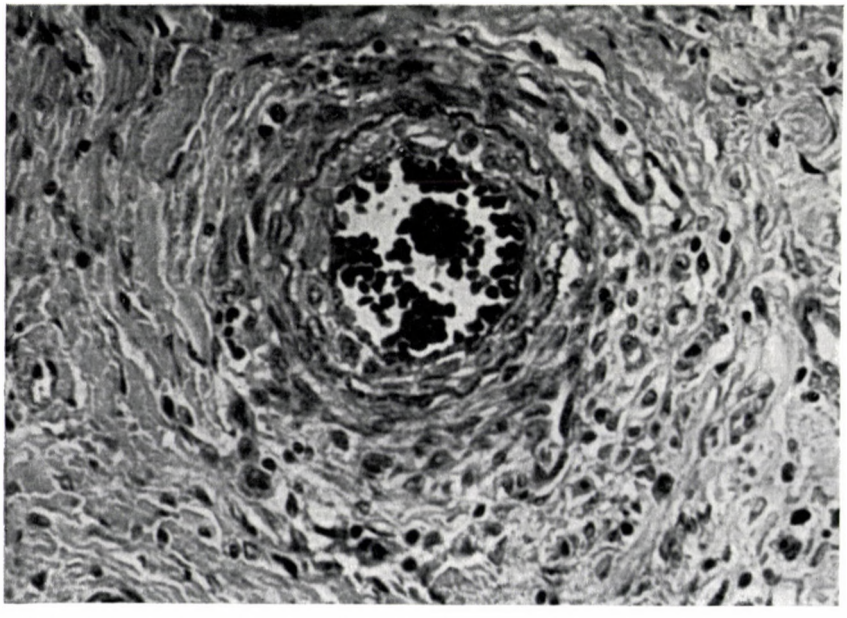

Fig. 4. Periarteritis nodosa. Male, age 30, femoral nerve. Subacute arteritis. Azan stain. ×360

identical morphological appearance of the two kinds of lesions. A moderate difference was encountered only in the composition and intensity of the mesenchymal tissue reaction associated with the fibrinoid necrosis of the vessel wall, in that in rheumatoid arthritis it was rather of exudative character composed chiefly of leukocytes or lymphocytes (Fig. 1), whereas in periarteritis nodosa there was a massive fibroblastic proliferation of the adventitia (Fig. 2) often with a proliferation of the intima and more intense fibrous tissue formation (Fig. 3). In the subacute phase, proliferation of smooth muscle cells of the media (Fig. 4) and the proliferative process in some cases resulted in a massive

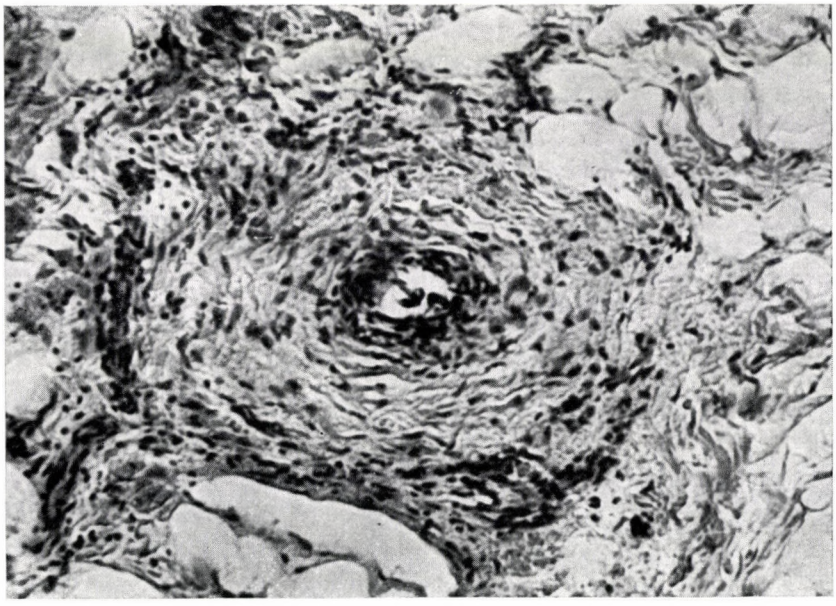

Fig. 5. Rheumatoid arthritis. Female, age 40, median nerve. Chronic arteritis. Haematoxylin-eosin. ×240

obliteration of the vascular lumen. This has never been encountered in rheumatoid vasculitis; its cause may be sought in the fact that in periarteritis nodosa vascular changes are often associated with thrombosis.

Comparison of chronic peripheral arteritis in rheumatoid arthritis and periarteritis nodosa showed little difference between the two lesions; it manifested itself chiefly in the microscopic picture of the intima. While in rheumatoid arthritis chronic arteritis manifested itself with a fibrous transformation of the media and a fibrous broadening of the adventitia with the intima relatively intact (Fig. 5), in periarteritis nodosa there was often a fibrous thickening of the intima which even obliterated the lumen (Fig. 6). Fibrous transformation of media and adventitia was also of a higher degree in periarteritis nodosa

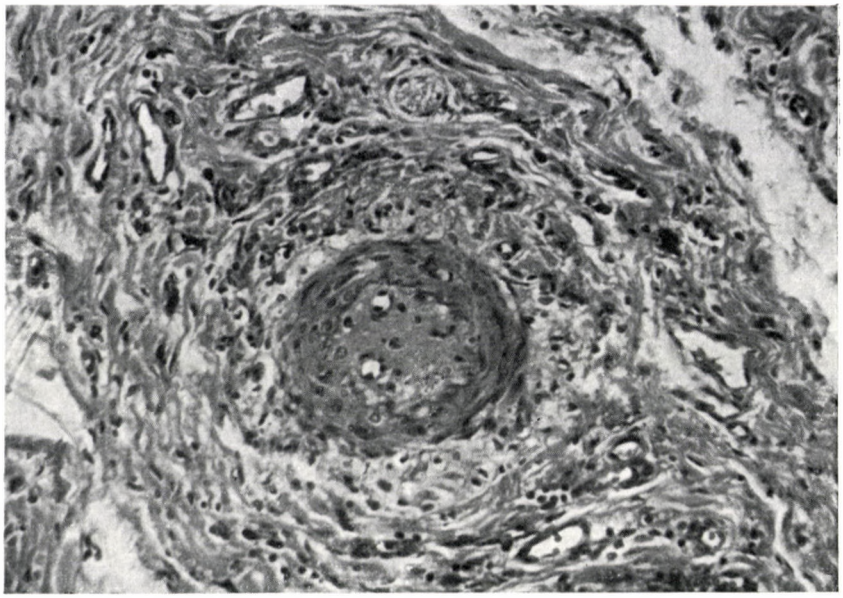

Fig. 6. Periarteritis nodosa. Male, age 21, median nerve. Chronic arteritis. Haematoxylin-eosin. ×240

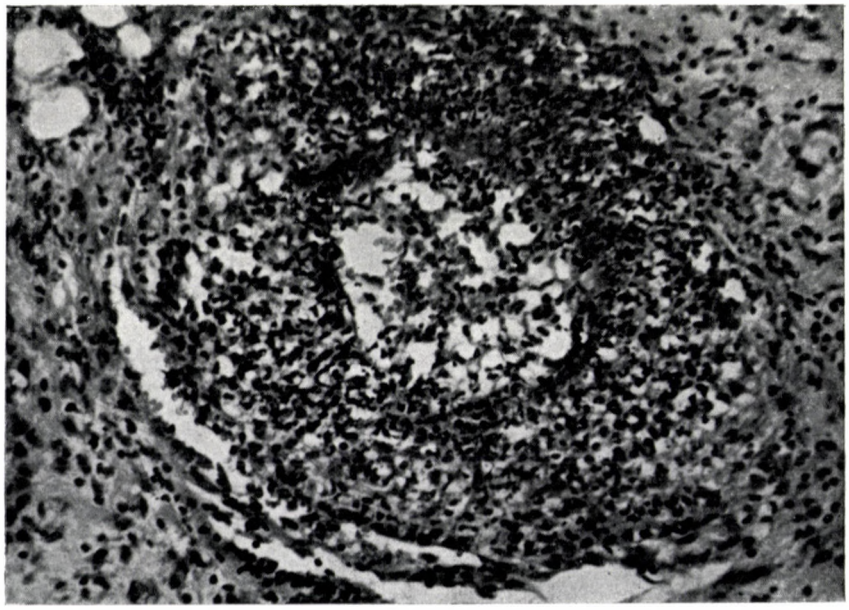

Fig. 7. Hypersensitive angitis. Female, age 64, ischiatic nerve. Acute arteritis. Haematoxylin-eosin. ×360

than in rheumatoid arthritis, but the difference was not so pronounced. Sometimes haemosiderin granules were seen in the media and adventitia, with either scarce remnants or marked regeneration of elastic fibres, as noted also in rheumatoid arthritis. The cicatrized vessel wall in some areas showed signs of recurrence, such as bleeding of the intima associated either with thrombosis, or fresh necrosis of the media, resp. of the thickened intima.

It is remarkable that chronic arteritis connected with fibrosis of the vessel wall — which occurs nearly in half of the cases of rheumatoid arthritis — was not found by us in periarteritis nodosa; in the latter condition we observed only the round-cell and mast-cell infiltration of the adventitia.

The histological picture of the so-called hypersensitive angitis (allergic arteritis) was characterized also in our material by generalized simultaneous fibrinoid necrosis of small arteries without involvement of the adjacent larger arteries. All layers of the vessel wall showed grave leukocytic and milder lymphocytic infiltration, without any sign of tissue regeneration or proliferation (Fig. 7). Vascular lesions were in a uniform stage both in the inner organs and the muscles and nerves; neither healing, nor chronic cicatrized lesions were found. In this respect the so-called hypersensitive angitis seems basically to differ from rheumatoid vasculitis.

The microscopic characteristics of necrotizing polyangitis and granulomatosis [11], or angitic granulomatosis [2] were described in detail by us in a previous paper [19]. Typical of this process are the occurrence of necrosis in certain organs (lung, spleen, kidney) independently of vessels, as well as of severe necrotic and proliferative vascular lesions; in the two cases encountered in our material severe vascular lesions were found in the kidneys, whereas no signs of arteritis were apparent in the vessels supplying the muscles and nerves.

Discussion

Our observations suggest the possibility of differentiating between small vessel lesions associated with rheumatoid arthritis and the other forms of "necrotizing arteritis". It seems that in rheumatoid arthritis and classical periarteritis nodosa lesions of the muscular and nervous vessels do not differ in nature only in extent and intensity. In contrast with the opinion of Skoloff and Bunim [38], in the acute and subacute phase this difference manifests itself not so much in the degree of destruction as in that of the reparative tissue process, which in the case of periarteritis nodosa appears as a massive proliferation of the connective tissue elements of the vessel wall.

If this assumption proves true, it will account for the contradictory behaviour of the two kinds of vascular lesion, viz. rheumatoid vasculitis and periarteritis nodosa, under corticosteroid treatment. It is known that in some cases

of rheumatoid arthritis steroid treatment either aggravates the existing vascular lesions [10, 21, 30], or elicits necrotising arteritis by activating the suspected latent lesions [14, 28, 42]. In contrast, in periarteritis nodosa, steroids have a favourable influence on the course of necrotizing arteritis. The different effect of steroids on the two vascular diseases may be interpreted as a proof of a basic difference in their nature. We believe that the disparity of steroid actions is due to the different tissular involvement of small arteries in the two conditions. Histologically, periarteritis nodosa differs from rheumatoid arthritis in the association of cell-rich intravascular or perivascular proliferation with necrotizing arteritis which is present in both diseases; this seems to account for the greater efficiency of steroids in periarteritis nodosa due to their suppressive action [22] on fibroblastic proliferation, granulation and fibrosis.

In the chronic stage, the rheumatoid vasculitis and the peripheral arteritis associated with periarteritis nodosa differ in the more distinctive thickening of the intima in the latter case, liable to cause a fibroid obliteration of the lumen. Further, adventitial fibrosis may also be more severe in periarteritis nodosa than in rheumatoid arthritis. Nevertheless, chronic arteritis manifesting itself exclusively with a slight round-cell infiltration of the adventitia without granular-cicatricial thickening of the vessel wall, does never occur in periarteritis nodosa.

Both rheumatoid arteritis and periarteritis nodosa are characterized by the simultaneous occurrence of lesions in various stages in the small arteries of muscles and nerves, as contrasted to allergic arteritis where the vascular lesions are in the same stage in all parts of the body.

In view of the microscopic characteristics of rheumatoid arthritis and periarteritis nodosa, we regard the relevant small arterial lesions a modification of necrotizing arteritis rather than a specific lesion. The nature of this modification and thus also the histologic picture of arteritis appear to depend on various factors. Thus in the pathogenesis of the more severe small arterial lesions associated with periarteritis nodosa, hypertension may play a role; it is known that an elevated blood pressure is more distinctive and frequent in periarteritis nodosa than in rheumatoid arthritis.

The inflammatory changes of medium arteries accompanied by necrosis and nodular fibrosis, so characteristic of classical periarteritis nodosa, do not usually occur in rheumatoid arthritis. In typical rheumatoid arthritis, the involvement of large arteries, sometimes also in the lungs, is only exceptionally of such an extent and intensity as to suggest the probability of its association, or coexistence, with periarteritis nodosa [4, 38]. These cases are considered clinically "malignant rheumatoid arthritis". On the analogy of this picture, the association or coexistence of rheumatoid arthritis with Takayasu's disease [15] or with temporal arteritis [23] has been reported.

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BEITRÄGE ZUR VERGLEICHENDEN MORPHOLOGIE DER VERÄNDERUNGEN KLEINSTER ARTERIEN BEI RHEUMATOIDER ARTHRITIS UND PERIARTERIITIS NODOSA

B. RADNAI

Anläßlich der Sektion an rheumatoider Arthritis oder Periarteriitis nodosa Verstorbener wurde die histologische Struktur der in der Skelettmuskulatur und in den peripheren Nerven beobachteten Veränderungen kleinster Arterien verglichen. Im Hinblick auf die periphere Arteriitis lassen sich zwischen den beiden Krankheitsbildern nur quantitative Unterschiede aufzeigen.

In der akuten-subakuten Phase der Gefäßveränderungen ist die Proliferation der Bindegewebselemente bei Periarteriitis nodosa ausgeprägter als bei rheumatoider Vasculitis. Diese Erscheinung mag als Erklärung jener Erfahrungstatsache dienen, daß die Kortikoidtherapie bei den beiden Krankheitsbildern auf die Gefäßveränderungen eine unterschiedliche Wirkung ausüben kann.

In der chronischen Phase der Gefäßveränderungen ist die Fibrose der Gefäßwand, insbesondere der Intima erheblicher, und sie kann zur Obliteration des Gefäßes führen. Es ist nicht ausgeschlossen, daß an der Pathogenese dieser Erscheinung die Hypertension beteiligt ist.

Obwohl bei rheumatoider Arthritis oder bei Periarteriitis nodosa die verschiedenen Arteriitiden nicht als spezifische Veränderungen betrachtet werden können, lassen sich die beiden Gefäßprozesse auf Grund der einheitlichen Betrachtung des klinischen, anatomischen und histologischen Bildes aller Wahrscheinlichkeit nach voneinander unterscheiden. — Jene Fälle, welche die morphologischen Zeichen beider Krankheitsbilder aufweisen, dürften dem klinischen Begriff »maligne rheumatoide Arthritis« entsprechen.

ДАННЫЕ К СРАВНИТЕЛЬНОЙ МОРФОЛОГИИ ИЗМЕНЕНИЙ МЕЛКИХ СОСУДОВ ПРИ РЕВМАТОИДНОМ АРТРИТЕ И УЗЛОВАТОМ АРТЕРИИТЕ

Б. РАДНАИ

На основе сравнения гистологической структуры изменений мелких сосудов наблюдаемых в скелетной мышце и в периферических нервах на вскрытии умерших от ревматоидного артрита и узловатого периартериита, между периферическими артериитами этих двух патологических процессов можно выявить лишь количественные разницы.

В острой-подострой фазах изменений сосудов, при узловатом периартериите пролиферация соединительно-тканных элементов стенки сосуда более выражена, чем при ревматоидном васкулите. Это явление может объяснять также вопрос, почему лечение кортикостероидами может оказать различное действие на изменения сосудов при этих двух патологических процессах.

В хронической фазе изменений сосудов при узловатом периартериите наблюдается более тяжелый фиброз стенки сосуда, в частности интимы, который может привести к облитерации сосуда. Полагается, что в патогенезе этого явления участвует гипертония.

Хотя различные артерииты, наблюдаемые в связи с ревматоидным васкулитом и в связи с узловатым периартериитом, нельзя рассматривать как специфические, на основе единого воззрения на клиническую, патолого-анатомическую и гистологическую картины, эти два патологических процессы по всей вероятности можно обособлять друг от друга. — Те случаи, которые показывают морфологические признаки обоих заболеваний, соответствуют, по-видимому, клиническому понятию «злокачественного ревматоидного артрит»-а.

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QUANTITATIVE HISTOCHEMICAL ANALYSIS OF CYTOMEGALIC NUCLEAR INCLUSIONS

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(Received May 10, 1968)

The concentrations of DNA, total proteins and basic protein have been determined cytophotometrically in the nuclear inclusions found in different organs of four subjects suffering from cytomegalic inclusion disease. The amount of DNA and proteins contained in the inclusions was in all cases 3 to 4 times higher than in the control nuclei. No basic protein of the histone type was found in the inclusions of two premature infants, while considerable amounts of it were revealed in those of a child and an adult.

The presence of DNA in cytomegalic nuclear inclusions has been proved by histochemical [35], photometric [12, 20] and fluorescence-optical [33] investigations. DNA-virus is now acknowledged as being involved in the pathogenesis of cytomegalic inclusion disease [35] and light microscopic observations are in harmony with this view. Electron microscopic examinations have already yielded information about the ultrastructure, crystalloid organization and intercellular development of the viral bodies [6, 11, 28, 36]. Both light microscopic and electron microscopic data agree in that inclusions from various organs contain not only DNA but proteins as well; quantitative measurements show that the amount of both substances is significantly increased [34].

So far, quantitative examinations have been concerned with single cases, with the inclusions of a particular organ so that no data are available to show differences in the amount of DNA and protein contained in the different organs of individuals belonging to different age groups. This has been studied in the present work. It was found that the nuclear inclusions of individuals of various ages invariably contained higher amounts of DNA and total protein than did the control nuclei, while no basic protein was found in the inclusions of premature infants.

Material and methods

The organs of four subjects of various ages who on histological evidence had died of cytomegalic inclusion disease were used for the quantitative photometric determination of DNA and protein.

Case 1. A male infant born at 7 months of pregnancy with a body weight of 930 g, became repeatedly grey, his heart sounds were weak, the respiration arrhythmic. He died

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after 20 hrs of postnatal life. The clinical diagnosis was intracranial haemorrhage (?). Histological examinations revealed cytomegalic giant cells in the *submandibular* gland.

*Case 2.** First child of Rh-incompatible parents. Her weight was 1900 g. The predominant symptom consisted in subcutaneous haemorrhages throughout the body. A systolic murmur was heard over the base of the heart. There was hepatic and splenic enlargement. Immediately after birth convulsions appeared, later the haemorrhages became more and more extensive, and the patient died at the age of four hours. Autopsy revealed a ventricular septal defect and cerebral haemorrhage into the ventricle. Histology revealed cytomegaly in *salivary glands*, *kidneys*, *liver*, *brain*.

*Case 3.*** (This case has been reported in detail; see reference 22.) A female patient of 36 years who had been hospitalized eight times during the previous 5 years on account of renal complaints. The clinical diagnosis was chronic pyelonephritis and uraemia. The gross necropsy findings did not admit of an unambiguous conclusion regarding the renal change; the kidneys weighed 80 g. The diagnosis of *renal cytomegalic disease* was only revealed by the histological examination.

Case 4. A male patient of 3 years whose illness, like that of his 9-month-old brother, had started with pneumonia 2 months before. The younger brother had died of bronchopneumonia after five days. The patient became more and more dyspnoeic and died with signs of respiratory failure two months later. The clinical diagnosis was bronchiolitis, bilateral bronchopneumonia, asthmatic bronchitis. Autopsy revealed chronic (viral?) pneumonia with interstitial emphysema. Histological examination revealed *cytomegalic inclusion pneumonia*.

The younger brother, who had died before our patient did, had the same kind of pneumonia. According to a subsequent statement of the parents, some pigs of the family had died of an unknown disease before the children fell ill.

We stained our sections with haematoxylin-eosin, PAS, Sudan black, gallocyanin, and toluidine blue of pH 4.

Detailed data about the microscopic findings and qualitative histochemistry in cytomegalic inclusion disease, the description of which is beyond the scope of this study, can be found in the literature [2, 16, 19, 23, 29, 30]; our findings were in complete agreement with them.

The DNA-contents of the cells were estimated by Feulgen's reaction with BLOCH's modification [3, 4] (λ : 570 mμ), while the histone-type and total proteins according to ALFERT and GESCHWIND [1] after staining with fast green of pH 8.1 and 2.2 (λ : 600 mμ). Cytophotometric quantitative determinations [21] were performed in 6 to 8 μ thick sections of the same cell fixed in 10% formalin and subjected to Feulgen's reaction. Several characteristic visual fields were photographed at 400 × magnification. The cytomegalic and the control cells were separately numbered on the prints and the DNA in the numbered individual cells was then determined cytophotometrically (obj. 100 ×). Extraction of the same sections by trichloroacetic acid was followed by staining with fast green. Then the DNA-tested cells were selected on the basis of the numbers on the prints and their content of basic protein and — after renewed staining — their total protein content were determined. About 25 to 30 cells were so examined at a time. Their DNA and protein contents were expressed in AU (arbitrary unit = extinction × surface μ^2).

Results

Differences between the inclusions in the individual cases were revealed already by light microscopy. In cases 1 and 2 (Fig. 1), the round or oval inclusions were clearly distinguishable from the nucleus. The non-staining halo between the Feulgen-positive inclusion and the finely granular nuclear membrane is well marked in the figure. The large cytoplasm of the cells was PAS and Sudan black positive, while neither the inclusion nor the cytoplasm stained with pH 8.1 fast green [Fig. 3a], which, however, stained intensely the Feulgen positive granules attached to the nuclear membrane. pH 2.1 fast green stained the inclusion, the cytoplasm and its granules intensely.

* We are indebted to Dr. Gy. KASZA for referring the material to us.
** We are indebted to Dr. I. KÁDAS for referring the material to us.

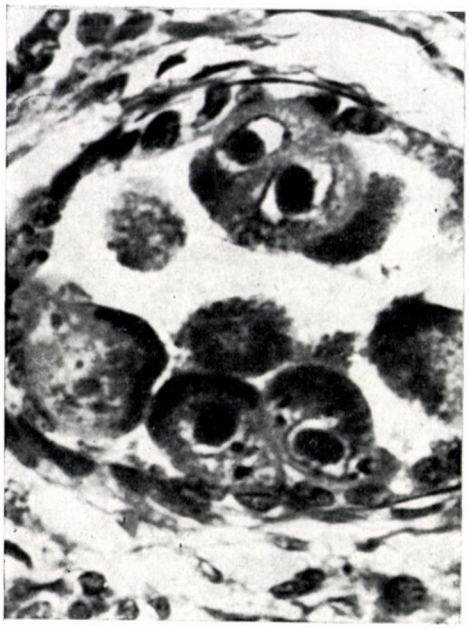


Fig. 1. Cytomegalic renal inclusion in a premature infant. PAS reaction. ×650

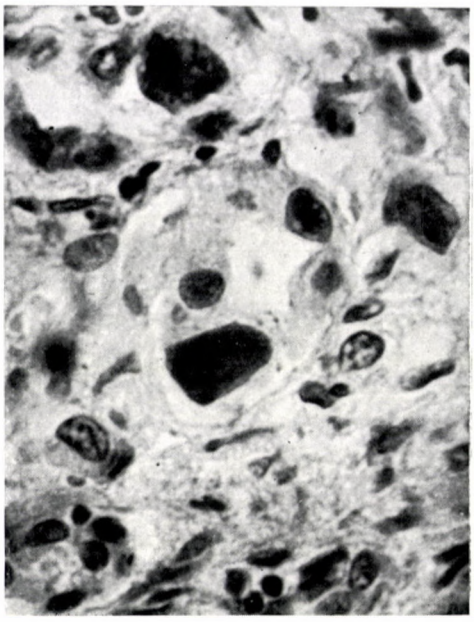


Fig. 2. Cytomegalic renal inclusions in an adult. Toluidine blue of pH 4.0. ×650

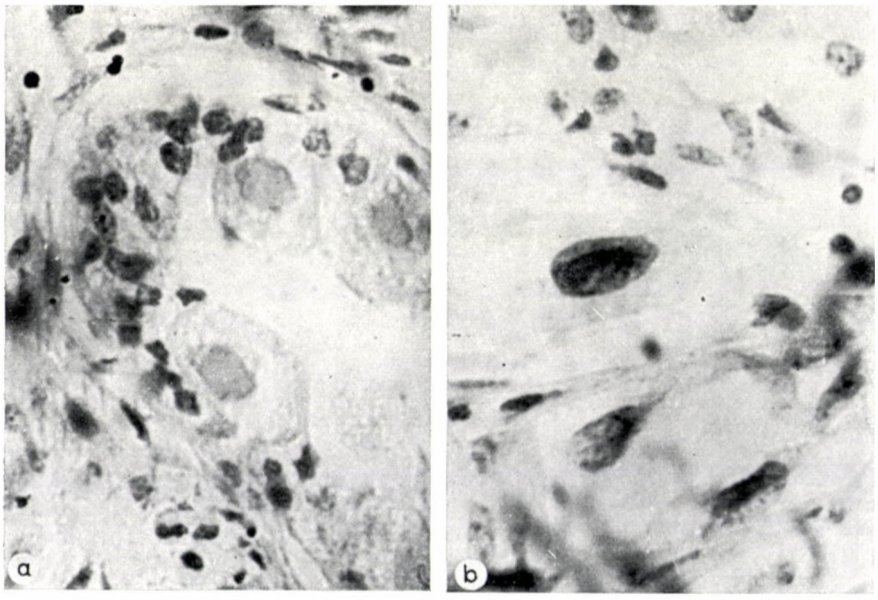


Fig. 3. Renal inclusions in cytomegalic infant (a) and adult (b). Fast green of pH 8.1. Basic protein was demonstrated in case (b) only. ×650

Table I

Amount of DNA, total proteins and basic protein in cytomegalic nuclear inclusions, expressed in arbitrary units

Case No.	Age, sex, specimen	1. DNA, AU			2. Total protein, AU			3. Basic protein, AU		
		Contr.	Cytomeg.	Contr. / Cytomeg.	Contr.	Cytomeg.	Contr. / Cytomeg.	Contr.	Cytomeg.	Contr. / Cytomeg.
1.	20 hours (930 g) m salivary gland	5.9	25.2	4.3	6.7	26.2	3.9	4.2	Ø	Ø
2.	4 hours (1900 g) f kidney	7.6	23.0	3.0	7.1	29.5	4.2	4.4	Ø	Ø
3.	36 years f kidney	9.9	46.1	4.5	8.3	36.0	4.3	5.7	12.8	2.2
4.	3 years m lung	10.6	29.8	2.8	12.4	45.7	3.7	8.3	13.4	1.6

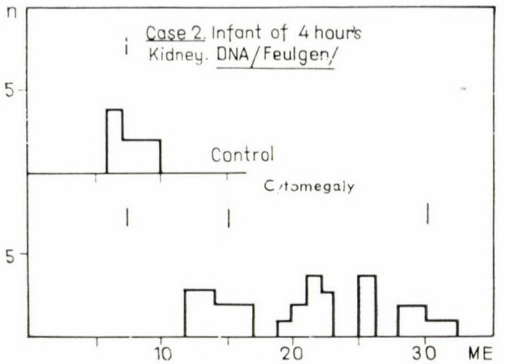
In contrast, the boundary between the ellipsoid inclusion almost completely filling the nucleus and the nuclear membrane was hardly visible in cases 3 and 4 (Fig. 2). Here the inclusion bodies were larger than in cases 1 and 2 (see the values for μ^2 in Table III). Feulgen-positive granulation of the nuclear membrane was absent. The cytoplasm failed to stain with PAS, Sudan black and pH 8.1 fast green, whereas the inclusions stained well with alkaline fast green (Fig. 3b). Both the nucleus and the cytoplasm gave a strongly positive reaction with acid fast green.

The results of quantitative cytophotometry are listed in Table I (its first column shows the Feulgen values of DNA). In all cases examined the DNA content of the inclusions was found to be 3 to 4 times that of intact nuclei in the same sections. The difference between average and mean values was relatively great, which was probably due to the widely variable size of the inclusions. Histograms 1 and 2 illustrate the distribution of DNA and basic protein (AU) in cases 2 and 3.

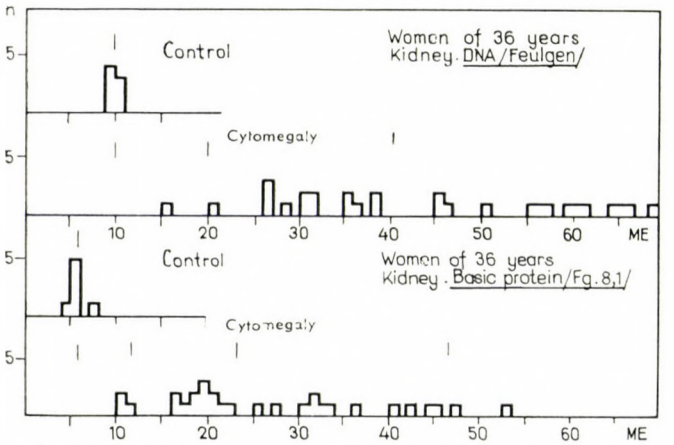
Staining with pH 2.2 fast green showed that the total protein content of the inclusions was in all four cases approximately four times higher than that of the control nuclei (second column of Table I).

While the values for DNA and total proteins were increased in all of the cases examined, those for basic protein showed considerable variations (third column of Table I). It was found in the course of the qualitative examinations already that only the inclusions of cases 1 and 2 failed to stain with pH 8.1 fast green. Quantitative determinations were, therefore, dispensed with in these cases, where the fast-green-positive granules adhering to the nuclear membrane were easy to measure. Their DNA content was approximately identical with

that of the control nuclei; the ratio Feulgen : fast green amounted to 1.6. The quantity of basic protein in the inclusions of cases 3 and 4 was, on the other hand, 1.5 to 2 times higher than that of the control nuclei. These data are particularly striking if the results obtained in cases 2 and 3 are compared, in which the cytomegalic kidneys originated from subjects of widely different ages.



Histogram 1. Relative DNA values of infantile renal inclusions are distributed over a comparatively narrow range



Histogram 2. Relative DNA and basic protein values of adult renal inclusions are distributed over a wide range

The quotients DNA : protein, computed from AU, are shown in Table II. In the control nuclei the quotient Feulgen : pH 8.1 fast green fluctuated around 1.5, a value characteristic of interphase nuclei. This quotient was about twice as high in the inclusions of cases 3 and 4 so that the ratio between the quotients DNA : basic protein in the controls and in the cytomegalic cases amounted to 1 : 2. Since the amount of both DNA and total proteins was about 3 to 4 times higher in the inclusions than in the control nuclei, the ratio of the quotients Feulgen : acid fast green amounted to 1 : 1.

Table II

The quotients of DNA : basic protein, and of DNA : total proteins computed from quantitative photometric values (Table I) of normal nuclei and nuclear inclusions

DNA/Basic protein [q_1]			Case No.	Age, sex, specimen	DNA/Total protein [q_2]		
Control q_{1-c}	Cytomegaly q_{1-cy}	$q_{1-c} : q_{1-cy}$			Control q_{2-c}	Cytomegaly q_{2-cy}	$q_{2-c} : q_{2-cy}$
1.4	0	1 : 0	1.	20 hours (930 g) m salivary gland	0.9	1.0	1 : 1.1
1.7	0	1 : 0	2.	4 hours (1900 g) f kidney	1.1	0.8	1 : 0.7
1.7	3.6	1 : 2	3.	36 years f kidney	1.2	1.3	1 : 1.1
1.3	2.2	1 : 2	4.	3 years m lung	0.9	0.7	1 : 1.3

Table III

DNA *(Feulgen) concentration in the surface of intact nuclei and renal inclusions of infants and adults with cytomegalic inclusion disease*

		Extinction E	Surface μ^2	$\frac{E}{\mu^2}$
Case 2 infant's kidney	Control	0.580	13.2	0.045
	Cytomegaly	0.390	54.5	0.007
Case 3 adult's kidney	Control	0.733	13.5	0.055
	Cytomegaly	0.613	77.8	0.008

Table III shows the DNA Feulgen concentration in the surface of the renal inclusions in case 2 (infant), case 3 (adult), and in the control nuclei. While the combined total amount of DNA and protein in the inclusions was in both cases many times higher than in the normal nuclei (Table II), the concentration of DNA was 6.5 to 7 times lower in the inclusions than in the control nuclei. Thus, the approximately fourfold increase in the total amount of DNA was out of proportion to the notable increase in the surface of the inclusions, a phenomenon in sharp contrast with that observed in likewise large tumour cells.

Discussion

Quantitative photometric estimations have shown that the total amount of DNA and protein contained in cytomegalic nuclear inclusions is 3 to 4 times higher than in diploid nuclei, a phenomenon in harmony with the results of

DICKMAN [12] and SANDRITTER [34], who also reported on increased DNA concentration in renal, salivary gland, and pulmonary inclusions. Similar observations were made by LEUCHTENBERGER [26], who found high nuclear concentrations of DNA in patients infected with DNA-virus.

The behaviour of basic protein was remarkable in our cases. SANDRITTER [34] was the first to describe that cytomegalic nuclear inclusions failed to stain with alkaline fast green. His patient was a baby, and also the further six cytomegalic salivary glands tested by him for staining with alkaline fast green were taken from infants. We, too, had two prematures with a negative histone reaction while the concentration of basic protein in the inclusions was significant in the two subjects of more advanced age. Thus, it seems that the amount of basic protein in nuclear inclusions is different in infants and older subjects.

Chemical [15], cytochemical [7, 8], immunological [9], autoradiographic [17], interference [25] and electron microscopic [31, 32] examinations showed the inclusions to represent intranuclear aggregates of viral particles. It is therefore safe to assume that infection induces a fundamental change in cellular metabolism [14, 18], which is supported by the observation that DNA isolated from DNA virus synthesizes not merely its own viral DNA but its specific proteins as well [10, 13]. The high DNA and total protein content found in our cases was in harmony with the results of these findings, which would further seem to be supported by the low DNA concentration in the inclusions. In pathological cells, e.g. in tumours, the DNA concentration is usually in linear relation to the number of chromosomes, a phenomenon we did not observe in the present material. Therefore, the extremely low concentration of DNA in the inclusions points to some intranuclear disorder, to a particular kind of DNA in the inclusions. According to DONNELLAN et al. [11] in the nucleus there are only incomplete viruses which have no ripe protein envelope; their final development takes place in the cytoplasm. Compared with intact nuclei, the amount of non-basic proteins was considerably increased in our four cases of cytomegalic inclusion disease. The increment might correspond to the pathologic protein of viral origin so that incomplete protein is represented by the non-basic protein fraction. It is, however, likewise probable that it is not the basic protein which completes the virus, for in none of our cases did we find proteins of the histone type in the cytoplasm, while their concentration was high in the inclusions of the individuals of more advanced age. Only KRISHAN et al. [24] have shown the presence of histone-type protein in atypical DNA nuclear inclusions. Thus, it is difficult to explain the phenomenon observed by us. The histograms of the infants and those of the older subjects showed essential differences, which were particularly conspicuous in the kidneys in cases 2 and 3 (see histograms 1 and 2). The fact that the values of DNA showed a relatively narrow distribution in the infants, whereas both DNA and basic protein were widely distributed in cases 3 and 4, suggests that the type of virus responsible for the disease in infants

might be different from that which causes it at a more advanced age. It is, however, likewise possible that one is simply dealing with inclusions at different stages of development. Again, viral inclusions may be brought about by the defence mechanism of the more developed organism or by prolonged drug treatment. It is further possible that, as a result of aging, the basic nuclear protein of the host cell is incorporated into the inclusion [27, 37]. All these suppositions are mere conjectures. What may be regarded as a fact, on the basis of the case reports, is that the two premature babies lived a few hours only, while the child of 3 years and the adult patient of 36 years were ill, and were treated, for months and years. It is therefore probable that, in addition to the factors discussed in the foregoing, the morphology, structure and chemical composition of inclusions depend not so much on the patient's age as on the duration and the general course of the disease.

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QUANTITATIVE HISTOCHEMISCHE ANALYSE DER KERNEINSCHLÜSSE BEI ZYTOMEGALIE

K. JOBST und M. KELLERMAYER

Bei vier Zytomegalie-Kranken verschiedenen Alters wurden in den Kerneinschlüssen die DNS, das Gesamteiweiß sowie die basischen Eiweiße cytophotometrisch bestimmt. Der DNS- sowie der Gesamtprotein-Gehalt war bei allen vier Kranken 3—4mal höher als in den Kontrollzellkernen. In den Kerneinschlüssen zweier Säuglinge wurden keine, in denen eines Kindes und eines Erwachsenen hingegen erhebliche Mengen von basischen Kerneiweißen vom Histontyp ermittelt.

КОЛИЧЕСТВЕННЫЙ ГИСТОХИМИЧЕСКИЙ АНАЛИЗ ЯДЕРНЫХ ВКЛЮЧЕНИЙ ПРИ ЦИТОМЕГАЛИИ

К. ЙОБСТ и М. КЕЛЛЕРМАЙЕР

В ядерных включениях четырех больных различного возраста с цитомегалией авторы проводили цитофотометрическое определение ДНК, общего белка и основных белков. Содержание ДНК и общего белка было в ядерных включениях у всех четырех больных 3—4 раза больше чем в контрольных клеточных ядрах. У двух больных грудного возраста не было найдено основных белков гистонового типа, в то время как у больного детского возраста и у взрослого их количество было значительным.

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RECENSIONES

Neurosecretion, IV

International Symposium on Neurosecretion. Strasbourg, July 25—27, 1966. Edited by F. Stutinsky. Springer Verlag, Berlin—Heidelberg—New York, 253 pages, with 87 figures and 1 portrait, 1967.

Volume I contains the lectures and discussion material of the IV. International Symposium on Neurosecretion, held in honour of Ernst Scharrer in July, 1966. After the opening lecture by Prof. J. Benoit there are 26 papers by H. A. Bern, F. Knowles, M. Herlant, M. Mazzuca, E. Follenius, J. Barry, J. Flament-Durand, A. Oksche, B. Vigh, I. Teichmann, J. C. van de Kamer, P. Disclos, A. Jasinski, A. Gorlman, T. Hara, J. C. Sloper, M. A. Karim, M. A. Richards, C. Kordon, A. Moskowska, H. Sachs, R. Portanova, E. W. Haller and L. Schare, K. Lederis, K. Fuxe and T. Hökfelt, W. W. Douglas, J. Taxi and B. Droz, H. Herlant-Meewis, J. Naisse and J. Mouton, I. R. Hagadorn, M. V. Angel, A. Oksche, M. Vaupel von Harnack and H. Wolff, G. Ohm and M. Vaupel von Harnack, P. Zimmermann, W. Bargmann. The papers dealt with (i) morphological changes induced experimentally in the neurosecretory system, and (ii) investigations into the hormonal effects of cellular processes taking place in the neurosecretory system.

All the papers rely upon investigations involving up-to-date methods (electron microscopy, biochemistry, autoradiography). By means of the electron microscope it is shown convincingly that in vertebrates the size of neurosecretory granules ranges from 1000 to 2000 Å. Further experimental evidence is offered to the physiological role of neurosecretion. Important data deal with the relationships of neurosecretory cells and the central as well as the peripheral nervous system. Studies have been made of the relation within the neurosecretory cells between nucleus and secretion granules.

The book should be read not only by experts of this relatively narrow field, but also by those interested in biology in general.

B. I. Baranyi

Senn, D. G.: Über das optische System im Gehirn squamater Reptilien

Suppl. 52—1 ad Vol. 65 »Acta Anatomica«, S. Karger AG, Basel/New York, 1966. IV + 88 Seiten mit 29 Abbildungen und 4 Tabellen. Preis: sFr/DM 23,—

Zur Kenntnis des allgemeinen Aufbaues des optischen Systems sowie dessen Evolution vermitteln die an Reptilien vorgenommenen Untersuchungen wichtige Angaben.

Vorliegende Arbeit untersucht in diesem Themenkreis nach einer vergleichenden anatomischen Behandlung der äußeren morphologischen Verhältnisse des Gehirns vom vergleichenden histologischen Gesichtspunkt zwei Probleme. Einerseits wird die Struktur des optischen Systems und der benachbarten Gehirngebiete bei verschiedenen squamaten Reptilien beschrieben, auf Grund welcher der Homologie mehrerer Regionen — mit besonderem Hinblick auf das Tectum opticum — eine Bedeutung zugeschrieben wird. Anderseits werden mit dem Vergleich der optischen Systeme die Verwandtschaftsbeziehungen von Echsen, Wühlschlangen, Boiden und höheren Schlangen besprochen.

Senns Monographie unterscheidet beim optischen System drei Teile: 1. Die diencephalen Anteile des optischen Systems, 2. Das tectum opticum und 3. Die Augenmuskelkerne.

Verfasser stellt fest, daß bei allen untersuchten Wühlschlangen und Boiden die Reduktion des optischen Systems als gestaltliche Modifikation des Gehirns augenfällig ist.

Die für Boiden und höhere Schlangen charakteristische Erscheinung der Einzelbündel im Abschnitt des Tractus opticus zwischen dem Augapfel und dem Chiasma, von denen jedes Bündel die zentrale Gliazellsäule enthält, hat sich bei Wühlschlangen in verschiedenem Grade als ancestrale Situation erhalten. Während die Fasern noch gleichmäßig (wie bei Echsen) im Tractus verstreut sind, zeigen die Gliazellen schon mehr oder weniger eine Anordnung in Säulen.

Die Ausprägung des Überkreuzungsmusters im Chiasma opticum hängt weitgehend von der quantitativen Möglichkeit ab. Doch geschieht die Aufteilung jedes Tractus bei den Echsen übersichtlich in wenige Blätter, während sie sich bei Schlangen als unregelmäßiges feines Muster darbietet.

Der Nucleus geniculatus lateralis, in welchem sich bei Echsen die mediale Zellplatte und das laterale Neuropil scharf voneinander abheben, ist bei extremen Wühlschlangen stark reduziert, bei anderen Schlangen in sich morphologisch durchdrungen. Mit Ausnahme des Nucleus posterodorsalis, der besonders bei Schlangen auffällt, sind die prätectalen Kerne bei Wühlschlangen nicht nachgewiesen; bei höheren Schlangen sind sie kaum verändert.

Im Tectum opticum verhalten sich die Schichtgruppen verschieden. Die periventriculären Schichten sind bei allen untersuchten Formen (Ausnahme: Varanus) in der Dicke konstant. Weiße und graue Substanz sind bei Echsen voneinander abgehoben, bei Schlangen morphologisch durchdrungen. Wühlschlangen stehen intermediär.

Die zentralen Schichten sind bei Echsen (Ausnahme: Varanus) und gewissen Wühlschlangen mehr in weiße und graue Zonen gegliedert als bei Boiden und höheren Schlangen. Bei Wühlschlangen sind diese Schichten reduziert.

Die Augenmuskelkerne entsprechen quantitativ dem Umfang des optischen Systems. Feinere Differenzierungen des Nucleus III wie bei Echsen sind bei Schlangen nicht erhalten. Die Reduktion des oculomotorischen Kernes bei Wühlschlangen und Boiden bewirkt eine Rostralverlagerung des Nucleus IV.

Eine vergleichende morphologische Untersuchung am Gehirn dieser Squamaten interessiert nicht nur funktionell und rein deskriptiv orientierte Morphologen und Neurologen, sondern verspricht auch Hinweise auf das Evolutionsproblem dieser Reptilien.

Einen besonderen Wert der Monographie stellt die von den ausgezeichneten Mikroskoppräparaten (Neurofibrillendarstellung nach Bodian und Kresylviolett-Färbung zur Darstellung der Nissl-Substanz und der Nukleinsäuren) verfertigte hervorragende Photodokumentation dar, zu deren Erfolg auch die erstklassige zeitgemäße Drucktechnik beiträgt.

T. DONÁTH

Bioluminescence in Progress

Edited by Frank H. Johnson and Yata Haneda. Princeton University Press, Princeton, New Jersey, 1966. Pp. 650.

The papers of this volume have been given at a Luminescence Conference which was held in Hakone National Park, Japan, September 12 to 16, 1965, under the auspices of the United States–Japan Cooperative Science Program with the joint support of the National Science Foundation and the Japan Society for the Promotion of Science.

The objectives of the Conference were fundamentally the same as those of an earlier conference devoted primarily to bioluminescence, held at Asilomar, California, in 1954, and supported by the National Science Foundation through the National Research Council's Committee on Photobiology, which "recognized the mutual advantages of bringing together a group of leading investigators for a critical appraisal of present knowledge, for the first-hand interchange of experiences and ideas, and for the projection of likely approaches to unsolved problems". The present Conference had the further objectives, in accordance with the purposes and policies of the U.S.–Japan Program, of promoting understanding and cooperation between Japanese and American scientists, with a view toward effecting a more intimate acquaintance with each other's points of view, methods, and research materials. The participants were thus almost wholly limited to Japanese and American investigators and their active collaborators.

The book embraces 35 papers of 48 researchers as well as the introduction of FRANK H. JOHNSON giving an excellent survey of the problematics of bioluminescence. The following subjects have been dealt with: J. R. TOTTER: Chemical Events Leading to Chemiluminescence of Lucigenine and Luminol; J. LEE, A. S. WESLEY, J. F. FERGUSON, III. and H. H. SELIGER: The Use of Luminol as a Standard of Photon Emission; J. W. HASTINGS and G. T.

REYNOLDS: The Preparation and Standardization by Different Methods of Liquid Light Sources; H.-D. STACHEL, E. C. TAYLOR, O. SHIMOMURA and F. H. JOHNSON: Synthesis and Properties of Some Indole Derivatives Related to Cypridina Luciferin and of Some New Indole Compounds in Dimethylsulfoxide; N. SUGIYAMA, M. AKUTAGAWA, T. GASHA and Y. SAIGA: Chemiluminescence of Indole Derivatives; Y. KISHI, T. GOTO, Y. HIRATA, O. SHIMOMURA and F. H. JOHNSON: The Structure of Cypridina Luciferase: Quantitative Measurement; Analysis of Inhibition by Urea; and Some Effects of Sodium and Potassium Ions; F. I. TSUJI and Y. HANEDA: Chemistry of the Luciferases of Cypridina hilgendorfi and Apogon ellioti; J. W. HASTINGS, Q. H. GIBSON, JOAN FRIEDLAND and J. SPUDICH: Molecular Mechanisms in Bacterial Bioluminescence: On Energy Storage Intermediates and the Role of Aldehyde in the Reaction; Q. H. GIBSON and J. W. HASTINGS: Light-initiated Bioluminescence; E. H. C. SIE, A. THANOS, and A. JORDON: Application of Luminescence in Aerospace Industry; R. L. AIRTH, G. ELIZABETH FOERSTER and PATRICIA Q. BEHRENS: The Luminous Fungi; M. J. CORMIER and J. R. TOTTER: The Apparent Phosphorescence of a Substance Extracted from the Mycelium of the Luminous Fungus, Omphalia flavida; S. KUWABARA and E. C. WASSINK: Purification and Properties of the Active Substance of Fungal Luminescence; E. C. WASSINK and S. KUWABARA: Some Notes on Semi-large-scale Cultivation of Luminous Fungi; HIROSHI NAKAMURA: A Note on Stimulatives of Bacterial Luminescence in Mass Culture; R. ECKERT: Excitation and Luminescence in Noctiluca miliaris; J. W. HASTINGS, M. VERGIN and R. DESA: Scintillons: The Biochemistry of Dinoflagellate Bioluminescence; B. M. SWEENEY and G. B. BOUCK: Crystal-like Particles in Luminous and Non-luminous Dinoflagellates; M. J. CORMIER, K. HORI and P. KREISS: Studies on the Bioluminescence System of the Sea Pansy, Renilla reniformis; M. J. CORMIER, P. KREISS and P. M. PRICHARD: Bioluminescence Systems of the Peroxidase Type: F. H. JOHNSON, O. SHIMOMURA and Y. HANEDA: A Note on the Large Luminescent Earthworm, Octochaetus multiporus, of New Zealand; O. SHIMOMURA, F. H. JOHNSON and Y. HANEDA: Isolation of the Luciferin of the New Zealand Fresh-water Limpet, Latia neritoides Gray; H. H. SELIGER and W. D. MCELROY: Quantitative Measurements of Luminescence; W. D. MCELROY and H. H. SELIGER: Firefly Bioluminescence; J. B. BUCK: Unit Activity in the Firefly Lantern; W. A. HAGINS, F. E. HANSON and J. B. BUCK: Statistics of Photon Emission and Control Mechanisms in Bioluminescence; O. SHIMOMURA, F. H. JOHNSON and Y. HANEDA: Observations on the Biochemistry of Luminescence in the New Zealand Glowworm, Arachnocampa luminosa; O. SHIMOMURA and F. H. JOHNSON: Partial Purification and Properties of the Chaetopterus Luminescence System; F. H. JOHNSON, H.-D. STACHEL, O. SHIMOMURA and Y. HANEDA: Partial Purification of the Luminescence System of a Deep-sea Shrimp, Hoplophorus gracilorostris; Y. HANEDA, F. H. JOHNSON and O. SHIMOMURA: The Origin of Luciferin in the Luminous Ducts of Parapriacanthus ransonneti, Pempheris klunzingeri, and Apogon ellioti. Y. HANEDA: On a Luminous Organ of the Australian Pine-cone Fish, Cleidopus gloria-maris De Vis; J.-M. BASSOT: On the Comparative Morphology of Some Luminous Organs; Y. K. OKADA: Observations on Rod-like Contents in the Photogenic Tissue of Watasenia scintillans through the Electron Microscope.

The papers themselves are noteworthy in both scope and content, for they range from purely chemical to purely biological in their approach to the central problem — the phenomenon of light emission by living organisms — and they include some new technical reports which constitute real milestones marking the advancing frontiers of this field. There are magnificent electronmicrographs and some colour plates among the more than 250 illustrations. It is reasonable to expect that the book will be found useful by anyone interested either in the background and status of special aspects or in a more comprehensive view of the subject as it is in progress.

T. DONÁTH

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Morphologia Normalis et Experimentalis

Pathologia

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Institute of Anatomy (Director: Prof. J. SZENTÁGOTHAI),
University Medical School, Budapest

EFFECTS OF MECHANICAL INSUFFICIENCY AND INCREASED PERIPHERAL OFFER IN THE MESENTERIC LYMPHATIC REGION

J. VAJDA, Erzsébet FEHÉR and K. CSÁNYI

(Received August 12, 1968)

The effects of a ligation of efferent lymphatics and of an increase of peripheral offer on the mesenteric lymphatic area have been investigated. Sudden obstruction of lymphatics led to congestion, gradually increasing the calibre of lymphatic vessels to 3—4 times the normal. The lymphatics can cope with the excess strain for a limited time only. The most important role is played by the zone of arcades and by the lymphatics of shunts, which in the course of compensation avert lymph flow toward adjacent, intact segments. This activity produces merely temporary results, because total mechanical failure develops in 4 to 5 hours.

The increase of peripheral offer affects the entire small intestinal efferent lymphatic system. Dilatation of the lumen spreads in the central direction. The excess lymph is carried away in a short time by the collecting and efferent lymphatics. Meanwhile, the thinner lymphatics of the intestinal wall dilate to 5 to 6 times, and the thicker mesenteric ones to 1—2 times the normal size.

With the two kinds of increased lymph volume, first the lymphatics of the arcades, then the shunt system, the capillary area and finally the reserve efferent lymphatics take part in compensation.

Introduction

The precise knowledge of the storing and transport capacity of mesenteric lymphatics is an important factor in the understanding of intestinal absorption and blood and lymph circulation.

Since the discovery of the chylus vessels, marking the onset of knowledge concerning the lymphatics, the problem has not been investigated directly. Detailed descriptions have been published on the morphology [18, 19, 29], innervation [2, 4, 8, 12, 25] and flow-maintaining elements [6, 7, 9, 10, 11, 13, 14, 17, 21] of the lymph vessels, but practically no data on lymphatic storage and transport following mechanical obstruction or increased peripheral offer. FÖLDI et al. [3, 19] were the first to evaluate functionally lymph circulation, as a complete system. They have introduced also the concept of "lymphatic insufficiency".

Storage by the mesenteric lymphatics was described by us earlier [23, 24]. In the present investigations we wished to confirm previous hypotheses, and to study where, when and to what extent the various compensatory mechanisms take part in the relief of insufficiency. A further aim was to study lymph distribution in the various mesenteric areas and the ability to distend of the lymphatic segments.

Materials and methods

Cats of either sex, identical in weight and age, were used. The following criteria have been observed.

1. The same segment of intestine was studied in every case. In the cat the abdomen was opened, the greater omentum was pulled up and the segment lying next to the surface, 27 to 30 cm from the duodenojejunal flexure before the jejunal-iliac juncture was located. This segment has been chosen for two reasons: partly because it is nearest to the surface and if manipulated, the resting state of other intestinal segments is hardly disturbed, and partly because the mesentery belonging to it is long and the bundles of lymphatic and blood vessels are regular and clearly visible.

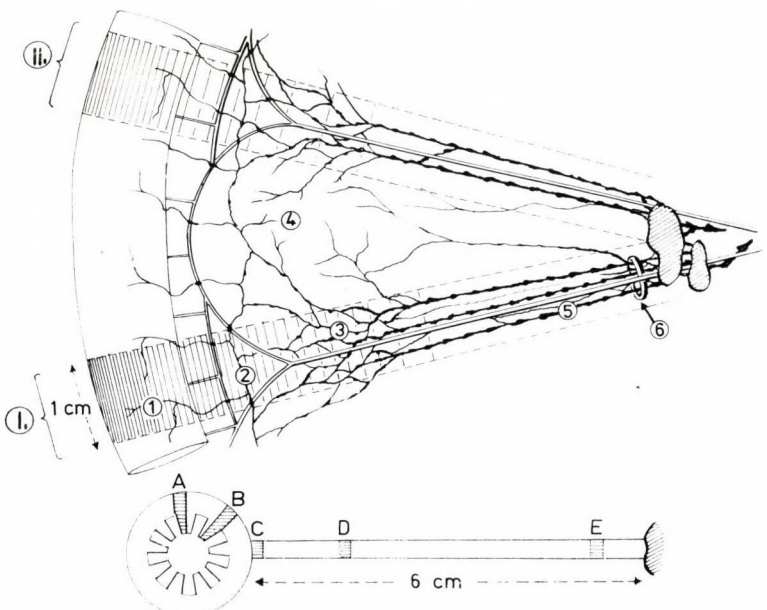

Fig. 1. Diagrammatic representation of mesenteric lymphatic system and sampling. I = ligated area; II = adjacent efferent segment; 1 = subserous lymphatics of intestinal wall; 2 = lymphatics of the zone of arcades; 3 = anastomosis (shunt) system; 4 = intersegmental capillary area; 5 = main efferent and reserve lymph vessels; 6 = site of ligation

2. When studying mechanical insufficiency, the arterial and venous supply to the ligated area was left intact. Only the efferent lymph vessels were ligated immediately before the lymph nodes, where they run alone. To study increased peripheral offer, after 1 day of food deprivation the cat was given as much cream as it could eat. This varied from 35 to 45 ml. In most animals this caused a slight diarrhoea in about 2 hours.

3. The specimen to be examined was a 15 cm long part of the intestine with intact mesentery, removed together with the mesenteric lymph nodes. It was fixed in formaldehyde for 16 hours and then subjected to study.

4. As illustrated diagrammatically in Fig. 1, 1 cm segment of the intestine was cut out together with its mesentery, up to the primary lymph node. When investigating mechanical insufficiency, the intestinal segments and mesentery lying orally and rectally from the ligated one were excised, to study lateral lymph outflow. In the longitudinal axis of the specimen are running the intestinal arterial and venous trunks and the efferent lymphatics accompanying them.

5. In the case of mechanical and peripheral overfilling the specimens were removed at 30 minutes, 1, $1^1/_2$, 2, 3, 4, 5 and 6 hours; the results of those periods were only considered where the difference was appreciable.

Cross sections, 4 mm thick each, were excised for microscopic study from the specimen at the following sites: A = from the intestinal wall 90° from the mesenteric insertion, B = 45° from the mesentery, C = from the part of the mesentery adjacent to the intestine, and E = from the mesenteric segment before the primary lymph node (Fig. 1). These sites had been chosen for the following reasons. In segment A (whole intestinal wall section) can be examined the valvular efferent lymphatics under the serosa and in the muscular layer. In segment B these, as well as the subserous lymphatic shunts can be studied. As the intestine has two sides, the results for segments A and B have been evaluated twice. On the other hand, no specimens were taken from the free, antimesenteric intestinal area, because previous investigations showed [22] that in this area there are no subserous and intramuscular valvular lymphatics of any importance in mesenteric storage. The lymph capillaries in the intestinal wall, as well as the side from which segments A and B originate, have been ignored, because previous investigations [22] demonstrated no major difference in this respect. In segment C the lymphatics of the so-called "arcade zone", ensuring connection with adjacent areas, could be examined. Segment D offered a possibility to study the anastomoses, from which arise the main efferent and reserve lymph vessels and which drains the efferent lymphatics of the intestinal wall and the intersegmental capillary lymphatics. In segment E the anastomoses lying before the lymph node draining the efferent lymphatics were examined. Into this "small anastomosis" system are emptying the central parts of the efferent lymphatics of the capillary area.

The 4 mm thick cross sections were embedded in celloidin-paraffin, as this has been found to cause the least shrinkage and damage. From the embedded material 8 μ thick serial sections were cut and stained with HE, Azan, PAS and by the Mallory—Farkas method. The four kinds of staining served to control the results of estimating and to add to the reliability of the results.

Technique of examination

The sections were examined by means of the PALKOVITS—CSAPÓ [15, 16] microprojection table, working on grounds of Chalkley's principle. In the projected areas we counted the thread crossings in the lumen of lymphatics, in 20 fields in one section, amounting to 75—80% of the whole section. Care was taken that the 20 fields be in the middle of the section. The sum of the 20 data showed the total number of thread crossings in the lumina of lymphatics in the entire projected area. In the next step it was calculated how many per cents of the area fall to the lymph vessels. The values were represented graphically, obtaining the curve shown in Fig. 2, used later as the basic state. The double of the values for segments

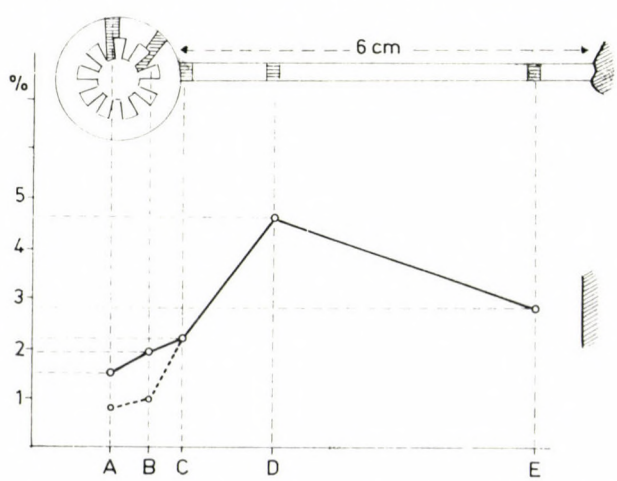

Fig. 2. Diameter of lymphatic lumina in percentage of the size of area examined. Range, basic: A = 1.5 to 1.6; B = 1.7 to 2.0; C = 2.1 to 2.4; D = 4.5 to 4.9; E = 2.7 to 2.9

A and B were taken. Among the segments, segment D contained the greatest total lumen, 4.6% of the projected area. This corresponds to the anastomosis system. Segment E, before the lymph nodes, yielded a lower value. Gradually decreasing values were obtained in segments C, B and A. These were considered to reflect the basic state and experimental results were related to them. The difference among the specimens stained by four methods was negligible, they served only to control the reliability of estimating.

Results

I. *Effect of mechanical insufficiency*

The increase in the lumen of lymphatics in response to congestion 30 minutes, 1, $1^1/_2$, 2, 3, 5 and 6 hours after ligation of the efferent lymphatics

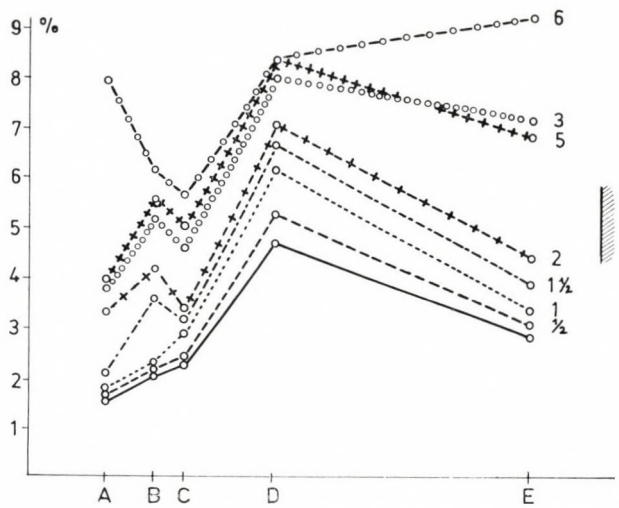

Fig. 3. Mechanical failure. Dilatation related to the basic state, at the times specified

is compared with the basic state in Fig. 3. The increase was not always proportionate to the length of time. The change was smaller in segment C at $1^1/_2$ and 2 hours, while the increase was great in segment E at 2 and 3 hours. The differences were more striking, if we took the basic values to represent 100% and related the percentual vascular dilatation to that figure. Such percentage changes are shown in Fig. 4.

It is seen that at a given time in segment C the increase was from 50% to 58%, while between 2 and 3 hours in segment E the increase was from 51% to 146%. The squares in Fig. 5 represent vascular dilatations above average at various points of time, while the shaded area shows the increases below average. It is visible that 30 minutes after ligation the entire mesentery was filled up, then 1 hour later the area near the intestine continued to fill. After

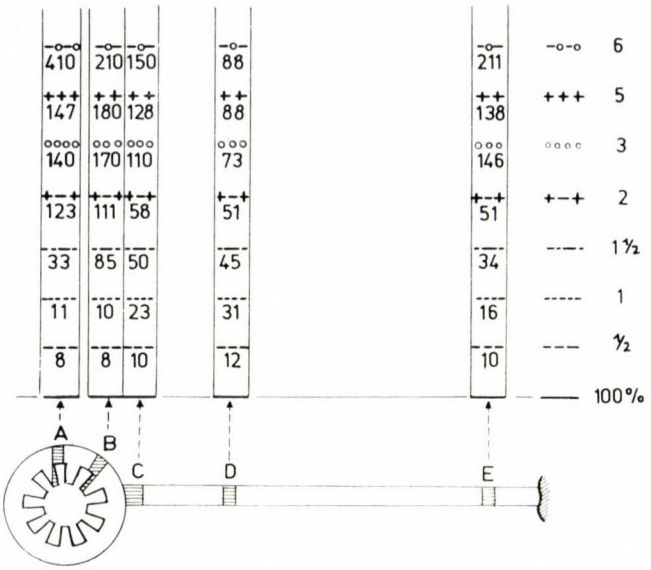

Fig. 4. The changes shown in Fig. 3, reckoned in percentage of basic state. On the right: time of examination

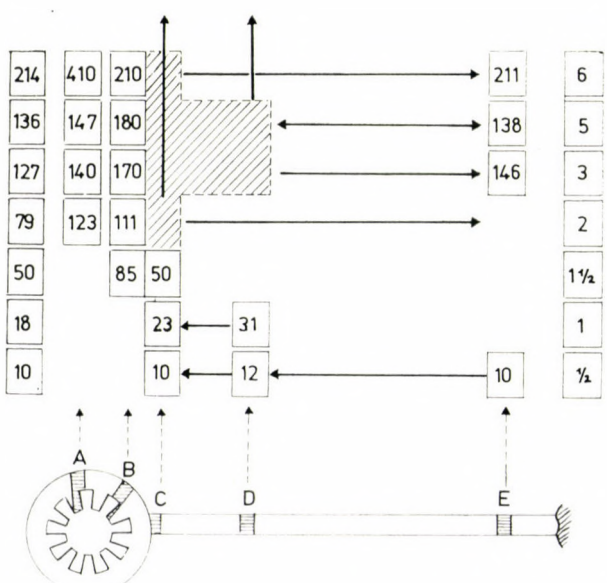

Fig. 5. Distribution of lymph in mesenteric region. Left column: average values measured, on the right: time of examination. Increases exceeding average are shown in the squares. In the shaded area the increases are below average. The arrows point in the direction of lymph flow

$1^1/_2$ hours of congestion outflow was blocked in segment B and dilatation occurred. For the slight increase in segment C between $1^1/_2$ and 2 hours, outflow in the lateral direction seemed to be responsible, the more so, as at that time there was a slight congestion in the entire mesentery, extending backwards to the intestinal wall. To clarify this point, measurements were made also in the adjacent segment C. The result is shown in Fig. 6. The solid line represents the increases in the ligated areas at various points of time, while the broken line illustrates those of the adjacent area. It may be observed that

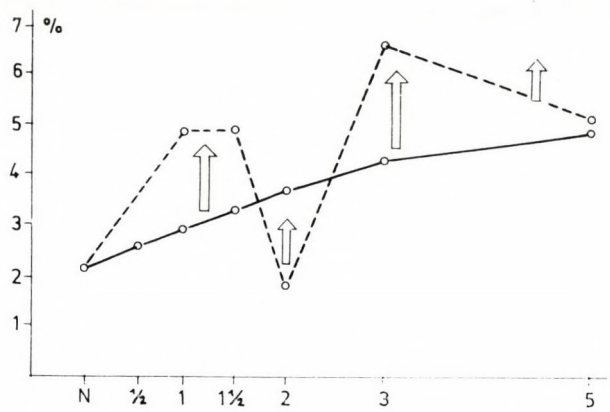

Fig. 6. Comparative examination of segment C, the zone of arcades. Solid line: dilatation occurring in clamped area. Broken line: changes in adjacent area. The arrows indicate direction of lymph flow, related to the ligated area. N = value for segment C in the basic curve

after 1 and $1^1/_2$ hours of clamping lymph began to flow sidewise through the arcades, but between $1^1/_2$ and 2 hours, the diameter of the lymphatic lumina decreased slightly below normal. At 3 hours another increase occurred, then congestion spread to more distant segments. Thus it was not the increase of lateral outflow which caused the increase to diminish in the examined segment. As further explanation, there remains the filling up of the capillary area, shown previously to be capable of lymph storage [23, 24]. If a certain pressure has been reached, the capillary area may become filled backward, because in the efferent lymphatics there are no bilamellar valves ensuring unidirectional flow. In support of this view is the fact that after the filling up of this area the reserve lymphatics open up and together with the efferent vessels of the capillary area produce the excessive increase in segment E after 2 hours of congestion. The vessels mentioned are namely drained by the small anastomosis system and were thus responsible for increase in dilatation from 51% to 146% in segment E. After 3, 5 and 6 hours of clamping the lymphatics continue to dilate in the intestinal wall, then, after the capillary area has become completely filled the mesenteric lymphatics continue to dilate again,

in the central direction. After six hours of congestion the intestinal wall was oedematous and there were ruptured lymphatics, so the results could not be evaluated.

Evaluation

In the first phase of mechanical insufficiency the entire mesenteric lymphatic system is slightly filled up, then congestion spreads backwards to the intestinal wall. Subsequently, congestion spreads sidewise, toward adjacent tissues. After 2 hours of congestion, pressure increases, the capillary area is filled, as the second compensatory system. As a result, the centrally running reserve lymphatics and the efferent lymphatics of the intestinal wall continue to dilate, until they are damaged and rupture.

II. *Effect of increased peripheral offer*

From the animals fed cream, tissue specimens were taken and treated at 1, 2, 3, 4, 5 and 6 hours. The values for the basic state are shown in Fig. 7. In the first hour following cream feeding, dilation of the lumen begins in the intestinal wall, then extends centrally up to the juxtaintestinal mesentery,

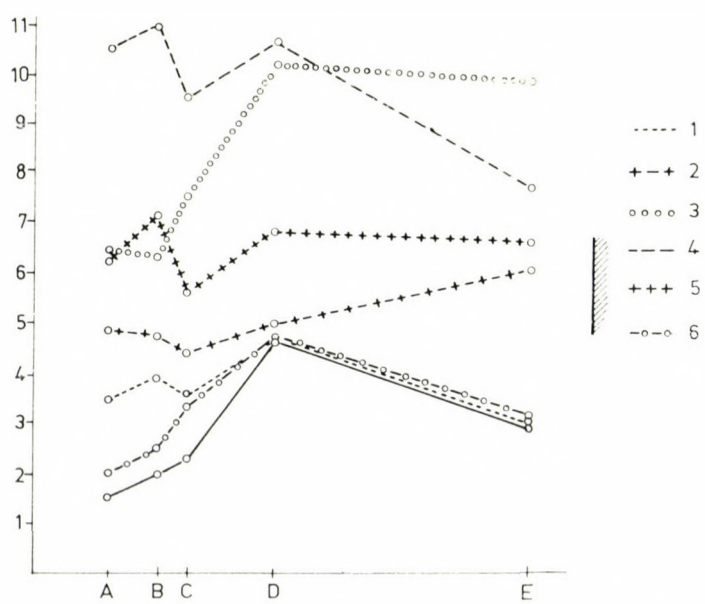

Fig. 7. Excessive periphera offer. Changes in diameters related to basic state. On the right: time of testing and symbols

i.e. to the lymphatic arcades. In this area vascular dilatation continues during the second hour when the anastomosis system becomes slightly filled (segment D). As a result of congestion before the lymph node, also the small anastomosis area becomes filled. At 3 hours the most marked increase is at the shunts. After filling the capillary area, segment E continues to dilate due to filling of the centrally efferent and reserve lymphatics. At four hours, segments A, B, C and D continue to increase though with a tendency to decrease, then the pre-lymph node segment E begins to empty. As related to the highest values,

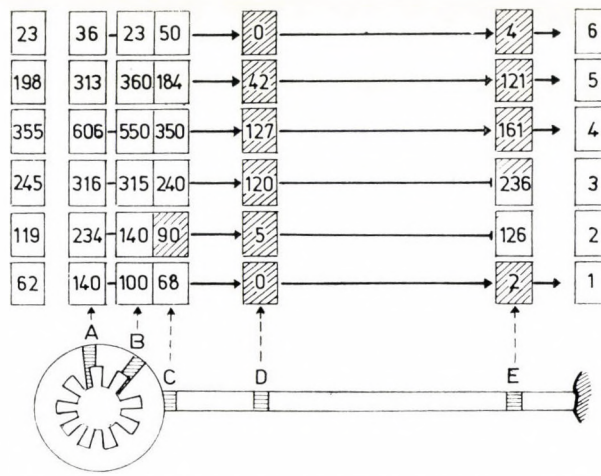

Fig. 8. Excessive peripheral offer. Changes in percentage of the basic value. Left column: average changes, right side: time of testing. Values below average are shaded. The arrows indicate direction of lymph flow

the lumina gradually decrease in diameter, as indicated by the 5-hour curve. Finally, the changes induced by feeding cream subside completely; with the percentage calculation for the dilatations exceeding the average at different points of time, we obtain Fig. 8.

This shows that in response to the increase in peripheral offer segments A, B, and with one exception segment C, underwent a dilatation of average or higher than average degree. The values for lateral outflow, i.e. those of segment C, gradually increased till 4 hours, after which they showed a declining tendency. Filling of the capillary system is indicated by the 2-hour values for segments C and D, and the 3 hour value for segment D. This filling then caused a considerable congestion before the lymph nodes, as indicated by the excessive increase of the 2 and 3-hour values for segment E.

Evaluation

The increase in peripheral offer was characterised by a dilatation beginning in segments A and B. At four hours the values for these segments reached

the maximum as a result of the increase in absorption rate when dilatation amounted to 606% and 550%, respectively. In segment C the 2-hour value was below the average level, because it is at that point of time that the capillary area begins to fill, and the maximum dilatation to nearly $3^1/_2$ times the original is reached after 4 hours of absorption. In the shunt area, the maximum (127%) increase was found at 4 hours, while in the pre-lymph node area the peak figure was reached 3 hours after feeding of cream.

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DIE WIRKUNG DER MECHANISCHEN INSUFFIZIENZ UND DES GESTEIGERTEN PERIPHERISCHEN ANGEBOTS IM BEREICH DER MESENTERIALEN LYMPHGEFÄßE

J. VAJDA, E. FEHÉR und K. CSÁNYI

Verfasser untersuchten den Einfluß der Ligierung der abführenden Lymphgefäße und des gesteigerten peripherischen Angebots auf dem Gebiet der mesenterialen Lymphgefäße. Der plötzliche Verschluß der Lymphgefäße bewirkt eine Stauung, die das Lumen der letzteren auf das 3- bis 4fache zu erweitern vermag. Die Lymphgefäße können diese große Belastung nur über eine kurze Zeitspanne bewältigen. Die wichtigste Rolle spielen darin die Lymphbahnen der Bogenzone und der Anastomosen, die im Verlauf der Kompensation den Lymphstrom in Richtung der benachbarten intakten Segmente umleiten. Diese Funktion ist jedoch nur von einem zeitweiligen Erfolg begleitet, denn in 4 bis 5 Stunden entwickelt sich die völlige mechanische Insuffizienz.

Der Anstieg des peripherischen Angebots betrifft den gesamten Lymphapparat des Dünndarms. Die Lumenerweiterung nimmt in zentraler Richtung zu. Durch das Zusammenwirken der speichernden und abführenden Lymphgefäße wird der angestaute Lymphüberschuß in kurzer Zeit weiterbefördert. Unterdessen erweitern sich die Lymphgefäße in den dünnwandigen Darmwänden auf das 5- bis 6fache, während sich die dickeren mesenterialen Lymphgefäße auf das 1- bis 2fache dilatieren.

Beim zweierlei Anstieg der Lymphmenge werden die Lymphbahnen der Bogenzone, das Anastomosensystem, das Gebiet der Kapillaren und schließlich die abführenden Reservelymphgefäße stufenweise in die Kompensation eingeschaltet.

ДЕЙСТВИЕ МЕХАНИЧЕСКОЙ НЕДОСТАТОЧНОСТИ И ПОВЫШЕННОГО ПЕРИФЕРИЧЕСКОГО ПОДВОЗА В МЕЗЕНТЕРИАЛЬНОЙ ЛИМФАТИЧЕСКОЙ ОБЛАСТИ

Й. ВАЙДА, Е. ФЕХЕР и К. ЧАНЬИ

Изучалось действие лигирования отводящих лимфатических сосудов и повышения периферического подвоза в лимфатической области брыжейки. Вследствие внезапного закрытия лимфатических сосудов развивается застой, постепенно расширяющий просвет сосудов до 3—4-кратного исходного размера. Лимфатические сосуды способны преодолеть большую нагрузку лишь на краткое время. Наиболее важную роль при этом играют лимфатические сосуды зоны дуг и анастомозов, отводящих лимфу в процессе компенсации к соседним, невредимым сегментам. Эта функция сопровождается лишь временным успехом, так как в течение 4—5 часов развивается полная механическая недостаточность.

Повышение периферического подвоза наблюдается во всей отводящей лимфатической системе тонкой кишки. Расширение просвета повышается в центральном направлении. Благодаря взаимодействию собирающих и отводящих лимфатических сосудов накопившаяся лишняя лимфа продвигается быстро. Между тем лимфатические сосуды стенки кишек с более тонкими стенками расширяются до 5—6-кратной величины, а более толстые мезентериальные лимфатические сосуды до 1—2-кратной величины.

При двух различных видах повышения количества лимфы в компенсацию постепенно включаются лимфатические пути дуговой зоны, система анастомозов, область капилляров и запасные отводящие лимфатические сосуды.

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RAPID MYELIN SHEATH STAINING IN PARAFFIN, FROZEN AND CELLOIDIN SECTIONS

A NEW MODIFICATION OF THE WEIGERT—LOYEZ STAINING METHOD

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(Received December 20, 1968)

A modification of Loyez's method has been found to allow a rapid staining of normal and pathological myelin sheaths, in paraffin, frozen and celloidin sections. A mordant has been prepared, containing alcoholic iron alum solution in which the sections are kept for 1 $^1/_2$—2 hours at 56°C. Staining is undertaken by a lithium carbonate—haematoxylin solution at 56°C for 1 hour, or at 42°C for 1 $^1/_2$—2 hours, followed by dehydration and conservation. The histological picture is similar to that obtained with the classical Spielmeyer method.

In neuropathological investigations, the myelin sheath forms a structural detail of great importance. In pathological processes of the nervous system, this sheath may undergo different alterations.

The staining techniques for myelin allow to study the normal and pathological myelin structures in man and experimental animals, and to follow myelination process in embryos.

Numerous methods have been elaborated for the staining of normal and altered myelin structures.

Elective myelin staining was introduced in neurohistological technique by WEIGERT [26] who presented the first myelin preparations in 1882 at the Eisenach Congress. The principle of his method consists in the application of a mordant solution, which fixes and prepares myelin for staining with haematoxylin. From the haematoxylin solutions still in current use, iron and lithium haematoxylin were first applied by WEIGERT, and practically every such method or modification as those of PAL [4], KULTSCHIZKY [10], WOLTERS [27], BENDA [2], FRANKEL [4], NAGEOTTE [21, 22], LOYEZ [18], LANDAU [11, 12], WRIGHT [cit. 16], SPIELMEYER [25], BACSICH [1], WEIL [cit. 16], HEIDENHAIN and HEIDENHAIN—WOELCKE [4], LILLIE [16], KLÜVER—BARRERA [7], LÁZÁR [14, 15], MARGOLIS—PICKETT [20], LOCKARD et al. [17], MANUS [19], ORA [23], KRUTSAY [8, 9], LAPHAM et al. [13] derive from the original Weigert method.

One of the most wide-spread neurohistological procedures is that of SPIELMEYER [25], which together with other myelin methods makes it possible to examine thicker sections, but has the disadvantage of being laborious and the frozen and celloidin sections are sometimes damaged in the course of the manipulation.

In current neuropathological practice the correct interpretation of a lesion often calls for the study of a certain region by several methods (Haematoxylin and eosin, van Gieson, Nissl, impregnation for neurofibrils and neuroglial structures, myelin stains, etc.), with the aim of evidencing different microscopic details. This can be achieved only by using sections of the same nervous tissue fragment [5, 6]. On the other hand, the method proposed by us allows myelin staining in series obtained from the same area.

Starting from LOYEZ's method, we use a combined alcoholic-iron alum mordant of 42 °C or 56 °C and staining is achieved at the same temperature.

The method can be applied in paraffin and celloidin sections, as well as in frozen ones. The frozen and celloidin sections must be differentiated in mordant solution, this treatment being superfluous with paraffin sections.

Description of the method

Stock solutions

A) 5% aqueous iron alum solution and 96% alcohol (ethanol), *equal parts.*
B) 10% alcoholic haematoxylin
C) Saturated lithium carbonate solution

1. Usual fixation of nervous tissue in 10% formalin. (Best results were obtained after fixation in this solution for 2—3 weeks, also after Bouin's fixative solution.)

2. Preparation of at least 10—15 μ thick paraffin section, 10—25 μ thick celloidin or 15—25 μ thick frozen sections.

3. Deparaffination, hydration in the usual way (to avoid the thicker paraffin section to become detached after treatment with 70% alcohol, the slides with the sections are immersed into 1% celloidin solution).

4. Treatment with solution "A" (the mordant) for $1^1/_2$ hours at 56°C, or 2—3 hours at 42°C.

5. Rapid washing (1—3 min.) in several times changed distilled water;

Stock solution B ..... 3 ml
Distilled water ..... to 100 ml, subsequently
Stock solution C ..... 2 ml.

The mixture should be kept at 42°C for $1^1/_2$—2 hours, or at 56°C for 1 hour.

This staining solution can be used only once!

6. Washing in tap water, to which a few drops of concentrated ammonia were added; change several times.

7. Dehydration in 70%, 80%, 96% and absolute alcohol series, 5 minutes each; if the paraffin sections were covered with celloidin between the 70% and 80% alcohol, the celloidin should be removed with a v/v alcohol—ether mixture.

8. Clear in two changes of xylol or toluol and mount in Canada balsam.

Note

If specimens obtained from small animals (guinea-pig, rabbit, rat, mouse) are studied, the mordant solution (A) must be diluted by the same volume of distilled water.

Results

On an uncoloured background, the myelin sheats stain dark-blue (Figs 4, 5, 6), while the blood erythrocytes from the vessels (Figs 3, 6, 7) of the

Fig. 1. Brain stem with cerebellum of the guinea-pig. Normal myelin structure. Modified myelin stain. Paraffin section of 15 μ. $\times 15$

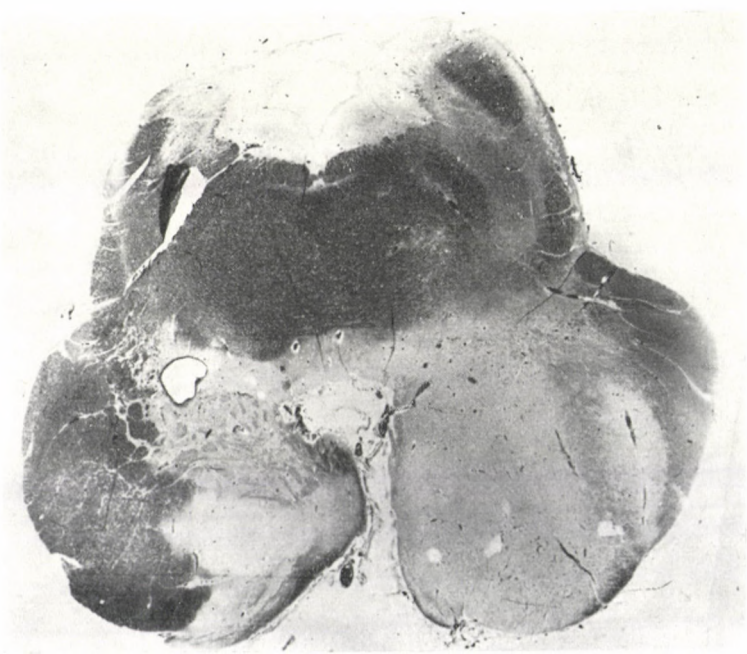

Fig. 2. Middle brain at the level of colliculus superior. A case of multiple sclerosis. Demyelinating areas in the pedunculus cerebri. Modified myelin stain. Paraffin section of 12 μ. $\times 6$

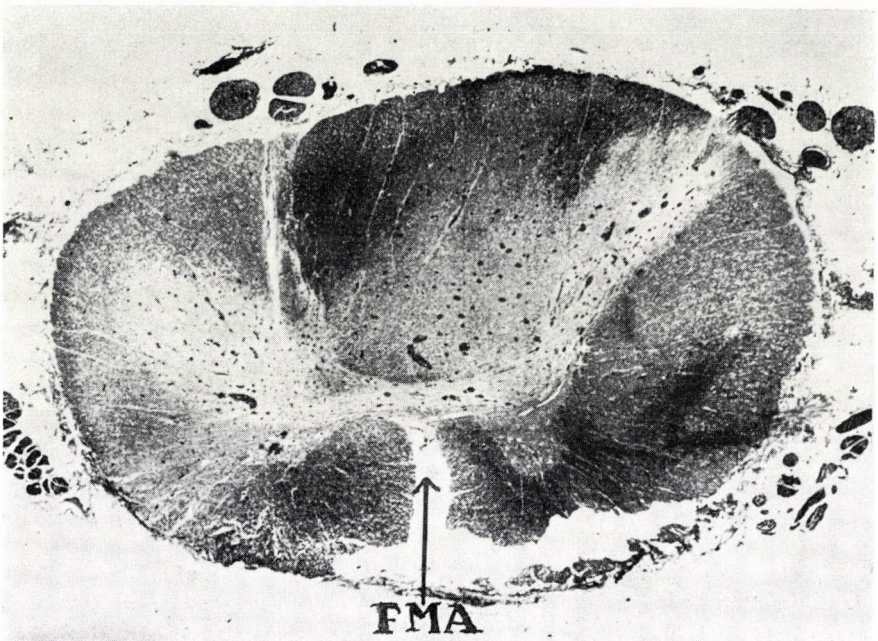

Fig. 3. Spinal cord of man with multiple sclerosis. Frozen section (gelatin-embedded material) of 20 μ. Demyelinating foci in the lateral and posterior fascicles. (FMA = fissura mediana anterior.) Modified myelin stain. $\times 10$

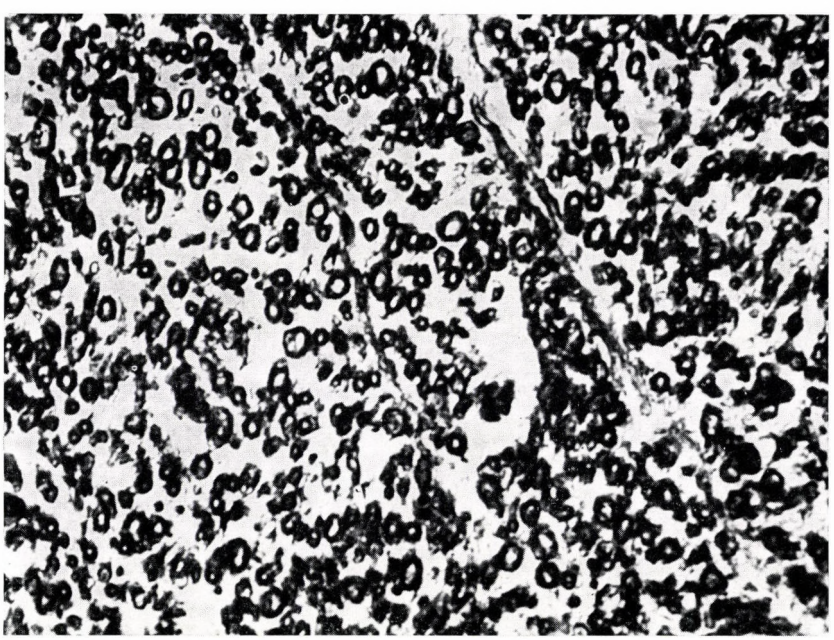

Fig. 4. The same case as in Fig. 3. Cervical segment of spinal cord. Picture of myelin sheaths of different diameters, in relatively intact areas. Paraffin section of 12 μ. Modified myelin stain. Photomicrograph with universal research microscope type MC_1. $\times 150$

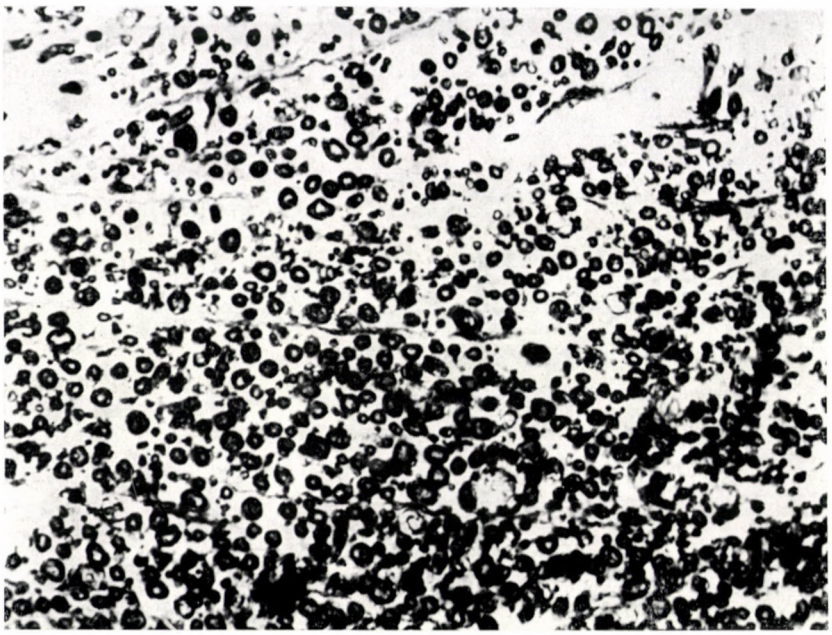

Fig. 5. The same case as in Fig. 3. Dorsal segment of human spinal cord. The limiting zone of partially demyelinated and intact areas in the posterior fascicles. Paraffin section of 12 μ. Modified myelin stain. Photomicrograph. $\times 150$

Fig. 6. Cerebral cortex of man (gyrus paracentralis). Normal myelin structure with the vascular network containing erythrocytes. Paraffin section of 12 μ. Photomicrograph. $\times 150$

Fig. 7. Cerebellar fragment from guinea-pig. Myelin and vascular picture. Paraffin section of 10 μ. Modified myelin stain. Photomicrograph. $\times 44$

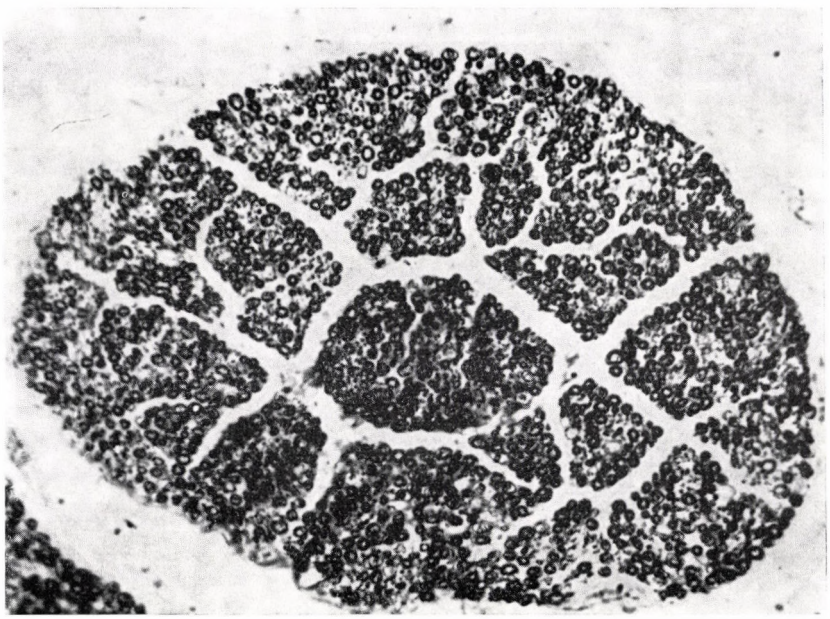

Fig. 8. Transversal sectioned human peripheral nerve trunk. (N. cutaneus antebrachii medialis.) Normal myelin picture. Paraffin section of 15 μ. Photomicrograph. $\times 44$

nervous parenchyma are black. Thus, in thicker sections the vascular network can be followed (Figs 6, 7), similar as with the Slonimschi—Cunge or Pickwort methods [cit. 24].

If the background is not sufficiently clear after differentiation, 0.05% of potassium permanganate solution, recommended also by ORA [23], should be applied.

*

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SCHNELLFÄRBUNGSMETHODE FÜR MYELINSCHEIDEN IN PARAFFIN-, GEFRIER- UND ZELLOIDIN-SCHNITTEN

J. KELEMEN und M. BECUS

Die Modifikation der Loyezschen Methode ermöglichte die Schnellfärbung von normalen und pathologischen Myelinscheiden in Paraffin-, Gefrier- und Zelloidin-Schnitten.

Die Schnitte werden für die Dauer von $1^1/_2$ bis 2 Stunden in eine alkoholische Eisenalaunlösung enthaltende Beize von 56°C gelegt und hernach mit einer Lithiumkarbonat-Hämatoxylin-Lösung von 56°C während 60 Minuten oder bei 42°C während $1^1/_2$ bis 2 Stunden gefärbt, mit nachfolgender Dehydratation und Konservierung. Das histologische Bild ist dem mit der klassischen Spielmeyerschen Methode erhaltenen ähnlich.

МЕТОД ДЛЯ БЫСТРОГО ОКРАШИВАНИЯ МИЕЛИНОВЫХ ВЛАГАЛИЩ В ПАРАФИНОВЫХ, ЗАМОРОЖЕННЫХ И ЦЕЛЛОИДИНОВЫХ СРЕЗАХ

Й. КЕЛЕМЕН и М. БЕКУС

Модификация метода Лойеза позволяет быстрое окрашивание нормальных и патологических миелиновых влагалищ в парафиновых, замороженных и целлоидиновых срезах.

Изготовляется протрава, содержащая спиртовой раствор железных квасцов, в которой срезы храняются в течение 1 1/2 до 2 часов при 56 °С. Для окрашивания применяется раствор карбоната лития-гематоксилина, при температуре 56° С в течение 60 мин. или при 42° С в течение 1 1/2 до 2 часов, при последующей дегидратации и консервировании. Гистологическая картина, получаемая этой методикой, подобна картине, получаемой классическим методом Шпильмейера.

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ADENOHYPOPHYSEAL CYTOLOGY IN NORMAL AND GONADECTOMISED PIGEONS

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The adenohypophyseal cytology in the common Indian pigeon of both sexes under normal as well as gonadectomised conditions was studied by histological and histometric methods. The pars distalis of the pigeon contains six types of chromophils — alpha, beta, gamma, delta, epsilon and eta cells and the chromophobes, spatially distributed in the caudal and cephalic lobes. The alpha and gamma cells are distributed in the caudal lobe, beta, epsilon and eta cells only in the cephalic lobe, whereas the delta cells along with the chromophobes occur in both lobes.

Gonadectomy in both sexes elicited a response of the gonadotrophs and the thyrotrophs. A significant hypertrophy of the gonadotrophs, the gamma and beta cells, was evident along with their enhanced mucoid reactivity. There was also a drastic response of the delta thyrotrophs. The acidophils, i.e., alpha, eta and epsilon cells, were apparently unchanged. Response of the gonadotrophs to gonadectomy is explained on the basis of the feedback mechanism. The striking behaviour of the delta cells in gonadectomised pituitaries seems to be a characteristic avian response.

Earlier investigations by a number of authors into the histology of the pars distalis in birds led to characterisation of the glandular cells into light staining and deep staining acidophils/basophils of two types. These and other outstanding features of avian adenohypophysis were studied under normal and experimental conditions (Schooley and Riddle [26], Rahn and Painter [24], Payne [18], Wilson [33], Wingstrand [34], Brown and Knigge [2], Mikami [16], Fayez and Rappay [6]). Recently, a critical evaluation of the terminology of the secretory cells of the vertebrate pars distalis with regard to functional connotation embodying Romeis' [25] nomenclature has been presented by Herlant [11]. He classified pituitary chromophils as serous (acidophils) and mucoid (mucoproteinaceous) cells. The first category comprised alpha (STH), prolactin (LTH) and epsilon (ACTH) cells; beta (FSH) and delta (TSH) cells were included into the second class. French authors studied the avian adenohypophysis based on this scheme of characterisation, utilising a number of trichrome and tetrachrome staining techniques and histochemical methods of differentiation [28, 29, 30, 32]. The application of modern methods allowed to identify six types of glandular secretory cells (alpha, prolactin, epsilon, beta, delta and gamma) in the Pekin duck and in a number of common birds (Tixier—Vidal [31]). Further, attempts were made to correlate the various cell types of the duck with the secretion of a specific hormone. Similar

investigations employing modern methods of analytical histology in a large number of avian species would certainly be a point of interest in comparative pituitary physiology. In the present study, the cytoarchitecture of the pars distalis of the adenohypophysis of the common Indian pigeon, *Columba livia*, is described and the cellular manifestations displayed after gonadectomy are reported.

Material and methods

Five groups of adult pigeons, each composed of ten animals of both sexes obtained during different periods of the year from natural population, served as the material for the study, of forty-four young adult male and female pigeons; thirty-two birds were divided into two groups, subsequently castrated and spayed, kept in different cages, while another cage containing twelve pigeons of each sex served as the respective controls. The period of castration and ovariectomy was 45 days. The animals for study were killed by cervical dislocation, the pituitaries were immediately dissected out and placed in fixatives.

For the purpose of differential study of the cell types, prolonged fixation for five days in Bouin—Hollande-sublimate was followed by dehydration, clearing, embedding in paraffin (m.p. 62°C), then 3 μ thick sagittal sections were prepared. The staining methods used were: Masson's trichrome, Heidenhain's azan, azocarminehaematoxylin-acid green orange G method (GURR [8]), GOLDBERG and CHAIKOFF's trichrome [1], and HERLANT's [10] acid alizarin blue. In addition, PAS-trichrome [21], PFAAB-PAS-orange G method of ADAMS [22], alcian blue-PAS [10] and CLARK's [4] aldehyde fuchsin staining were also applied to distinguish the mucoid cell types.

For differential count of the tinctorially distinguishable cell types, the method of BRISENO-CASTREJON and FINERTY [1] was followed. In a Herlant-stained (instead of the usual azocarmine-stained) midsagittal (instead of horizontal) section the cells were counted under oil immersion in each 3rd field of every 3rd row, and the total percentage was calculated. Moreover, in view to the bilobular pattern of the tissue, a vertical plane of observation was used instead of the recommended horizontal plane. About 1800—2000 cells were counted from each midsagittal section and at least three sections from each bird were considered. Four birds from each group were taken for these measurements. The diameter of the cells was measured with an ocular micrometer at 1250× magnification, in at least four birds in each group.

Observations

A. *Normal pigeon*

Six types of chromophilic cells are observed in the pars distalis of normal pigeons of both sexes in addition to the chromophobes. The staining behaviour of the various cell types is shown in Table 1, the percentage counts in Table 2. The cytology of the pars distalis in the pigeon was described by SCHOOLEY and RIDDLE [26] and others [6, 24].

Cell types of the caudal lobe

Gamma. In the pigeon, these cells are voluminous and large ovoid with a dense granular cytoplasm (Figs 1 and 5). Vacuoles are rare, but some cells contain a large basal vacuole containing a conspicuous colloid droplet. The nuclei are large, round, with a prominent central nucleolus.

Table 1

Staining characteristics of pituitary cell types in the pigeon

	Caudal lobe		Cephalic lobe			Both lobes	
	Alpha (serous)	Gamma (mucoid)	Beta (mucoid)	Eta (serous)	Epsilon (serous)	Delta (mucoid)	Chromophobe
Azan (Heidenhain)	light orange	deep red	deep blue	weak orange	rose	blue	faint blue
Azocarmine light green	orange	deep red	green	orange	pink	light green	faint green
Goldberg and Chaikoff's trichrome	weak orange	deep red	bluish violet	light orange	reddish orange	blue	weak blue
Herlant's tetrachrome	weak orange	purple	deep blue	orange	brick red	blue	weak blue
PAS-trichrome	orange	dull orange	magenta	orange	orange	magenta	
AB-PAS		faint magenta	deep magenta			deep blue	
PFAAB-PAS-OG	orange	faint magenta	deep magenta	orange	orange	blue	
Aldehyde fuchsin			blue violet			blue violet	

Table 2

Percentage of pituitary cell types in normal and gonadectomised pigeons

	Alpha	Beta	Gamma	Delta	Eta	Epsilon	Chromophobe
Male	2.6 ± 0.6*	1.7 ± 2.1	12.0 ± 2.6	43.1 ± 1.0	7.9 ± 2.1	2.8 ± 1.7	28.1 ± 0.8
Castrate	3.0 ± 2.6	2.9 ± 1.0	14.4 ± 0.2	49.2 ± 1.2**	3.9 ± 3.0	1.1 ± 0.2	25.1 ± 1.2
Female	2.3 ± 1.2	2.0 ± 0.6	11.2 ± 2.5	39.2 ± 6.4	3.6 ± 0.6	1.0 ± 0.0	40.1 ± 2.9
Spayed	2.3 ± 0.4	3.0 ± 1.0	13.7 ± 4.3	39.5 ± 5.9	4.3 ± 1.6	2.9 ± 0.0	36.1 ± 0.1

* Standard error of the mean
** $P < 0.05$

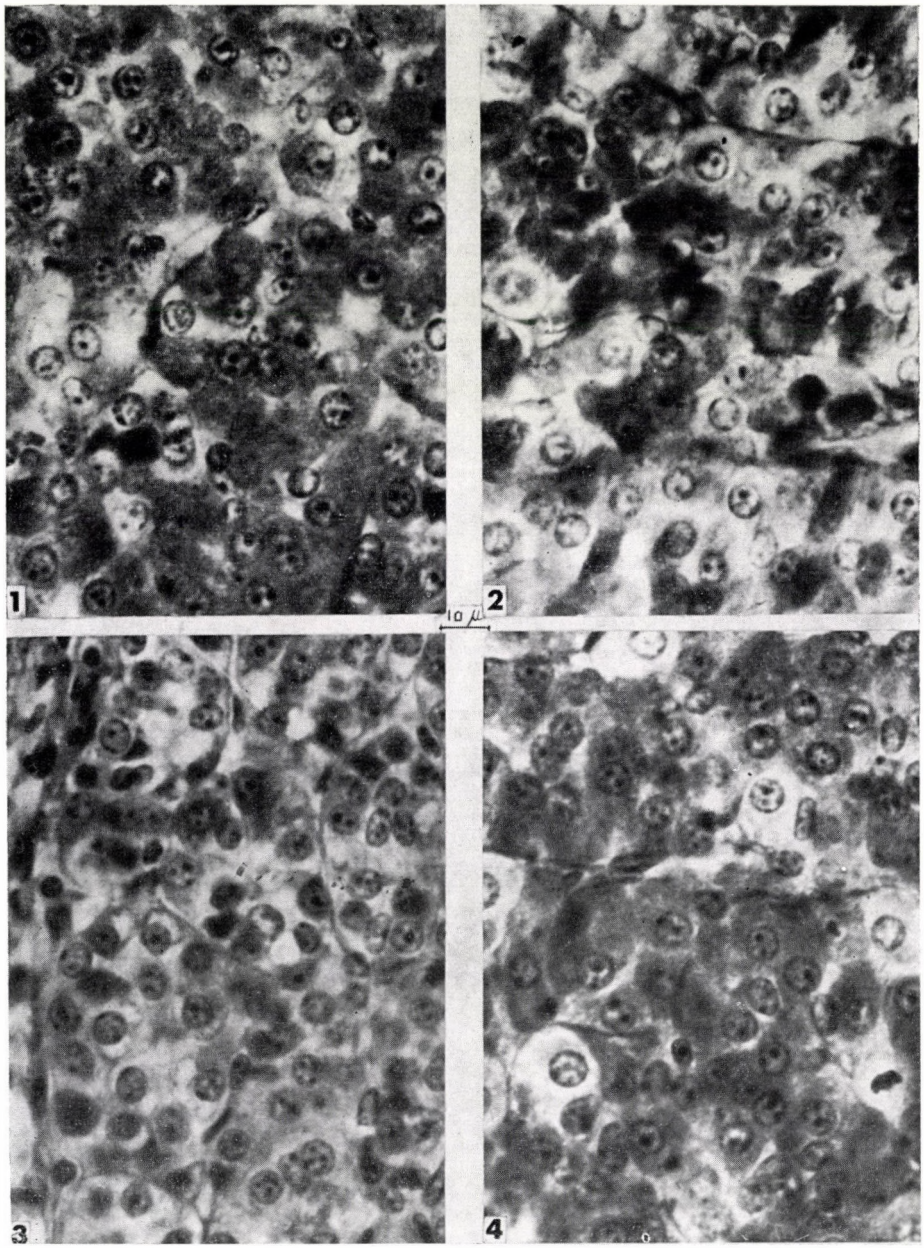

Fig. 1. Caudal lobe of the pars distalis of a normal male pigeon. Herlant's stain
Fig. 2. Caudal lobe of a castrate pigeon. Note disorganised acini. Herlant's stain
Fig. 3. Cephalic lobe of an intact bird. Herlant's stain
Fig. 4. Cephalic lobe in a castrate pigeon showing an abundance of clear delta cells one of which contains a mitotic figure. Herlant's stain

Alpha. These cells are identified by their organgophilic character and very small size, and frequently they have a close association with gamma cells (Figs 1 and 5). The central nuclei are somewhat oval in shape with a distinct nucleolus. The cells do not show any specific topographic pattern and their number is relatively small.

Cell types of the cephalic lobe

Beta. The beta cells are of a typical angular shape and medium size with a densely granular homogeneous cytoplasm. They are PAS and AF positive, brickrose with PAS-orange G and rose colored with AB-PAS stain. The cells frequently contain one or more clear and distinct small vacuoles (Fig. 7). Pituitaries fixed in different months round the year indicated that beta cells display a conspicuous seasonal variability in their staining with PAS. In the pigeon, where two reproductive cycles occur in a year [5], these mucoid cells probably maintain a cyclic phase.

Eta. These cells are small, roundish and nonvacuolated (Fig. 7) with unevenly scattered sparse granules staining a faint orange with Herlant's tetrachrome. They are distributed in clusters throughout the cephalic lobe, but more concentrated along the border.

Epsilon. The epsilon cells are identified by their granular densely homogeneous cytoplasm (Fig. 7), staining a deep red with Herlant's dye. These cells, like the eta cells, are very small and roundish, and some contain one or more vacuoles. Sometimes, they occur within the clusters of eta cells and these two cannot be distinguished without the best staining differentiation.

Cell type in both lobes

Delta. The delta cells are the largest of all cell types and they form the most abundant cell group in the pars distalis of the pigeon (Table 2). Among the various morphotypes exhibited by them, the most prominent variety is the round and voluminous one, densely packed with coarse cytoplasmic granules (Figs 5 and 7) and staining with aniline blue. The cells give a weak reaction with PAS and a stronger one with aldehyde fuchsin. They can be identified by their strong reaction with alcian blue after permanganate oxidation. A second variety of the delta cells of the same size and shape shows a finely granular, less dense cytoplasm, light blue in colour. The other forms are characterised by the presence of large vacuoles filling the whole of the cytoplasm or one totally devoid of any stainable material. The nuclei are large, with a central nucleolus or without a distinct nucleolus.

Chromophobe. Chromophobes form the second most numerous group among the cell types (Table 2) of the anterior lobe being distributed throughout. Heaps of chromophobes occur at the centre of acini or cell cords.

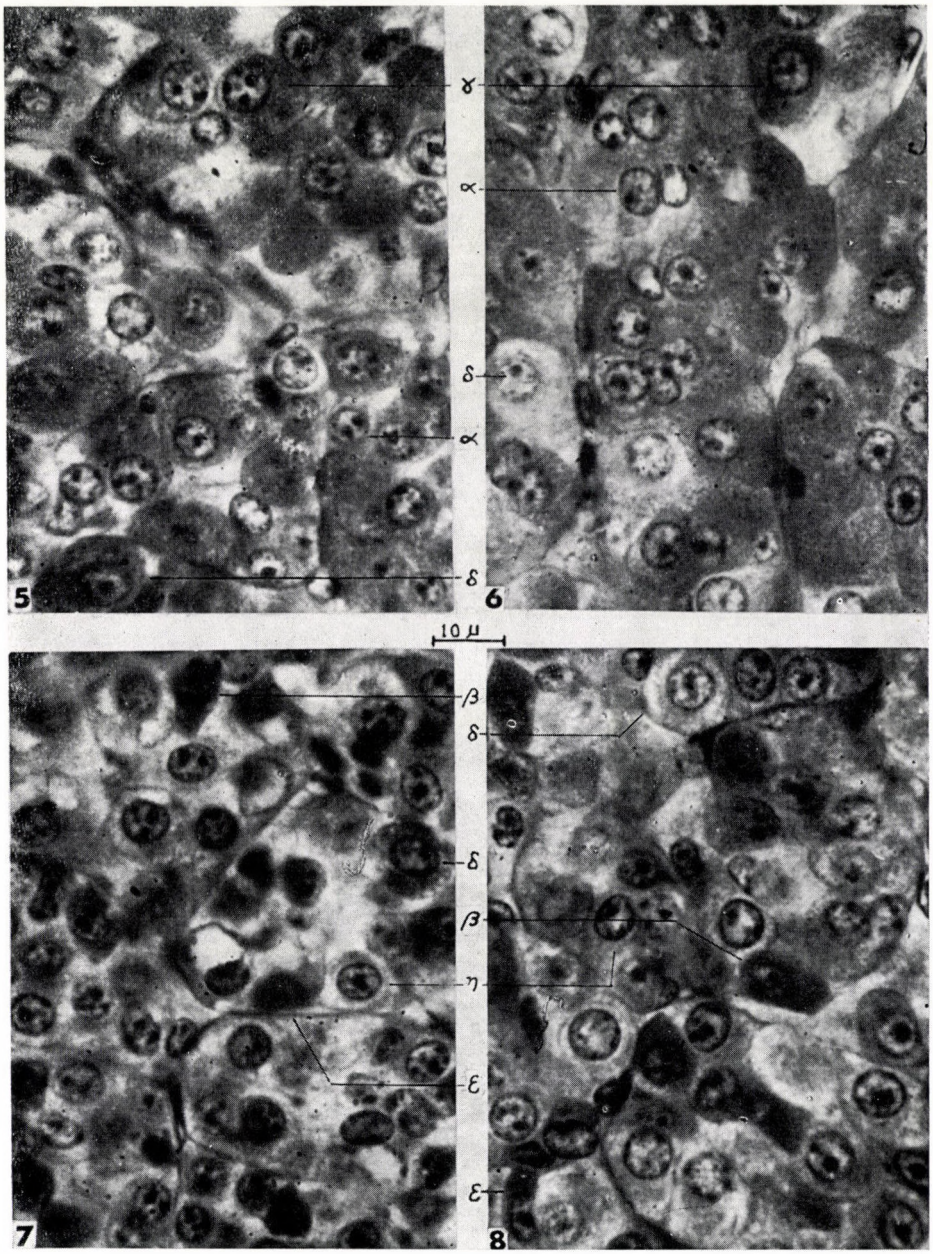

Fig. 5. Caudal lobe of a normal male pigeon
Fig. 6. Same lobe following castration. Observe hypertrophied gamma and delta cells
Fig. 7. Cephalic region of a control bird
Fig. 8. Cephalic region in a castrated bird. Note abundance and hypertrophy of beta and delta cells (Figs 5—8 stained by Herlant's method)

The cytoplasmic outline around each chromophobe nucleus is indistinct. The nuclear chromatin condensation is very high as compared to the chromophils. Intermediate stages between chromophobes and ether cells are frequently found.

The above description is based on the study of normal adult male and female pigeons. In young birds the pars distalis is more chromophobic, particularly in the cephalic lobe, but cellular pyknosis is uncommon. The follicles

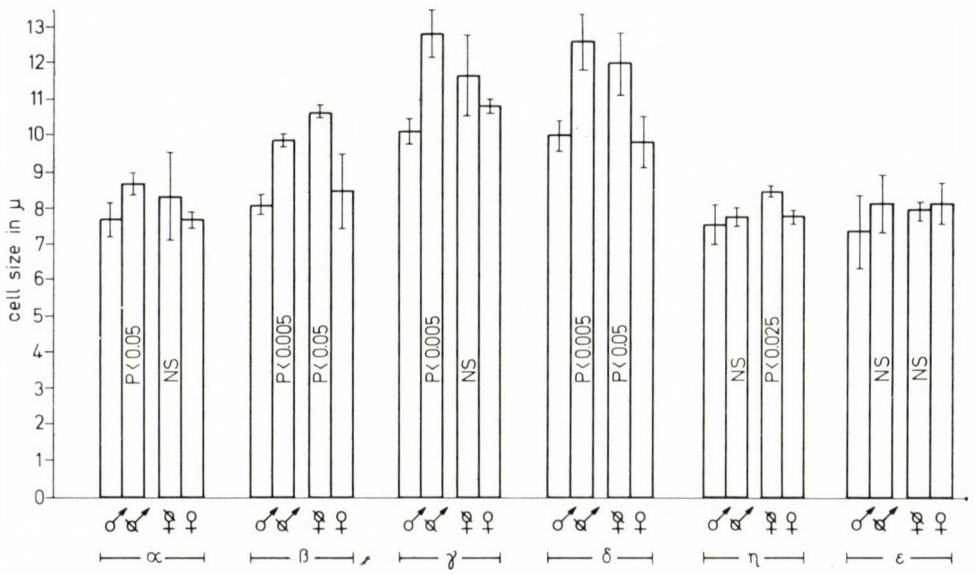

Fig. 9. Histogram representation of the size of chromophils (alpha, beta, gamma, delta, eta and epsilon cells) in control, castrated and spayed birds. Vertical deflections denote standard error

are very few and small with a little amount of colloid inside. Shape, size and general features of the cell types are almost the same as in adult birds.

B. *Gonadectomised pigeons*

Gonadectomy produced almost identical changes in hypophyseal cytology in both sexes; the response to castration was, however, qualitatively more marked.

Castration

Castration for a time period of 45 days induced an intense hypertrophy and hyperplasia of the whole gland (Figs 2 and 4). As a consequence, the regular orientation and pattern of parenchymal acini were completely lost.

Gamma. These cells became heavily granulated and at the same time manifested a moderate hypertrophy (Figs 6 and 9). Sometimes, gamma cells were observed to coalesce into peculiar nests at the centre of acini; they

also became elongated with a shifting of nuclear polarity away from the basement membrane.

Alpha. These cells did not show a striking response to spaying, though statistically there was an increase in cell size in castrated birds.

Beta. A considerable increase in chromophilic intensity together with a slight hypertrophy was observed in the beta cells. The presence of small discrete vacuoles, a characteristic feature of these cells in the normal pituitary, became less common. In contrast to their restricted distribution along the more dorsal side of the cephalic lobe, these cells became dispersed throughout. The increase in PAS and AF positive granulation was a notable feature of the castrate hypophysis.

Eta and Epsilon. These cephalic acidophils did not present any notable cytomorphic alteration in gonadectomised pigeons, except for an increase in size of the eta cells in the spayed group.

Delta. The delta cells exhibited the most striking behaviour following gonadectomy. There was extensive hypertrophy (Fig. 8) and hyperplasia (Table 2) and most cells became degranulated. The glandular section appeared to be consisting of numerous clear cells due to their prolific abundance (Fig. 4).

Chromophobe. Gonadectomy failed to elicit any change (Table 2).

Spaying

The cytomorphic pattern encountered in the castrate pituitary was distinctly obvious in the pars distalis of the spayed group; the intensity of stimulation was, however, qualitatively less in needs elucidation. The identification of these two cell types with parallel staining methods (i.e., beta: PAS+, AF−; delta: PAS+, AF++) as envisaged by HALMI [9] and PURVES and GRIESBACH [23] does not permit differentiation of thyrotrophs and gonadotrophs in a way similar to that in the rat. The AF plus reaction of the gonadotrophs in the pigeon has its parallel in the hamster and the human [3, 27].

The apparent hyperactivity of the gonadotrophs (beta and gamma) in both sexes of gonadectomised pigeons is easily explained on the basis of the classical feedback mechanism. Earlier workers reported marked mucoid basophil activity following castration in male birds [2, 16, 18, 19, 20, 26]. Castrated cockerels release and synthesize hypophyseal LH faster than do intact birds [17]. The vigorous response of the delta cells, however, is a striking phenomenon, almost unique in birds, as remarked by TIXIER-VIDAL and BENOIT [30] in relation to their findings in castrated ducks. In birds, there is evidence of a reciprocal relationship or antagonism between sex and thyroid hormones [12, 13, 14, 26] and this explains the thyrotrophic activity in gonadectomised birds. Probably, stimulation of pituitary thyrotrophins and gonadotrophins occurs simultaneously and independently of each other.

The adenohypophyseal cellular response of the ovariectomised pigeons was similar to that of castrated birds, but the intensity of stimulation was somewhat weaker. It has been observed that gonadotrophin stimulation is slower in females than in male birds [15].

*

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DIE ZYTOLOGIE DER ADENOHYPOPHYSE VON TAUBEN IN DER NORM UND NACH GONADEKTOMIE

T. K. BHATTACHARYYA, M. SARKAR

Die Zytologie der Adenohypophyse von gewöhnlichen indischen Tauben beiderlei Geschlechts wurde in der Norm und nach Gonadektomie mit Hilfe histologischer und histometrischer Verfahren untersucht. Die Pars distalis der Taube enthält sechs verschiedene chromophile Strukturen, α-, β-, γ-, ϑ-, ε- und η-Zellen sowie chromophobe Strukturen, die in den caudalen und cephalen Lappen räumlich verteilt sind. Die α- und β-Zellen sind im caudalen Lappen, die β-, ε- und η-Zellen nur im cephalen Lappen verteilt, während sich die ϑ-Zellen wie auch die chromophoben Strukturen in beiden Lappen befinden.

Gonadektomie rief bei beiden Geschlechtern eine Reaktion der gonadotropen und thyreotropen Hormone hervor. Eine signifikante Hypertrophie der gonadotropen Strukturen — γ- und β-Zellen — war bei gleichzeitig gesteigerter Mucoidenreaktion nachweisbar. Auch die thyreotropen ϑ-Zellen reagierten heftig. Die acidophilen Strukturen, d. h. die α-, η- und ε-Zellen blieben scheinbar unverändert. Die Reaktion der gonadotropen Hormone auf die Ablation der Gonaden läßt sich auf Grund des feed-back-Mechanismus erklären. Das auffallende Verhalten der ϑ-Zellen in der Hypophyse nach Gonadektomie scheint eine charakteristische Reaktion der Vögel zu sein.

ЦИТОЛОГИЯ АДЕНОГИПОФИЗА ГОЛУБЯ В НОРМЕ И ПОСЛЕ УДАЛЕНИЯ ПОЛОВЫХ ЖЕЛЕЗ

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Цитология аденогипофиза обыкновенных индейских голубей обоего пола в норме и после удаления половых желез была изучена при помощи гистологических и гистометрических методов исследования. Pars distalis голубя содержит шесть типов хромофильных структур — α-, β-, γ-, ϑ-, ε- и η-клетки —, и хромофобные структуры, распределенные в каудальных и головных долях. α- и γ-клетки распределены в каудальной доле, β-, ε- и η-клетки располагаются исключительно лишь в головной доле, в то время как ϑ-клетки, так же как и хромофобные структуры встречаются в обеих долях.

Удаление половых желез вызывало у обоих полов реакцию со стороны гонадотропных и тиреотропных гормонов. Достоверная гипертрофия гонадотропных структур (γ- и β-клетки) была выявляема, при одновременном повышении их мукоидной реактивности. Наблюдалась также сильная реакция со стороны β-тиреотропных структур. Ацидофильные, то есть α-, η- и ε-клетки, по-видимому, были неизменными. Ответ гонадотропных структур на удаление половых желез объясняется механизмом обратной связи (feed back). Поразительное поведение ϑ-клеток в гипофизе после гонадектомии, по-видимому, является характерной реакцией птиц.

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ANNULAR CALCIFICATIONS IN BENIGN MAMMARY DISEASES (TUMOURS)

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Ring-shaped calcification occurring in benign diseases of the breast may, according to literature, develop in two ways. It is held by some authors that the wall of ducts is necrotized by intraluminarly accumulated cellular or acellular secretion which invades the periductal tissues, causes fat necrosis and undergoes calcification, while — according to others — fat necrosis is the primary phenomenon; the fat passing into the nearby ducts, causes ectasis. It is suggested that, in addition, annular calcifications may occur in mastopathies in the following manner.

(1) Hyalin is deposited between the epithelium and myoepithelium in ducts with apocrine metaplasia.

(2) Deposition of hyalin is presumably due to the dysfunction of the myoepithelial cells and the basement membrane among others. The resulting molecular-biological disturbance leads to lesions that become subsequently visible under the microscope. These processes impair the mural circulation of the ducts and lead thus to the deposition of hyaline material.

(3) It is in the circularly deposited hyalin that calcification takes place. The annular shadow, seen in the X-ray pictures, contains a less intensive calcium shadow if also part of the intraluminar secretion is calcified. Complete calcification of the mural and intraluminar hyaline matter results in the formation of homogeneous calcareous nodules.

Introduction

LEBORGNE [7] was the first to present mammograms to show characteristic microcalcifications in some cases of breast cancer, and — according to the literature available — he was the first to describe a differing calcification in ductal ectasis and fibroadenoma [8]. An annular-linear calcification is the most general late radiographic phenomenon in secretory disease of the breast [3], and ring-shaped calcifications have furthermore been observed in some cases of adenosis and epithelial hyperplasia associated with ductal ectasis [2]. In all these benign processes the ducts are distended by their cellular or acellular contents, and secretion leads to mural ulceration so that the ductal substance invades the adjacent tissues. The consequential periductal mastitis is slight if the affected tissues are fibrous, while fat necrosis results if the periductal tissues are adipose [1]. The necrotic areas become calcified and the calcium deposited in the ductal wall appears as a linear or annular shadow [3], but similar calcareous deposits may appear in the periductal tissues also [9]. LEBORGNE [8] postulated a different mechanism for the development of ring-

shaped calcifications. According to this author, the primary phenomenon is a fat necrosis of traumatic, inflammatory or unknown origin which undergoes calcification; the content of the resulting cyst passes into and dilates the nearby ducts. Our observations have, however, shown that annular calcifications, developing in benign processes of the female breast, may be formed in other ways as well.

Material

The material of this study consisted of 162 patients whose surgically removed mammary tissue (following mammography) had been histologically analysed. The lesions were benign in 93 and malignant in 69 cases. The benign cases included 55 mastopathies of diverse

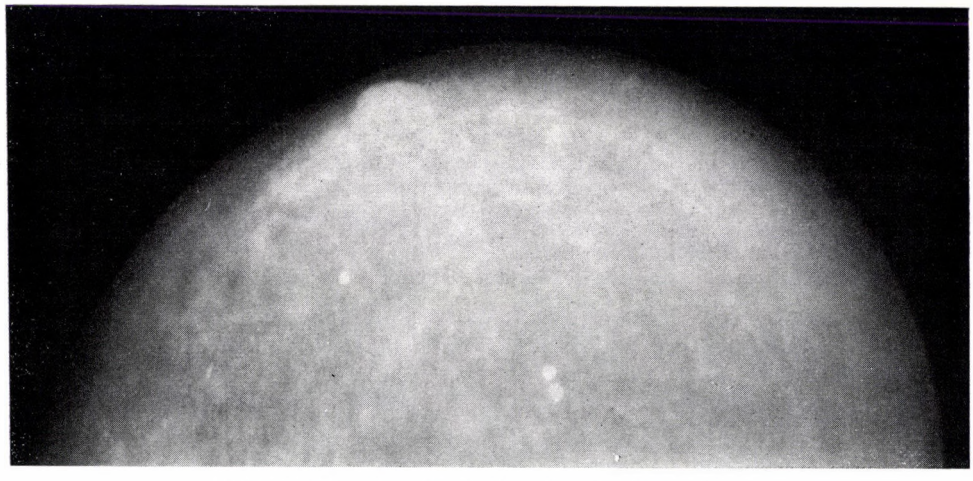

Fig. 1. Ring-shaped calcifications in the breast

types, 32 fibromas, 3 abscesses, 2 lipomas and one foreign-body granuloma, while all malignancies were carcinomas. The radiograms showed ring-shaped calcifications (Fig. 1) in 6 cases of mastopathy and in 2 cases of carcinoma.

Results

In none of these eight cases did we observe ulceration of the ductal epithelium, passage of the ductal contents into the periductal tissues or an invasion of the substance from the area of fat necrosis into the ducts, whereas the annular calcification shown by the X-rays was found in the ductal wall.

The pictures showed two types of annular calcification. In the first type (Fig. 2), opacity was the same within and outside the ring, while in the second type (Fig. 3) the shadow cast by the ring-encircled area appeared to be less intensive.

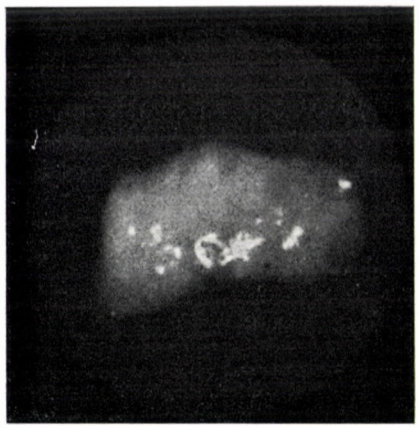

Fig. 2. A type of calcareous ring shadow (biopsy specimen). Note small nodules of calcium around the circular shadows

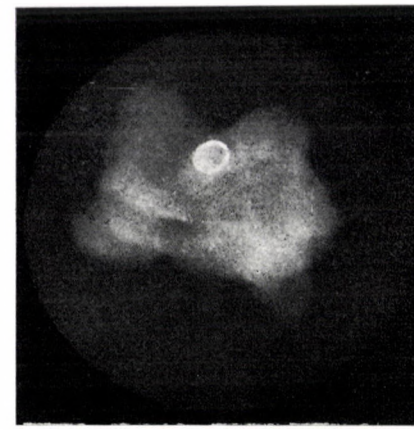

Fig. 3. Another type of calcareous ring shadow (biopsy specimen taken from breast shown in Fig. 1)

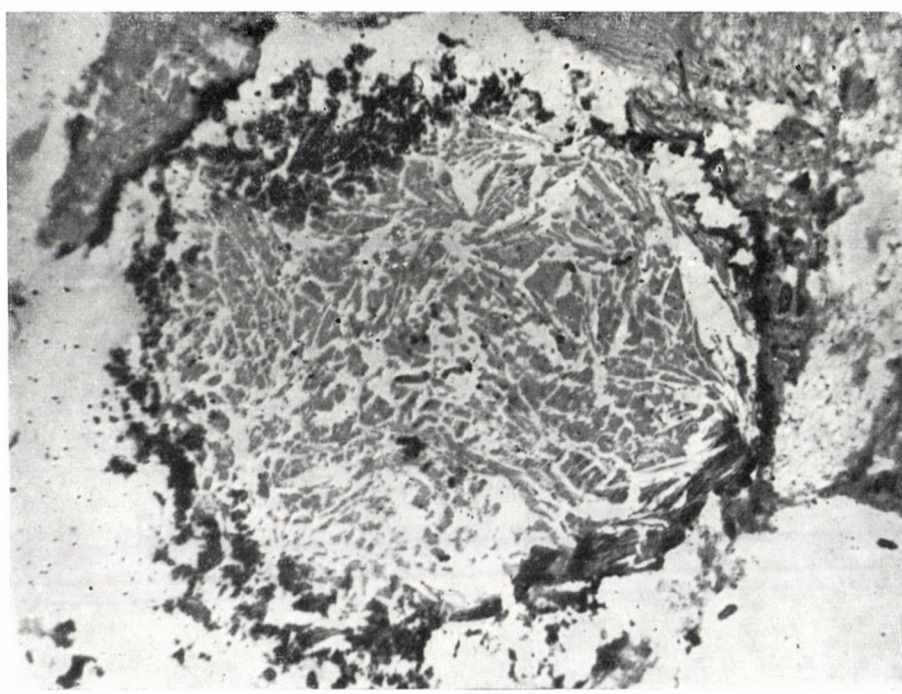

Fig. 4. Circular calcification in ductal wall and initial stage of calcification of intraluminar secretion. (Photomicrograph of lesion shown in Fig. 3)

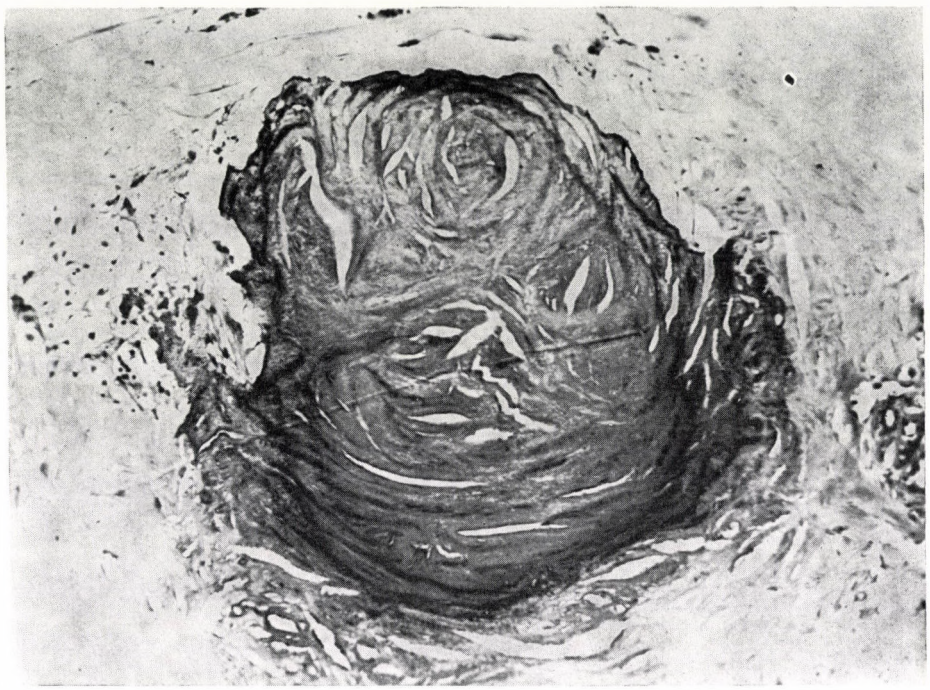

Fig. 5. Complete calcification of ductal wall and intraluminar secretion. (Photomicrograph of a nodule shown in Fig. 2)

Histological examinations of breasts exhibiting calcifications of the described kind revealed a bulky hyaline ring between the epithelial and the myoepithelial layer of the ducts; this hyaline zone showed calcified areas in accordance with the ring-shaped shadow in the X-rays. Apocrine metaplasia in the epithelium and acellular secretion in the lumen of the ducts were invariably registered. The mural ring-shaped calcification and initial calcification of the luminar contents can be seen in Fig. 4. The numerous small calcareous nodules around the annular calcification, presented in Fig. 2, are the result of a total homogeneous calcification of the mural hyaline and the congested luminal substance. The photomicrograph of a nodule of this kind is shown in Fig. 5. Calcifications as seen in Figs 2 and 3 are, thus, different degrees of the lesions occurring in the last phase of the mural hyalin and the luminal secretion. It is only in this last phase that the process can be detected by X-rays. Although the pictures showed features closely similar to that described by LEBORGNE [8] as microcystic fat necrosis, no break-through of the subepithelial hyaline substance into the surrounding tissues, no ulceration of the ductal epithelium and no fat necrosis were observed in any of the examined cases. The ring-shaped calcifications were at some distance from the cancerous and surrounded

by non-tumorous tissue in both of the examined carcinoma cases. These calcareous deposits showed neither radiomorphological nor histological differences from those observed in cases of mastopathy. The apocrine metaplasia registered in carcinomatous and non-carcinomatous cases alike was in agreement with literary data; 65 such cases were observed, and it was in eight that the X-rays howed annular calcification.

Discussion

It is universally agreed that most benign changes of the female breast are due to hormonal disturbances. Diseases of this category are termed mastopathy by some and mammary dysplasia or mammary distrophy by other authors. Also the term chronic mastitis has been used but we consider this name misleading since there is no inflammation in such cases. Several radiologists have tried to set up classifications based on radiographic symptoms but these show fairly great differences. We have adopted the nomenclature and definitions proposed by INGLEBY and GERSHON-COHEN [5].

Histologically, the following changes belong to the category at issue: cysts, apocrine epithelium, sclerosing adenosis, intraductal epithelial hyperplasia and ductal ectasis [12].

Apocrine epithelium, as encountered in our material, consisted of large, tall, columnar secretory cells with a small nucleus and abundant, clear eosinophilic cytoplasm which often contained yellowish-brown aggregates [1, 4].

The ducts of normal non-lactating breasts contain an outer layer of myoepithelial and an inner layer of epithelial cells. The cytoplasmic membrane of the myoepithelial cells contains histochemically demonstrable adenosine diphosphate and nucleoside diphosphate so that the membrane seems to play an important part in selective transport of materials [10]. The presumable function of the myoepithelial cells consists in facilitating the expulsion of secretion owing to their contractility; besides, they act as endocrine receptors by conducting hormonal impulses to the epithelium [6]. In mammary dysplasia the basement membrane becomes abnormal, its myoepithelial components are irregularly oriented or disappear altogether [11]. The hyaline substance was arranged subepithelially and the number of myoepithelial elements was diminished in our material.

Deposits of intercellular hyalin are known to be due to circulatory or inflammatory disturbances. Hyaline tissue is particularly susceptible to calcification [4], a fact which explains the appearance of ring-shaped calcium shadows in the ductal walls. Neither literary data nor our own observations have allowed definite statements as to the correlation between the metaplasia of the ductal epithelium and the appearance of the hyaline substance or that

between the dysfunction of the myoepithelial cells and the apocrine metaplasia. It is in any case probable that changes in the basement membrane and the myoepithelial layer, caused by primary dysplasia and disturbing the exchange of fluid in the wall of ducts, play an important part in the deposition of hyalin.

Summarized, it is in mammary dysplasia that the ring-shaped calcareous deposits of 2 to 4 mm diameter appear. Calcification occurs either in the manner described in literature or in that described in the foregoing. X-ray pictures without a thorough examination of clinical manifestations provide no reliable information on the pathomechanism. Round, oval or circular calcium shadows in the mammogram point to a benign process in the first place, especially if they appear bilaterally. Calcifications as shown in our pictures can reliably be distinguished from typical carcinomatous microcalcifications. Biopsy may be necessary if the calcification is less characteristic [9].

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NEUE BEITRÄGE ZU DEN URSACHEN DER BEI ERKRANKUNGEN DER WEIBLICHEN MAMMA AUFTRETENDEN KALKARTIGEN RINGSCHATTEN

GY. LISZKA, I. KISS und D. CSIFFÁRY

Auf Grund der Literaturangaben entstehen die bei benignen Mammaprozessen wahrnehmbaren kalkartigen Ringschatten nach zwei verschiedenen Mechanismen. Nach dem einen usuriert das sich im Ductuslumen anspeichernde zellulare oder azellulare Exkret die Ductuswand, letztere nekrotisiert, und der in die umgebenden Gewebe durchbrechende Ductusinhalt bewirkt eine Fettnekrose und verkalkt. Nach der anderen Auffassung ist der primäre Vorgang die Fettnekrose, die nach Einbruch in den Ductus den letzteren dilatiert und eine Ductusectasie zustande bringt. Auf Grund ihrer eigenen Untersuchungen stellen die Verfasser fest, daß ein kalkartiger Ringschatten in der mastopathischen Mamma auch in folgender Weise entstehen kann:

1. In jenen Gängen, deren Epithel apocrine Metaplasie aufweist, lagert sich zwischen dem Epithel und dem Myoepithel eine hyaline Substanz ab.

2. Eine wahrscheinliche Ursache der Ablagerung der hyalinen Substanz besteht darin, daß in der mastopathischen Mamma die Myoepithelzellen und die Basalmembran — aus bisher unaufgeklärten Gründen — ihrer Funktion nicht gerecht werden. Die entstehende molekularbiologische Störung führt im weiteren Verlauf zu mikroskopisch nachweisbaren Veränderungen. All diese Veränderungen schädigen die Säftezirkulation in der Ductuswand, was die Ablagerung der hyalinen Substanz nach sich zieht.

3. Die Verkalkung kommt in der hyalinen Substanz zustande, die in der Ductuswand zirkulär abgelagert ist. Dies kommt in Form von kalkartigen Ringschatten zur Darstellung. In jenen Fällen, in denen auch ein Teil des im Ductuslumen vorhandenen Sekrets verkalkt ist, sehen wir in den Ringschatten einen kalkigen Inhalt von geringerer Intensität. Die völlige Verkalkung der hyalinen Substanz in der Ductuswand und im -lumen ergibt einen homogenen kalkigen Knoten.

НОВЫЕ ДАННЫЕ О ПРИЧИНАХ ВОЗНИКНОВЕНИЯ ИЗВЕСТКОВЫХ КОЛЬЦЕВИДНЫХ ТЕНЕЙ, ПОЯВЛЯЮЩИХСЯ В СВЯЗИ С ДОБРОКАЧЕСТВЕННЫМИ ЗАБОЛЕВАНИЯМИ ЖЕНСКОЙ ГРУДНОЙ ЖЕЛЕЗЫ

Д. ЛИСКА, И. КИШ и Д. ЧИФФАРИ

Согласно литературным данным, известковые кольцевидные тени образовываются при доброкачественных процессах грудной железы по двум различным механизмам. Одна возможность, та, что накопляющееся в просвете протока клеточное или неклеточное выделение изнашивает стенки протока, которые некротизируют, причем, прорывающееся в окружающие ткани содержимое протока вызывает жировой некроз и обызвествление. По другому пониманию первичным процессом является жировой некроз, который, после прорывания в проток расширяет последний и обуславливает эктазию протока. На основе собственных исследований авторы устанавливают, что в грудной железе с мастопатией известковая кольцевидная тень может возникнуть также по следующему механизму:

1. В тех протоках, эпителий которых показывает апокринную метаплазию, между эпителием и мисэпителием отлагается гиалиновое вещество.

2. Одной причиной отложения гиалинового вещества, по всей вероятности является, что при мастопатии в грудной железе мисэпителиальные клетки и базальная мембрана — по до сих пор еще невыясненной причине — не выполняют своей функции. Возникающее молекулярно-биологическое расстройство приводит в последствии к микроскопически выявляемым изменениям. Все эти изменения нарушают сокообращение в стенке протока, и это влечет за собой отложение гиалинового вещества.

3. Обызвествление происходит в гиалиновом веществе, отложившемся циркулярно в стенке протока. Это видно в форме известковых кольцевидных теней. Если одна часть секрета, находящегося в просвете протока, также обызвествляется, то в кольцевидных тенях видно известковое содержимое меньшей интенсивности. Полное обызвествление гиалинового вещества, находящегося в стенке и в просвете протока, приводит к образованию однородного, известкового узла.

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THE INNERVATION OF THE JUXTAGLOMERULAR APPARATUS*

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By a combined approach using the catecholamine reaction and an electron microscopic analysis of experimental fibre degeneration, an attempt is made to elucidate the innervation of the juxtaglomerular apparatus in the cat, the rat and the mouse. Two types of terminal axon have been identified: one (A_1) containing numerous dense core vesicles 6—800 Å in diameter and another (A_2) having only small light synaptic vesicles. The majority of the A_1 type axons originates from sympathetic ganglion cells located more distally than the coeliac ganglion. A_2 type axons either originate from the coeliac ganglion or from some place above it, they probably belong to the vagal (cholinergic) system. A few sensory fibres can be traced back to the spinal ganglia Th_{10}–L_3, but could not be distinguished structurally from A_1 type axons.

The fundamental role of the juxtaglomerular apparatus (JGA) in volume and osmoregulation, exercised by renin — a hormone-like substance secreted by the juxtaglomerular cells (JGC) — has been fully established. Besides an influence on the renin secretion by haemodynamic and humoral (Na-level) factors, the possibility of a neural control has recently arisen. According to GÖMÖRY and OLTVÁNYI (1951) nerve bundles running along the afferent arteriole become loose near the vascular pole and encompass the juxtaglomerular cell-group in a characteristic manner. It is undoubtedly the dense nerve plexus of the afferent arteriole described by SMIRNOV (1901) from which the abundant innervation of the JGC derives. On the basis of light microscopic innervation studies with conventional neurohistological methods a vegetative and possibly a sensory innervation of the JGA has been discussed by ÁBRAHÁM (1943, 1969). In an electron microscopic study, BARAJAS (1964) differentiated "dark" and "light" axons around the afferent arteriole, the efferent arteriole and the JGA, in the monkey and the rat. HARTROFT (1966) recorded in a short paper lacking illustrations the presence of nerve terminals around the cells of the macula densa. The general problem of the vascular region's innervation has been summarized by SZENTÁGOTHAI (1966) corresponding to the level of knowledge around 1964—65. Using the paraformaldehyde reaction of FALCK and HILLARP, VAGERMARK et al. (1968) demonstrated adrenergic fibres around the

* On the basis of a lecture delivered at the 8th Hungarian Biological Congress (1968), completed with recent literary data.

juxtaglomerular cells. In a recent survey, ENDES (1968) referred to the studies of GOMBA (in press) which have revealed a rich adrenergic innervation in the JGA.

The aim of the present study was a further elucidation of the innervation of the vascular pole region of the renal corpuscles and of the JGA in particular. A combined approach by histochemical and electron microscopic methods and by the experimental degeneration procedure was therefore performed.

Material and methods

In normal material obtained from the kidney of the cat, the rat and the mouse, FALCK and HILLAR's paraformaldehyde reaction was applied for the demonstration of catecholamines (CA) by fluorescence microscopy. For this purpose the material was prepared according to the slightly modified cryostate procedure of CSILLIK and KÁLMÁN (1967). Further normal material was subjected to electron microscopic examination. For the purposes of experimental degeneration in cats and mice, the coeliac ganglion and in another group of animals the spinal ganglia Th_{10}–L_3 of the left side were removed. Material from the left kidney obtained 20 hours postoperatively was examined electron microscopically following prefixation in glutaraldehyde and postfixation in osmic acid. After orientation in semithin sections of tissue blocks embedded in Durcupan, ultrathin sections were contrasted with lead citrate according to REYNOLDS [19] and examined with a Tesla BS 413 electron microscope.

Results

The nerve fibres entering the kidney establish a rich plexus of preterminal and partly terminal nerve fibres around the renal artery and its branches. The plexus continues undiminished along the interlobular arteries and the afferent arterioles (Figs 1 and 2). It is seen clearly that the catecholamine reaction

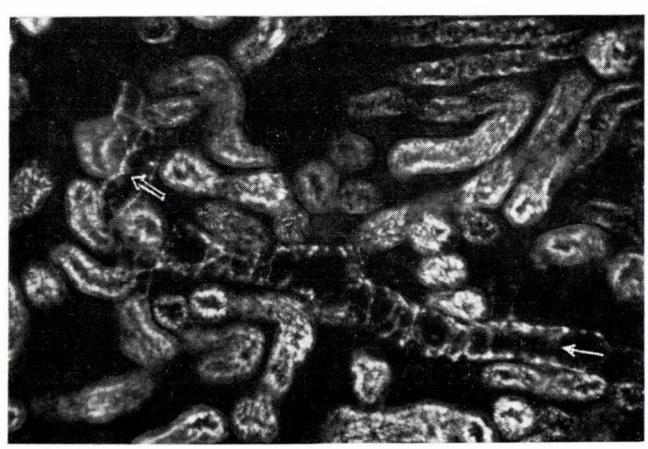

Fig. 1. Terminals giving catecholamine reaction around the interlobular artery (simple arrow) and the afferent arteriole (double arrow). Kidney of cat. ×120

becomes stronger and spots of bright green fluorescence are concentrated at the vascular pole of the glomerulus. At some places the fibres may be traced over to the efferent arterioles and around the Bowman capsule, while no reaction could be seen within the glomeruli (Figs 2 and 3). The striking structural similarity between glomerular and smooth muscle cells is shown in an electron micrograph (Fig. 4); the myofilaments are well discernible also in the JGC.

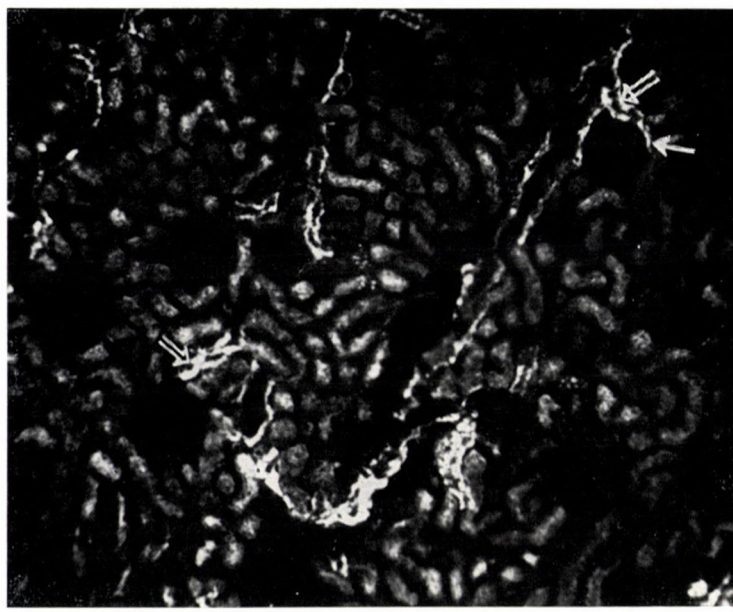

Fig. 2. General view of the cat kidney. Catecholamine reaction becoming more intensive in the neighbourhood of the JGA (double arrow), nerve fibre curving around Bowman's capsule (simple arrow). ×80

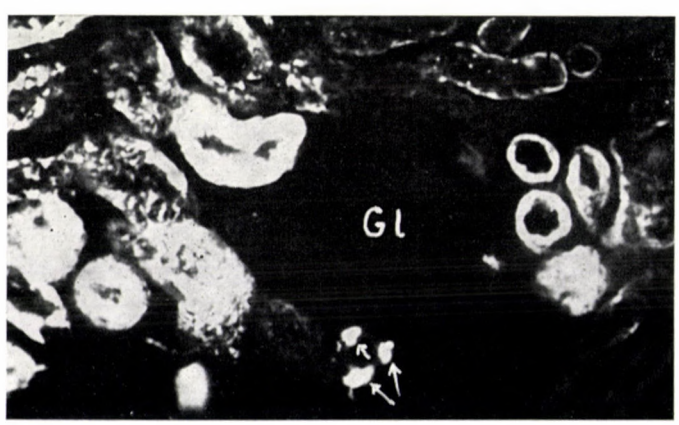

Fig. 3. Vascular pole of glomerulus (Gl); arrows point to sites of catecholamine reaction becoming more intensive close to the JGA. Cat. ×200

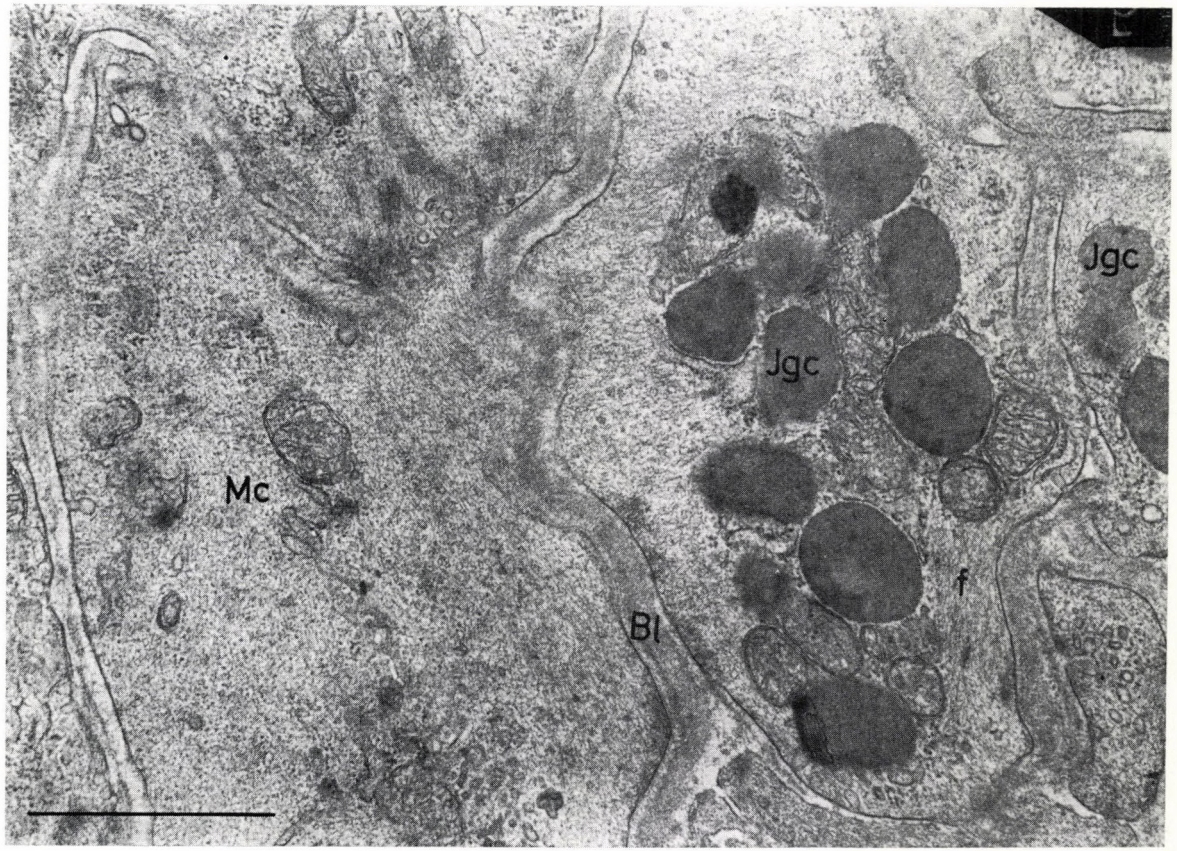

Fig. 4. Smooth muscle cell (Mc) arranged side by side in the wall of the afferent arteriole with juxtaglomerular cell containing specific granules (JGC); basal lamina surrounding the JGC (Bl), myofilaments (f). Electron micrograph. Mouse

One group of the axons closely arranged around the JGC contains dense-core vesicles (A_1), while another group of axons (A_2) contains exclusively empty vesicles about 300 Å in diameter. At some places the two kinds of axon occur together, at others they run separately (Figs 5 and 6). Both kinds of axon become closely attached to the JGC separated only by a thin basement mem-

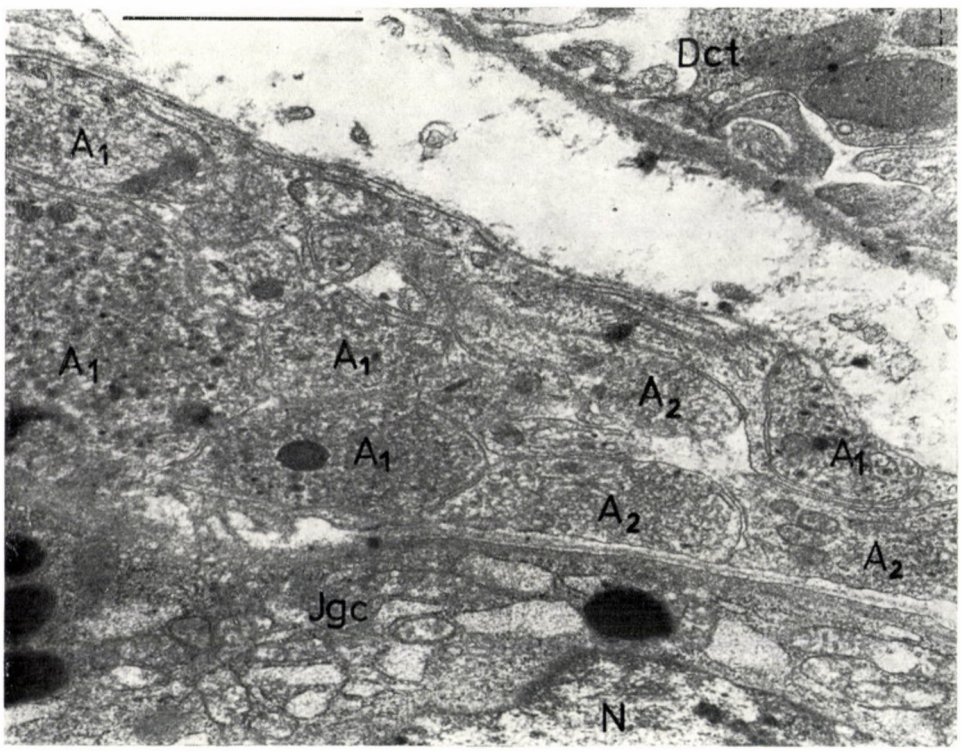

Fig. 5. Group of axons near a juxtaglomerular cell (JGC). Nucleus of the JGC (N). (A_1) Axons containing dense core vesicles; (A_2) axons with empty vesicles; (Dct) basis of the epithelium of the distal convoluted tubules. Electron micrograph. Mouse

brane; this appears to be much thinner than the general basement membrane of the granulated cells. A frequent electron microscopical finding is the A_1 type axon, containing dense-core vesicles, situated near the efferent arteriole and the capsule of the glomerulus (Fig. 7). Fig. 8 shows in the same area a degenerating axon after the removal of the coeliac ganglion. After the removal of the spinal ganglia (Th_{10}–L_3) of the mouse, a degenerating axon was observed adjacent to the glomerular capsule (Fig. 9).

Discussion

A considerable increase of the blood renin level upon stimulation of the renal plexus and sympathetic excitation has been found by VANDER (1965) and by BUNAG et al. (1966). WATHEN (1965) observed the same phenomenon after catecholamine infusion, and DUNIHUE et al. (1961) after the infusion of a small

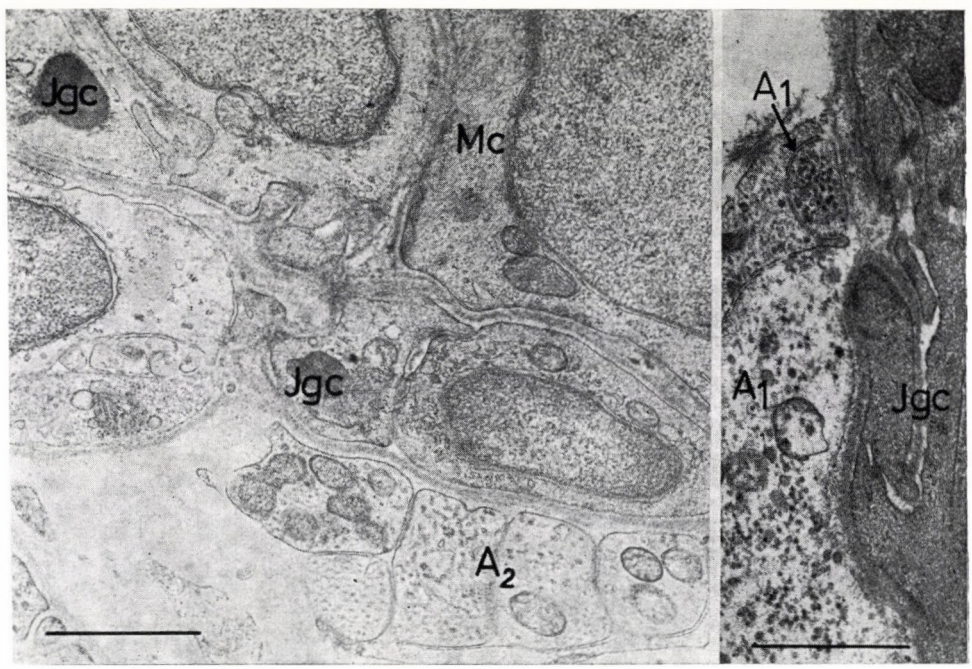

Fig. 6. (A_2) Group of axons with empty vesicles adjacent to juxtaglomerular cell (JGC). Muscle cell (Mc). At the right side of the figure, a type A_1 axon emerges from a Schwann cell, and another type A_1 axon is closely attached to a JGC. At this site the basal membrane became thinner. Electron micrograph. Mouse

dose of adrenaline, while KAMURA (1966) recorded a decrease of the juxtaglomerular index after the subcutaneous infusion of adrenaline. Although none of these authors ascribed these results to a direct effect on the JGA, their close relationship with the reported observations is strongly suggestive of a direct action of catecholaminergic nerves on the JGA. Both the intensive catecholamine reaction of nerve elements around the JGA, as well as the terminals containing dense-core vesicles and being in intimate contact with the JGA are well in accord with this notion.

The electron microscopical analysis of the axons around the JGA groups permits their classification according to the type of vesicle contained, rather

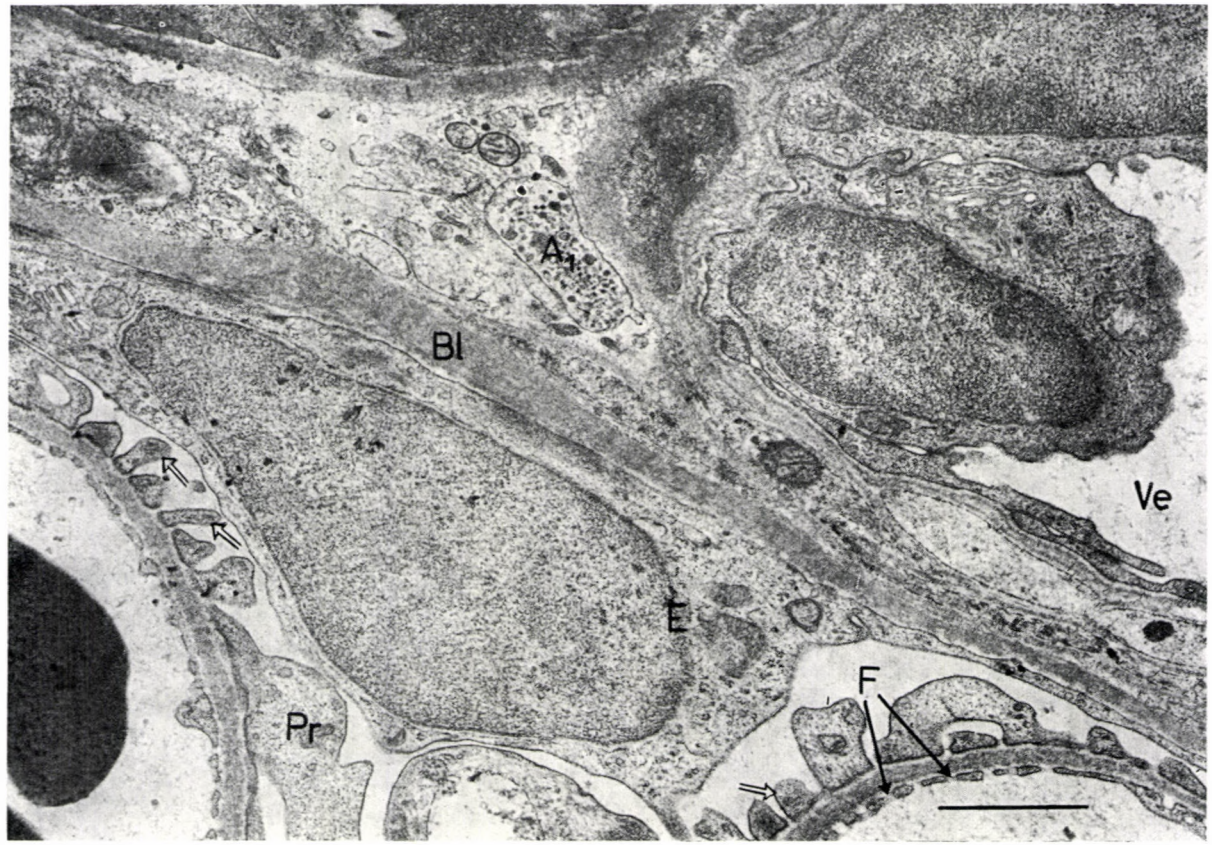

Fig. 7. Axon containing intact dense core vesicles (A_1) in the angle between the glomerulus and the vas efferens (Ve). Epithelium (E), basal lamina (Bl), process of the podocyte (Pr), fenestrated endothelium (F). The double arrow points to the peduncles of the podocytes. Electron micrograph. Cat

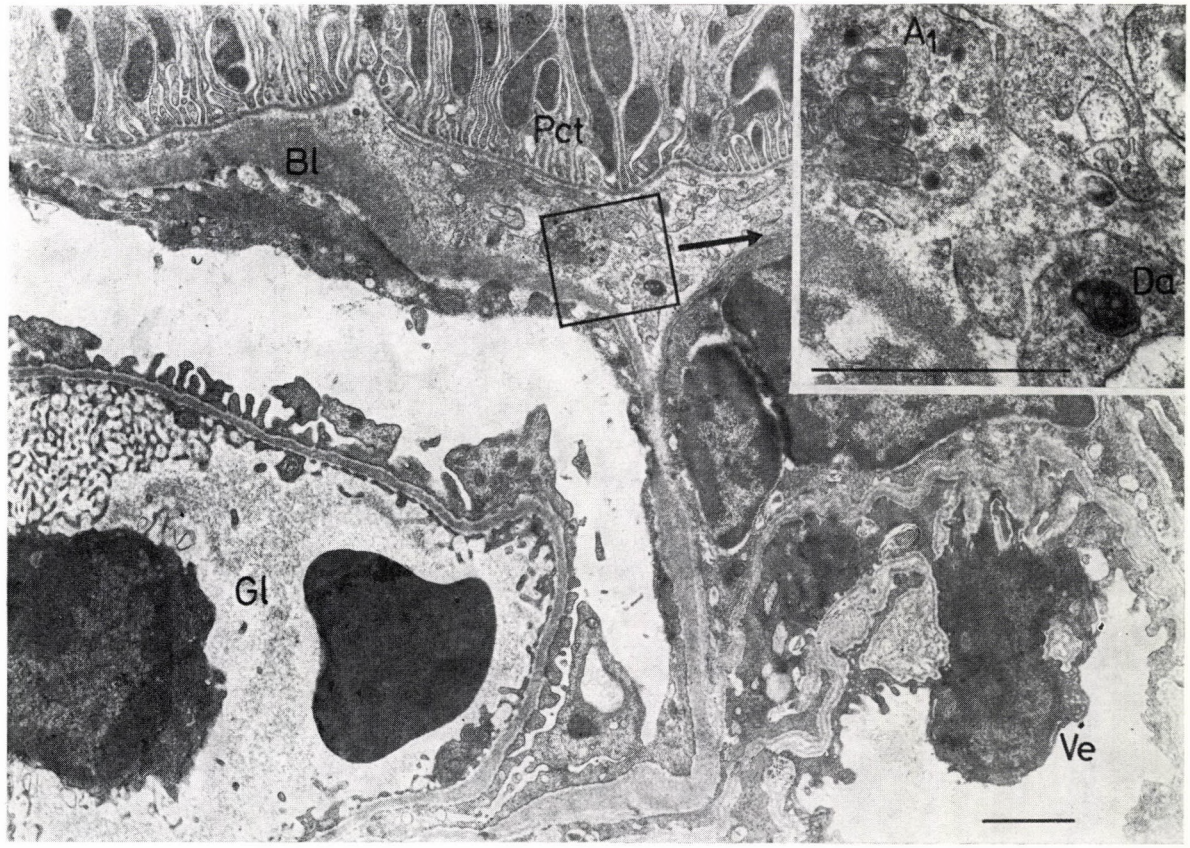

Fig. 8. Intact axon containing dense core vesicles (A_1) and degenerating axon (Da) in the angle between glomerulus (Gl) and vas efferens. Right upper inset shows some details at higher magnification. (Bl) Basal lamina of Bowman's capsule; (Pct) basis of the epithelium of the proximal convoluted tubules. Coeliac ganglion removed 20 hrs previously. Electronmicrograph. Cat

than the subdivision of BARAJAS (1964) based on other criteria the functional significance of which is much less established than that of the character of vesicles.

The degeneration analysis of the nerve elements participating in the innervation of the vascular pole region of the renal corpuscles offers some

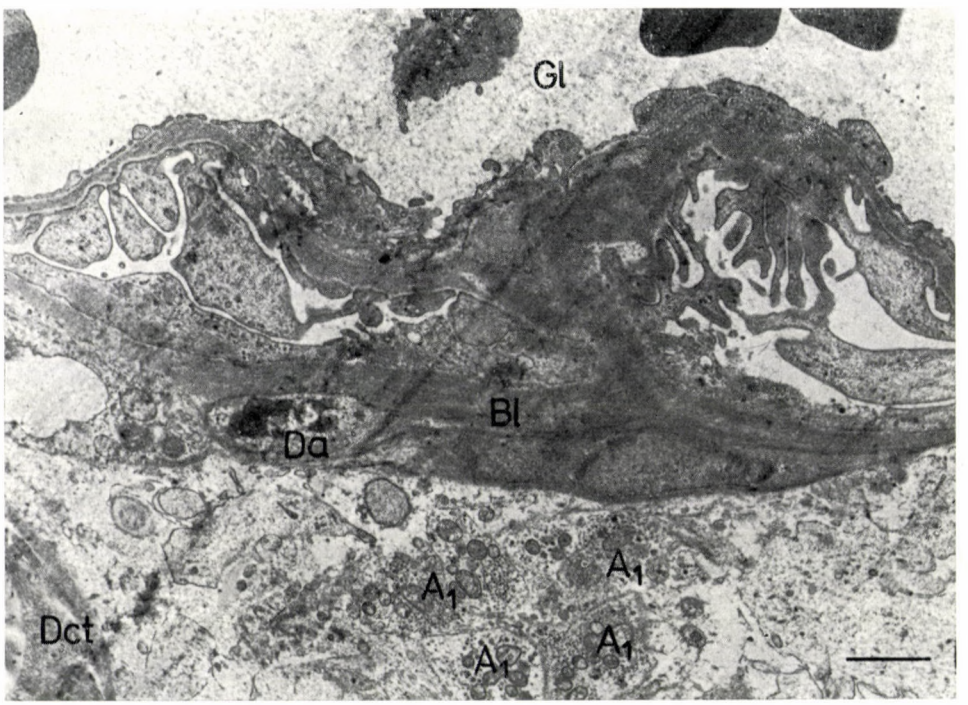

Fig. 9. Degenerating axon (Da) adjacent to the basal lamina (Bl) of Bowman's capsule in the kidney of a mouse 20 hrs after removal of the spinal ganglia Th_{10}–L_3. (A_1) Intact axons containing dense-core vesicles arranged side-by-side; (Gl) glomerulus; (Dct) distal convoluted tubules. Electron micrograph

information concerning the origin of various elements. It has, however, to be admitted that the picture is not yet conclusive. After removal of the coeliac ganglion, the type A_2 axons (having only empty vesicles) completely disappear from the area of the JGA. On the other hand, under similar conditions, signs of axonal degeneration may be observed in the angle between Bowman's capsule and the efferent arteriole, where normally only type A_1 axons occur. As upon removal of the spinal ganglia giving the sensitive nerve supply to the kidneys, signs of axonal degeneration were observed at the same site, one may conclude with certain reservations that some A_1 type fibres might also be sensitive. Since the sensory fibres have to pass through the coeliac ganglion

the axon degeneration around the efferent arteriole might be explained in both cases as being due to the interruption of sensory fibres. The considerable number of A_1 type axons having dense-core vesicles that remain intact after removal of the coeliac ganglion have undoubtedly to originate from ganglia, for example the renal plexus, situated nearer to the kidney. They are, therefore, obviously of sympathetic origin. The coeliac ganglion may be the site of origin of the A_2 type axons, or at least they have to pass through the ganglion. They might in both cases belong to the vagal system. Apart from the observation of sensory fibres, which have to be considered preliminary and require further elucidation, a degeneration analysis would support the general notion according to which in the vegetative peripheral innervation, the axons (A_1) containing numerous dense-core vesicles 6—800 Å in diameter correspond to the catecholaminergic, and the axons (A_2) with light vesicles to the cholinergic nerve endings.

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ÜBER DIE INNERVATION DES JUXTAGLOMERULÄREN APPARATS

CS. LÉRÁNTH, GY. UNGVÁRY und T. DONÁTH

Der juxtaglomeruläre Apparat von normalen Katzen, Ratten und Mäusen sowie nach Entfernung des Ggl. coeliacum und des Ggl. spinale wurde mit der Katecholamin-Nachweisreaktion und im Elektronenmikroskop untersucht. Im JGA wurden um den JGS herum der Anstieg der KA-Reaktion sowie dense-core-Bläschen bzw. leere Bläschen enthaltende Axonendigungen gefunden. Nach Exstirpation des Ggl. coeliacum wurde im Gefäßpol, nach Exstirpation des Ggl. spinale an der Bowmanschen Kapsel Axondegeneration nachgewiesen.

ИННЕРВАЦИЯ ЮКСТАГЛОМЕРУЛЯРНОГО АППАРАТА

Ч. ЛЕРАНТ, Д. УНГВАРИ и Т. ДОНАТ

Юкстагломерулярный аппарат кошек, крыс и мышей был изучен в норме и после удаления чревного и спинномозгового узлов реакцией для выявления катехоламинов и в электронном микроскопе. В юкстагломерулярном аппарате, около JGS были установлены повышение реакции катехоламинов и терминальные аксоны, содержащие пузырьки dense core или пустые пузырьки. После экстирпации чревного узла в полюсе сосуда, после удаления спинномозгового узла на капсуле Боумена была выявлена дегенерация аксонов.

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CHANGES IN THE VASCULAR STRUCTURE OF THE LIVER FOLLOWING SUBTOTAL HEPATECTOMY IN THE RAT

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The rate of regeneration following subtotal hepatectomy in the rat has been studied by the planimetric evaluation of portograms and by means of semiquantitative injected corrosion preparations. The latter method seems to be suitable for the determination of the extent and quality of vascular capacity and — possibly — of vascular regeneration, and also for the estimation of vascular capacity in any organ. Repeated resections allowed reliably to follow lobular changes.

The capacity and possibly the regeneration of the vessels was found — with reference to the wet weight — to lag in the first week behind the rate of the organ's regeneration in both the portal and the hepatic venous system. Portal and hepatic capacity did not change in parallel. No qualitative changes in the characteristic vascular structure of the liver were observed.

It has been shown that hepatic regeneration consists in the hypertrophy of existing and the formation of new lobules.

Partial resection or necrosis of the liver is followed by a process of regeneration. Disturbances of liver function caused by subtotal resection do not endanger the animal's life if only owing to the rapid rate of regeneration. The most important findings in this respect were the recognition of the liver's great regenerative capacity, its functional reserves, and the detection of certain general biological laws.

CRUVEILHIER [5] was the first to describe hepatic regeneration in 1833. He found that removal of 75% of the liver was followed by complete regeneration of the organ in eight weeks in dogs and in three weeks in rats.

The liver's regenerative power was best illustrated by the experiments of MANN [17] and those of BOLLMAN and MANN [2]. A lobe was removed from the liver of dogs every tenth day, and at the termination of the experiment the weight of the remaining lobe exceeded the original weight of the whole liver.

Recent works are mainly concerned with the rate of regeneration [6, 14, 23], the factors involved in the process of regeneration [3, 4, 7, 9, 11, 16, 23] and the histochemical and ultrastructural changes which occur in its course [1].

The available literature failed to provide information about changes occurring in the vascular apparatus of the regenerating liver. Surprisingly, it was in a speculative manner that certain authors tried to decide the question as to whether regeneration consists in the hypertrophy of existing [10, 14, 18, 21] or the formation of new complex lobules [8].

The present experiments were designed to provide information on the following questions.

1. Which method or methods are best suited for the quantitative assay of intrahepatic blood vessels?

2. What qualitative and quantitative changes are occurring in the vascular structure of the regenerating liver?

3. Is it by means of lobular hypertrophy or by the formation of new lobules that regeneration occurs?

Material and method

Two hundred white male rats of 150 to 200 g body weight were used. We removed the two large lobes (lobus magnus and lobus fissus), i.e. 65 to 70% of the entire weight of the liver in 130 animals, while 30 unoperated rats served as controls. Forty animals were subjected to renewed surgery three weeks after the first resection: we removed the anterenal and retrorenal lobes (which amounted to about 70% of the regenerated organ). Twenty of these 40 animals were once more operated upon at the end of the third week following the second operation: we removed the anteventricular lobe (i.e. 50 to 60% of the tissue that had regenerated since the second resection). As a result, only one of the original six lobes was left in these animals. Two per cent of the rats died after the first, 30% after the second, 10% after the third resection. The body weight of the animals was determined in grams, that of the resected lobes and the removed livers in centigrams at every surgical intervention and at the time when the rats were killed. Animals that had undergone one resection were divided into groups of 25 and sacrificed 1, 2 and 7 days, 1 and 3 months after the operation. The livers of 10 animals of each group were injected for 4 min. with a 5% solution of PVC *via* the portal and the hepatic veins at a constant pressure of 20 mm Hg. Contrast material was injected into the liver of 5 animals through a cannula inserted in the mesenteric vein and at constant tube distance X-rays were taken of the organ. By projecting the roentgenograms on graph paper at a scale of 1 : 1 we were able to compare the size of the differently aged regenerated organs (to be called "regenerates" in the following) as expressed in surface units. A mixture of Indian ink and gelatin was injected through the portal vein and the inferior V. cava into the liver of 5 animals which had been left to survive for 3 months; the livers were fixed in formalin, sectioned, and prepared according to Spalteholtz's thick preparation technique.

The livers of half of the twice and thrice resected animals were injected with 5% PVC through the portal vein or through the inferior V. cava and the hepatic veins.

Livers injected with PVC were corroded by means of concentrated hydrochloric acid, washed in water and dried at room temperature. The dried preparations were weighed on an analytical balance with an accuracy of ± 5 mg. Data for the once resected animals were analysed for significance by Student's *t*-test, and the standard deviations were computed. A stereomicroscope was used for the examination of the corrosion preparations and those injected with Indian ink and gelatin.

Results

Fig. 1 presents the roentgenogram of the liver of a control animal. The lobes are well outlined after the injection of 1 ml of adipiodone into the mesenteric vein. Fig. 2 shows a liver with a one-week-old regenerate; the persisting four lobes are clearly visible. Fig. 3 shows that, while regeneration had been rapid until the end of the first week, a certain setback was registered thereafter.

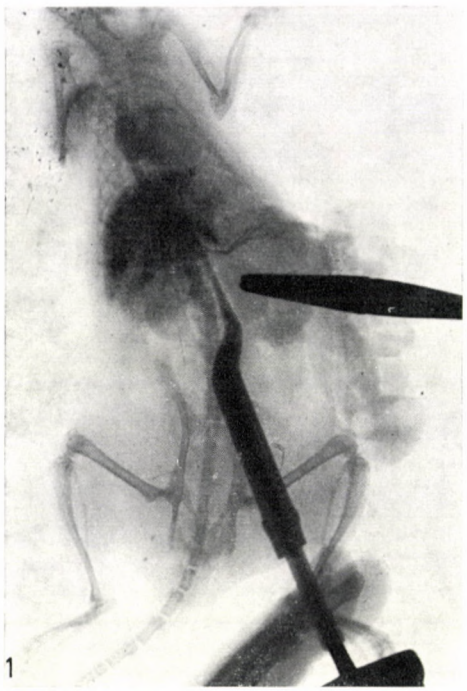

Fig. 1. X-rays of liver of control animal

Fig. 2. X-rays of liver with one-week regenerate

The wet weight (Fig. 4D) of the livers showed a significant increment between the second and the seventh postoperative day, and this gain was followed by a significant loss of weight up to the end of the first postoperative month. The casts of the hepatic vein revealed a significant growth of weight between the second and the seventh day (Fig. 4E), but the columns showed a moderate increase even thereafter. The weight of the portal casts (Fig. 4F) pointed to a highly significant increase between the second and the seventh day; a tendency to decrease was registered thereafter but the decrement failed to reach statistical significance.

It is evident from the upper half of Fig. 5 that the weight of the one-week-old regenerate had approached the original weight of the liver; the regenerate grew less thereafter, but after three months it exceeded the computed liver weight by 20%. By expressing the weight of the liver in per cents of the body weight, the one-week regenerate showed a considerable increase, while the liver of animals was somewhat larger than the original after the first and somewhat smaller after the third postoperative month (3 and 5%, respectively; lower half of Fig. 5).

The relative weight of the hepatic vein preparations (i.e. their weight in per cents of the liver weight) showed the lowest value on the seventh day,

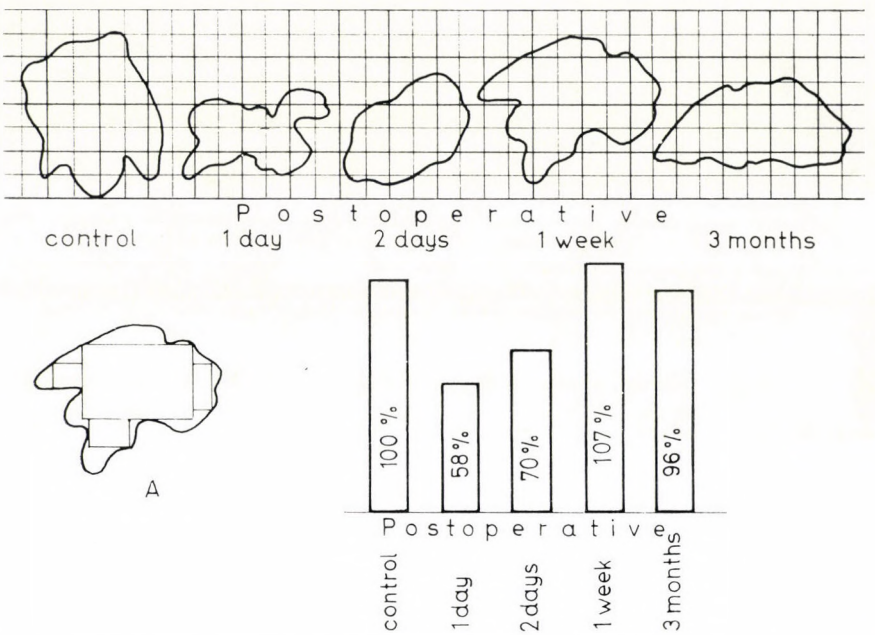

Fig. 3. **Planimetric evaluation of the roentgenograms. The control area is taken as 100% and postoperative results are referred to it**

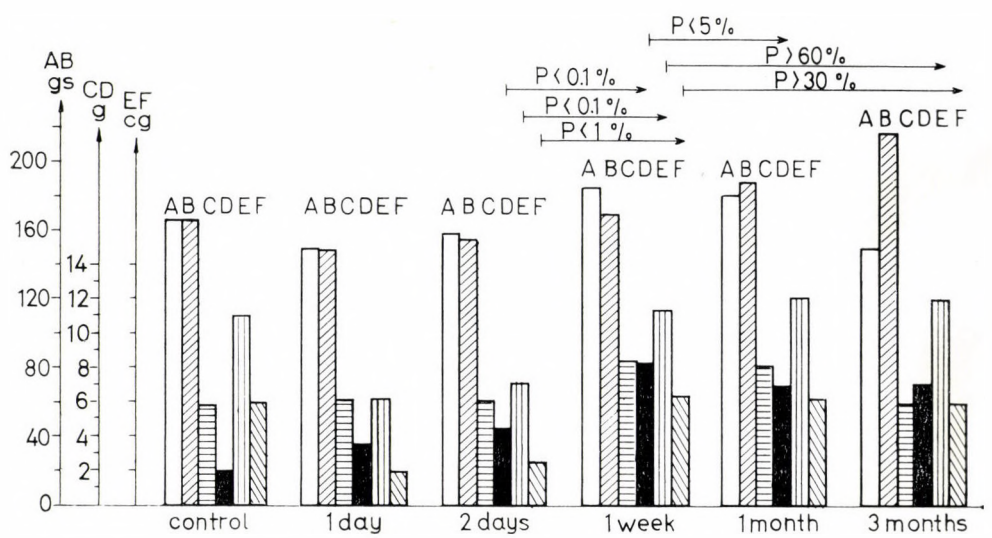

Fig. 4. **A = operative body weight in grams. B = body weight in grams, at sacrifice. C = computed liver weight (g). D = weight of the four small lobes (regenerates) (g). E = weight of corrosion preparation of the hepatic venous apparatus in 0.01 g. F = weight of the corrosion preparation of the portal vein (cg). Standard deviation: ±5% in every column**

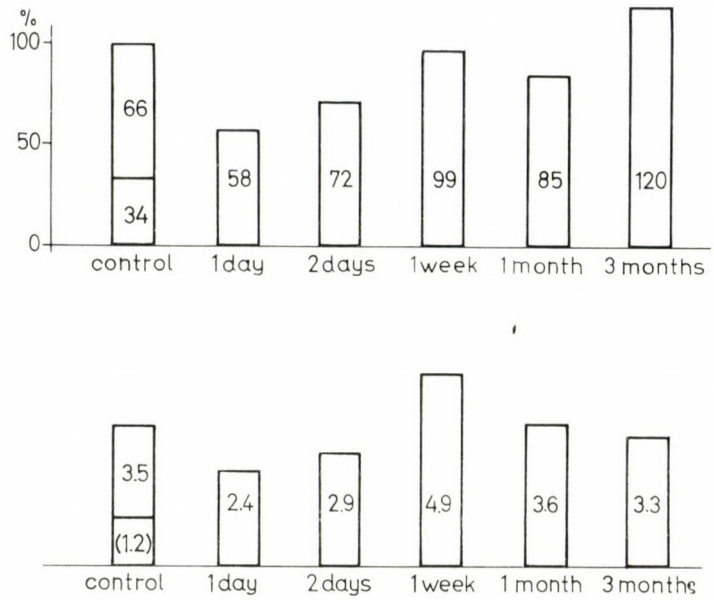

Fig. 5. Above: weight of regenerate in per cent of control liver weight. Below: weight of regenerate in per cent of body weight

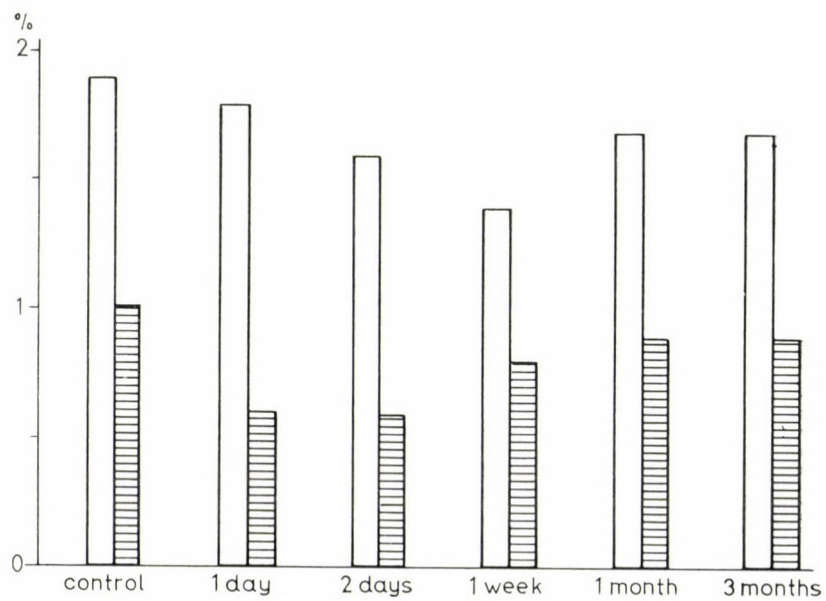

Fig. 6. Empty columns = weight of corrosion preparation of the hepatic venous system in per cent of liver weight. Shaded columns = weight of corrosion preparation of the portal venous system in per cent of liver weight

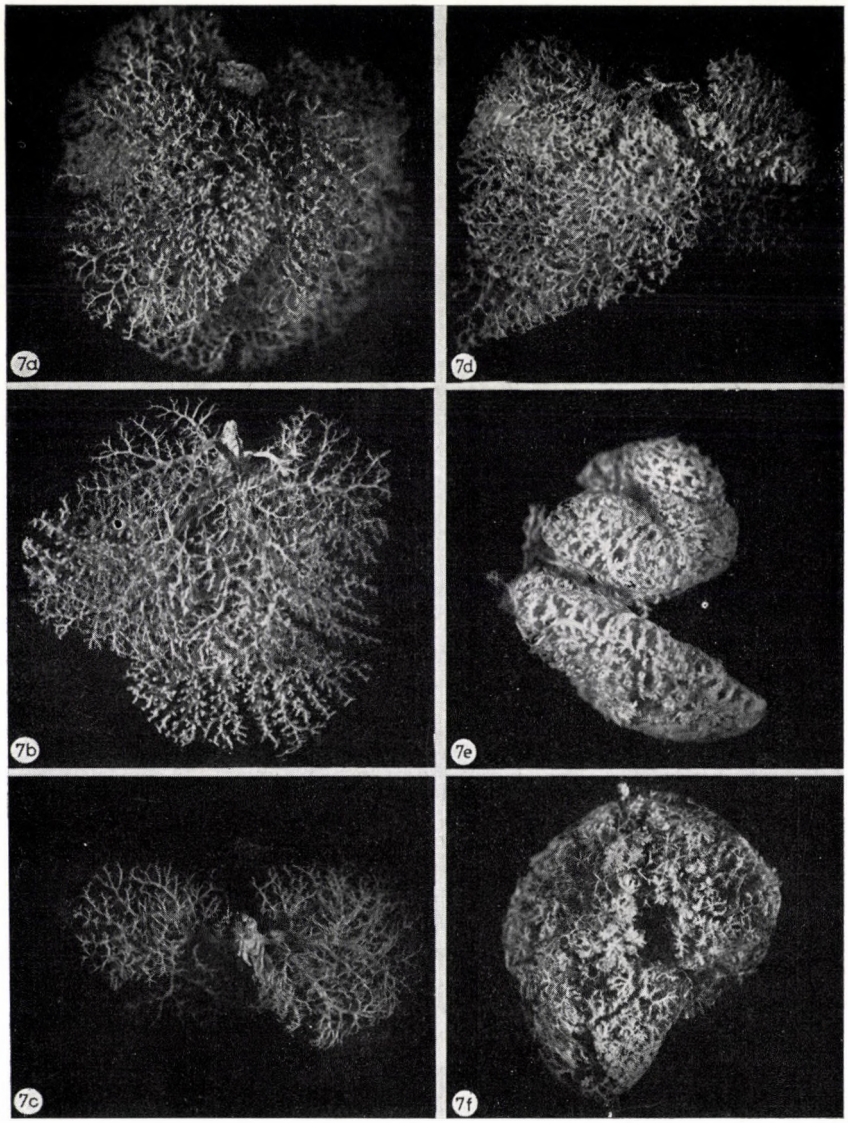

Fig. 7. a = portal cast of normal liver; b = portal cast of the two large lobes to be resected; c = portal cast of the remaining four small lobes; d = portal cast of the four small lobes 4 weeks after the resection of the large lobes; e = portal cast of the two small lobes 4 weeks after the other two small lobes had been removed; f = portal cast of the single lobe 4 weeks after the third resection at which one of the remaining two small lobes was removed

and returned thereafter to a level slightly lower than the normal. The portal preparations behaved differently in that they displayed the lowest absolute and relative value in one- and two-day-old regenerates; it had considerably

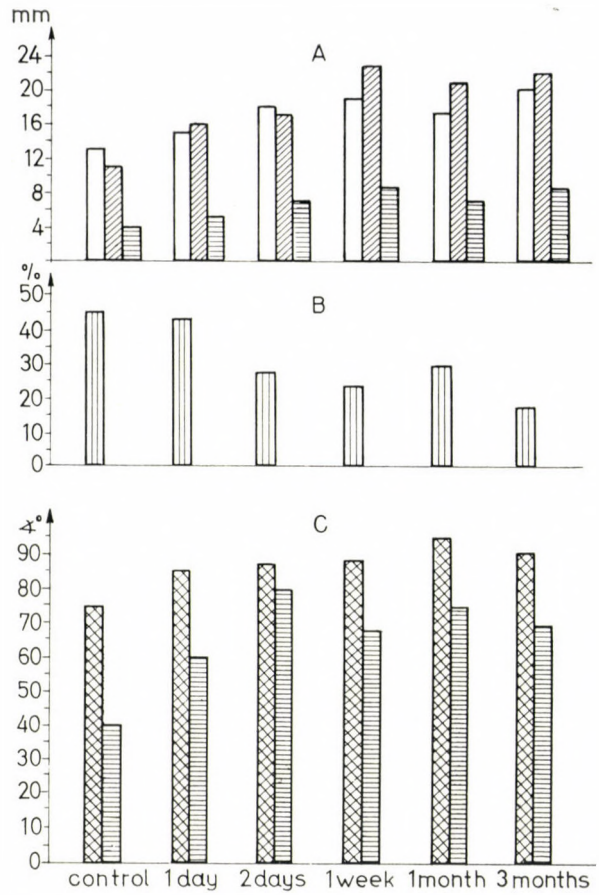

Fig. 8. A = length of anterenal lobe (mm); width of anterenal lobe (mm); thickness of anterenal lobe (mm). B = trunk of hepatic vein of the anterenal lobe in per cent of the cranio-caudal (long) diameter of the lobe. C = change of angle formed by the inferior V. cava and the hepatic venous trunk of the anterenal lobe; change of angle formed by the first ramification of the portal vein. Standard deviation = $\pm 5\%$ in every column

increased by the seventh day and continued to increase but failed to reach the original level (Fig. 6).

The lobes of the regenerating liver changed their form in a characteristic manner. Fig. 7 shows a normal portal cast (a), the two large lobes to be resected (b) and the remaining four small lobes (c) with apparently poor vascularization. The lobes are thin with a sharp edge. The remaining four lobes of once resected animals are still sharp edged but their casts are more bulky and show more abundant ramifications (d). Corrosion preparations of twice resected livers (i.e. those with two remaining lobes) and three times resected organs (with a single remaining lobe) whose weight usually reached the computed original

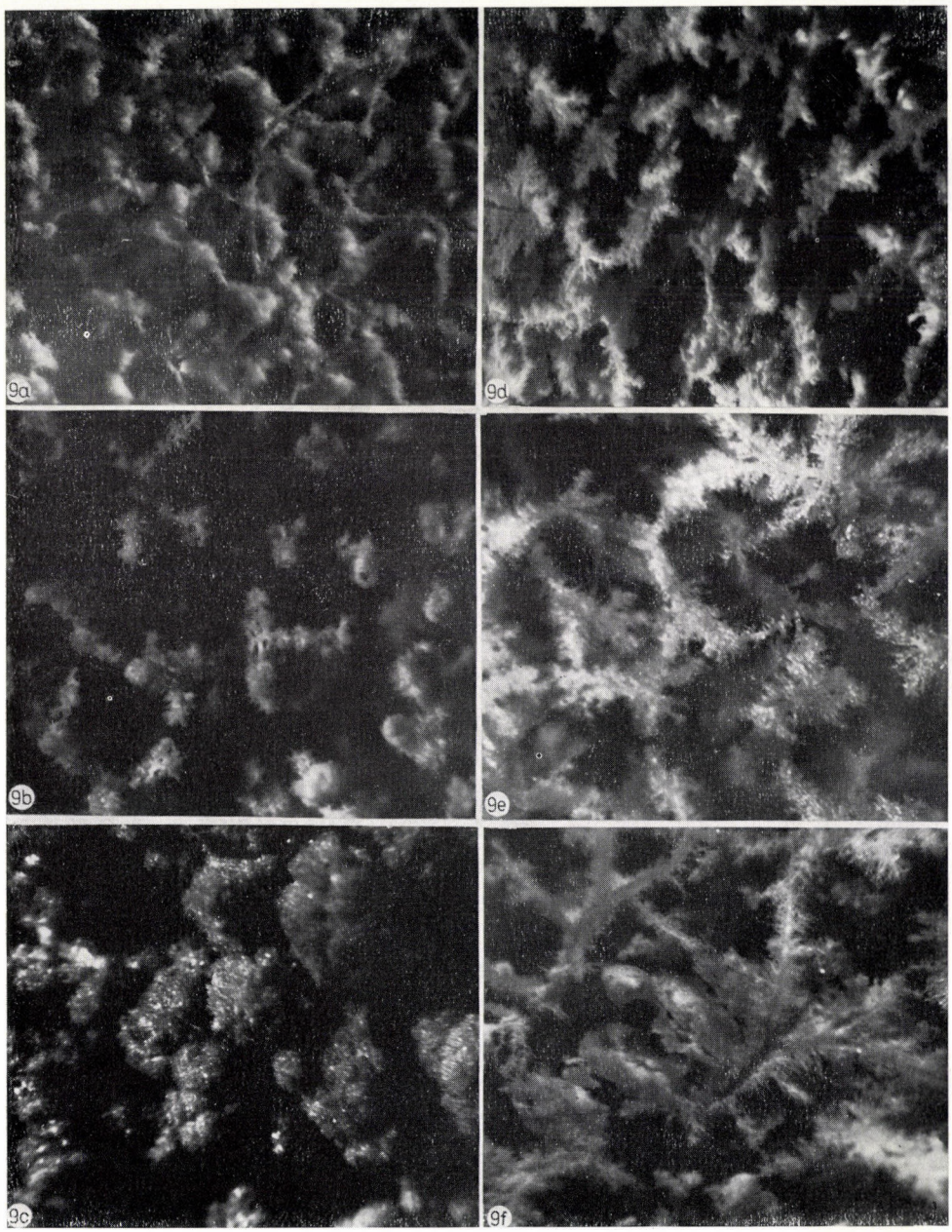

Fig. 9. a = normal portal cast; b = portal cast prepared after single resection; c = portal cast prepared after second resection; d = corrosion preparation of normal hepatic vein; e = preparation of hepatic vein after a single resection; f = preparation of hepatic vein after second resection. Stereomicroscopic pictures. $\times 50$

weight, are rounded, more densely ramified, so that the shape of the original lobe is no longer identifiable in them (e—f).

Fig. 8A illustrates millimetric changes in the length, width and thickness of the anterenal lobe. The originally second longest (latero-lateral) diameter became the longest in the course of regeneration, thus indicating a widening of the lobe. Changes of the angles formed by the last portion of the hepatic vein with the inferior V. cava and those formed at the first ramification of the portal vein were characteristic (Fig. 8C). Fig. 8B presents the ratio between

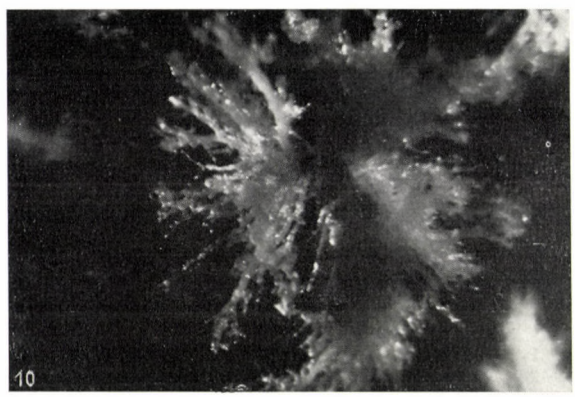

Fig. 10. Corrosion preparation of Kiernan's lobule removed from one-lobe liver. ×80

the trunk of the hepatic vein of the anterenal lobe (i.e. of the segment between the most cranially opening branch of the hepatic vein and the inferior V. cava) and the cranio-caudal diameter of the lobe. This ratio fell from 45 to 25% by the end of the first week, rose to 30% after a month, and was less than 20% after three months.

Observing the divisions of the portal vein in the corrosion preparations as far as the sinusoids, we registered 6 to 7 ramifications in the controls and 9 to 10 in three-month-old regenerates, thus not only the length and width of the vessels had increased but also the number of ramifications. The portal vessels and the branches of the hepatic vein of the regenerates were similar to those of the controls.

Stereomicroscopic pictures of normal, once, twice and thrice resected livers, made at identical magnifications, are highly instructive. Figs 9a—c present the tufts of the portal vein which increased along with the number of resections. Figs 9d—f demonstrate the hepatic venous system; the increase in number of the central and sublobular veins and the sinusoids opening into these vessels is conspicuous. Fig. 10 presents the picture of Kiernan's lobule removed from a one-lobe liver; some of the elongated sinusoids may have been suitable for developing new central or sublobular veins in the three-day animal.

Discussion

The portograms and their planimetric evaluation allowed to observe the rate of regeneration after subtotal hepatectomy. Portography is, however, too laborious and affords but approximate data regarding the extent of regeneration so that its use cannot be recommended. The method is justified only if one wishes to follow the course of regeneration in vivo.

Changes in the wet weight of the liver, as observed in the present experiments, were in fair agreement with those reported in literature. The rapid gain of weight was to some extent obviously due to increased water contents, as is the case with all rapidly growing organs, tissues, hypertrophic kidney [15] or muscle [14]. The liver contains a large volume of water 48 hrs after resection according to ZAKI [25], 72 hrs thereafter according to HARKNESS [12].

According to literature, the liver needs three weeks for restoring its water contents to normal, to accomplish the process of regeneration. The diagram illustrating liver weight shows, however, an apparent decrease in the one-month group. This was probably due to the fact that the body weight of the members of this group was less than in the other test animals. No significant decrease in weight is apparent in the one-month group if the regenerate's weight is referred to the computed weight of the liver, a result in agreement with literary data. As regards weight, the process of regeneration can, therefore, be regarded as accomplished. (It is for this reason that the body weight of the animals and the weight of the removed liver should always be determined.)

The capacity of the hepatic veins increased at a rapid rate until the seventh day; although the rate was slower thereafter, a continued slight increase was registered nevertheless. A comparison of this change with that observed in wet weight is indicative of a relative insufficiency of the hepatic venous system during the first days: it reached the minimum on the seventh day, i.e. the very day on which the weight of the liver had reached the maximum. (Constriction of the efferent channels or oedema?)

The capacity of the portal venous system, too, increased in the course of regeneration; the increase was moderate on the first two days, pronounced between the second and the seventh day, and once more moderate thereafter. It would seem that, at first, the capacity of the portal apparatus is inferior even to the normal capacity of the small lobes despite the relatively increased supply, a phenomenon due to the inability of adaptation caused by the high water content of the surrounding parenchyma and vessel wall. A comparison of our data with the findings of MENYHÁRT and SIMON [19] is instructive: they found the mass of the liver to amount to 55% after 24 hours and 75% after 72 hrs, while the corresponding values of resistance were 65 and 55%, respectively. (We computed these values from curves that had not been analysed by the authors in this respect.) The changes in resistance did not, thus, follow

that of weight increment, a phenomenon which may be explained by a slower growth of portal capacity or, possibly, by the slower regeneration of portal vessels.

The changes observed in the shape of lobes following hepatectomy were presumably due to several factors. The lobes assumed more and more rounded forms. Of course, the liver's connective tissue, capsule, the juxtaposed lobules growing in all directions, and the topography of the abdominal organs were all contributory factors. The new special arrangement and the new form of the lobes had modified the angles at which the vessels meet and ramify so that considerable changes in blood flow resulted. Analysis of Fig. 8B justifies the supposition, a speculative one since our results were not significant statistically and revealed certain tendencies only, that intralobular regeneration is not uniform: changes occur first in the peripheral and subsequently in the central area. What can confidently be concluded from the present experiments is that the growth of hepatic tissue in the course of regeneration is the result of two processes, i.e. the formation of new lobules and the hypertrophy of existing ones. As to the process of hypertrophy, JATROPULOS [14] showed by exact measurements that the distance between Glisson's triangles and the central vein varied from 280 to 520 μ in normal livers; the most frequent values were between 370 and 420 μ. The diameter was found to grow at a rapid rate in regenerating livers; the most frequent values were 460 μ in the second, and 490 μ in the tenth week. Although JATROPULOS observed a renewed diminution of the diameter after the 20th week, he refrained from interpreting this phenomenon by a lobular re-formation owing to the inadequacy of his method. It is easy to settle the problem by the corrosion method which showed that divisions of the portal vessels were considerably more numerous in the regenerated than in the corresponding control lobules. Considering that the portal lobule contains not only terminal branches (which divide into sinusoids) but also tertiary and quaternary branches, the hepatic lobe must obviously contain more than the original number of lobules. Thus, the formation of new lobules must be accepted as proven. This conclusion was confirmed by the results of repeated resections. Certain sinusoids of Kiernan's growing lobule shown in Fig. 10 are evidently more elongated than the adjacent ones. It is presumably from these sinusoids that the new central veins develop, and their primordial side branches indicate the formation of new sinusoids.

The above observations and considerations justify the conclusion supposed by HIGGINS and ANDERSON in 1931 and supported but not proved by JATROPULOS in 1965, that, in contradiction to PFUHL's statement [20], in the course of compensatory hypertrophy, and possibly even independently thereof, new hepatic lobules are formed during postnatal growth.

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STRUKTURVERÄNDERUNGEN DER GEFÄSSE NACH SUBTOTALER LEBERRESEKTION AN WEISSEN RATTEN

GY. UNGVÁRY, J. DEMETER, A. HUDÁK und J. TARI

Das Tempo der nach subtotaler Hepatektomie erfolgenden Regeneration wurde an weißen Ratten mittels planimetrischer Bewertung der Portogramme sowie mit Hilfe einer semiquantitativen Injektions-Korrosionsmethode verfolgt. Letzteres Verfahren ist zur Bestimmung des Ausmaßes und der Qualität der Gefäßkapazität (evtl. der Gefäßregeneration) geeignet. Es sei erwähnt, daß es auch für die Messung der Veränderungen des Gesamtvolumens sämtlicher Organe anwendbar ist. Mittels mehrmaliger Resektion konnten die Veränderungen der Leberläppchen demonstriert werden.

In der ersten Woche erreicht die Gefäßkapazität (evtl. die Gefäßregeneration), bezogen auf das feuchte Gewicht, weder im Vena-portae-System, noch im Vena-hepatica-System, das Tempo der Organregeneration. Die Kapazität der V. portae und der V. hepatica verändert sich nicht parallel. In der für die Leber charakteristischen Gefäßstruktur kommt keine qualitative Veränderung zustande.

Es wurde der überzeugende Beweis erbracht, daß im Verlauf der Leberregeneration zum Teil die bereits vorhandenen Lobuli hypertrophieren, zum Teil aber neue Läppchen entstehen.

ИЗМЕНЕНИЕ ВАСКУЛЯРНОЙ СТРУКТУРЫ ПЕЧЕНИ ПОСЛЕ СУБТОТАЛЬНОЙ РЕЗЕКЦИИ ПЕЧЕНИ У БЕЛЫХ КРЫС

Д. УНГВАРИ, Й. ДЕМЕТЕР, А. ХУДАК и Й. ТАРИ

Темпы регенерации после субтотальной гепатектомии были прослежены у белых крыс посредством планиметрической оценки портограмм и полуколичественной инъекционно-коррозионной методикой. Последняя оказалась подходящей для определения размера и качества сосудистой емкости (возможно и регенерации сосуда). Следует отметить, что методика авторов пригодна для измерения всех изменений общего объема, происходящих в любом органе. При помощи повторной резекции удалось демонстрировать изменения печеночных долек.

Установлено, что емкость сосудов (возможно и их регенерация), на первой неделе после резекции, в переводе на влажный вес, не достигает темпа регенерации органов, ни в системе печеночной, ни в системе воротной вен. Изменения емкости воротной и печеночной вен происходят не параллельно. В характерной для печени структуре сосудов не наблюдается качественного изменения.

С помощью этой методики удалось убедительно доказать, что в процессе регенерации печени отчасти наблюдается гипертрофизация уже существовавших долек, а отчасти образование новых долек.

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HISTOCHEMICAL EXAMINATION OF SUPPORTING TISSUES BY MEANS OF FLUORESCENCE

II. FLUOROCHROMES AS AN INDICATOR OF LAMELLAR BONE MINERALIZATION

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A new method of marking has been elaborated for the examination of ossification, bone reorganization and mineralization. In vivo fluorescence produced by combined alizarin red S, tetracycline and fluorexon treatment yielded reliable results; it inhibited calcification to a lesser extent and was, moreover, suitable for determining the direction of these biological processes. Measurement of the diameter of the Haversian lamellae is recommended for statistical evaluation.

Introduction

Sensitivity, selectivity, sharp contrasts and simplicity are well known advantages of histochemical fluorescence methods. Secondary fluorescence offers further facilities for the histochemical investigation of calcium salts in bone. Most of the fluorochromes used for these procedures can be employed in vivo, a fact which makes it possible to register conditions of mineralization at predetermined intervals. By using standard methods, parenterally introduced fluorescent substances appear like annual rings in the skeleton of the test animal indicating the development of the Haversian system as well as periosteal and endosteal ossification from the time of the first injection to the sacrificing of the animal. No other morphological method reveals the dynamics of ossification with equal accuracy. Although attempts have been made to employ lead acetate as indicator [35, 57, 73] it has failed to find general acceptance.

The more important fluorescence methods for the demonstration of calcium salts are as follows.

1. *Tetracycline*

In 1951, REGNA et al. [60] discovered that tetracycline formed a chelate with calcium, MILCH et al. [53, 54] utilized this phenomenon for the localization of calcium in bone. The method has since been generally adopted. Tetracycline derivatives are especially suitable for the study of bone growth in animals [8, 14, 15, 16, 21, 26, 29, 32, 39, 47, 56] but can be used in human material as

well [18, 22]. The procedure is moreover useful for quantitative histological examinations [22, 23, 24, 27, 31, 43, 48, 67, 70], for the study of embryonic ossification as also for that of tooth growth [4, 7, 30]. As regards skeletal anomalies, the method has chiefly been used in cases of osteoporosis [17, 25, 68], rickets [64] and tumours [55]. By comparison to and combination with other histochemical and histophysical procedures, the method at issue has been perfected [15, 30, 36, 42, 43, 65]. TAPP et al. [66] employed tetracycline in vitro as a histochemical reagent for calcium.

Tetracycline serves furthermore for the study and demonstration of calcifying cartilage [21], various tumours [46, 52], atherosclerotic lesions [44], fish scales [5], spermatozoa [19] and the mitochondria of living cells [13].

Various theories have been advanced in connection with the binding of tetracycline derivatives in bone. HARRIS et al. [33] demonstrated the existence of an interaction between tetracycline and collagen, while URIST and MCLEAN [69] suggested that apatite crystals and mucopolysaccharides may be involved in this respect. Formation of a chelate complex with calcium may in any case serve as a working hypothesis.

IBSEN et al. [40] elaborated a method for the quantitative determination of deposited tetracycline. This method, if generally adopted for biological xaminations, may yield valuable information about the mechanism of binding.

2. *Alizarin red S*

The use of this dye is one of the oldest methods in calcium histochemistry. In 1900 GRANDIS and MAININI [28] dyed bones with purpurin, and they subsequently studied ossification by means of sodium alizarin sulphonate, the active principle of purpurin [9, 10, 41, 45, 49, 50, 58, 62, 63] and used this compound to demonstrate calcium in sections [9, 10, 51]. HOYTE's investigations [37, 38] gave the main impetus for developing the application of alizarin red S into an exact method for the study of bone growth. DIXON and HOYTE [12] applied autoradiography to determine the selectivity of alizarin red for calcium.

Although an eminent fluorochrome, alizarin red S was not utilized in fluorescence microscopy until recent times. HARRIS [31], HARRIS et al. [34] and ADKINS [1] were the first to apply the dye for fluorescence microscopic examinations.

Especial credit is due to HONG et al. [36] for having instituted comparisons between the alizarin red method with the tetracycline, lead acetate, trypan blue and ^{45}Ca autoradiographic techniques. Confirming earlier findings, they demonstrated that alizarin red inhibited the development of bone [1, 31, 34].

Fig. 1. Tibia, middle third, transverse section; tetracycline fluorescence. ×100. Filters: BG + OG. Six-month-old dog.The animal received one injection. Note yellow fluorescence in Haversian system produced by tetracycline bound to formed bone

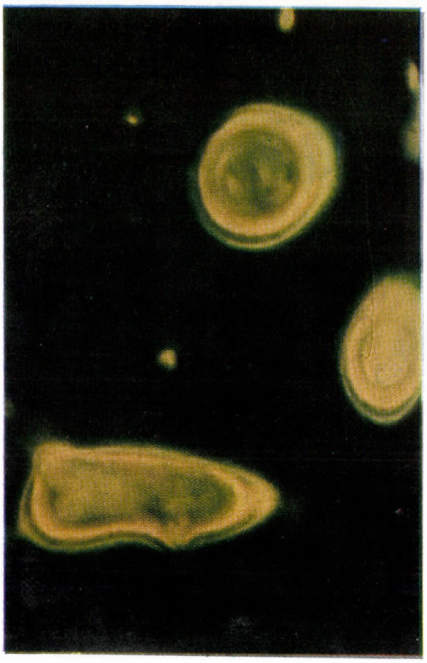

Fig. 2. Tibia, middle third, transverse section; fluorexon + tetracycline fluores cence. ×100. Filters: BG + OG. Six-month-old dog. The animal received first fluorexon and a week later tetracycline. A green and a yellow fluorescent ring in Haversian system indicate growth during this time

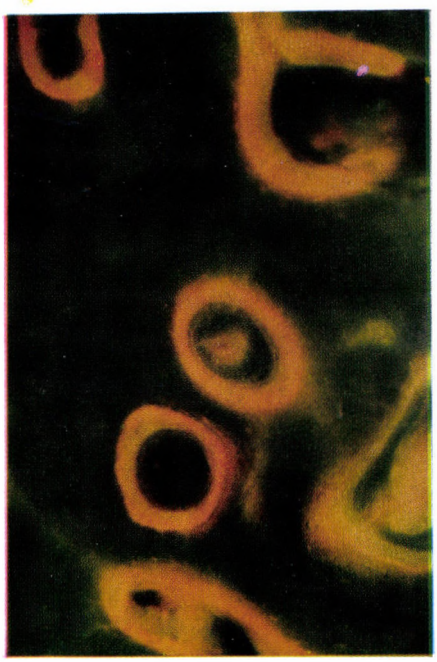

Fig. 3. Tibia, middle third, transverse section; alizarin red S fluorescence. ×100. Filters: BG + OG. Six-month-old dog. The animal received a single injection and was sacrificed a week later. Note brickred fluorescence

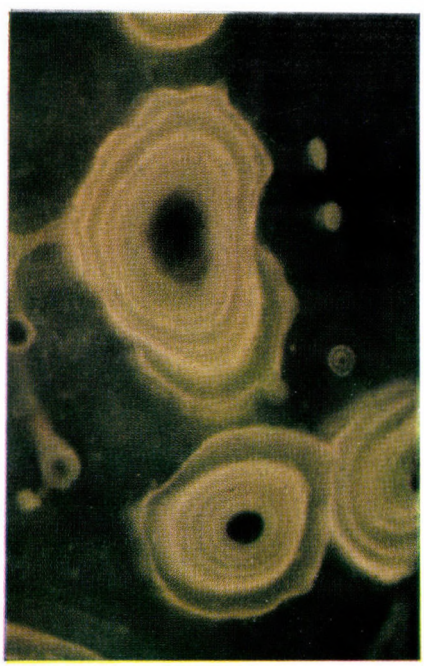

Fig. 4. Tibia, middle third, transverse section; tetracycline fluorescen ×100. ce. Filters: BG + OG. Six-month-old dog. The animal received a total of 5 injections at weekly intervals. The Haversian system shows five fluorescent rings whose distance from one another is gradually decreasing

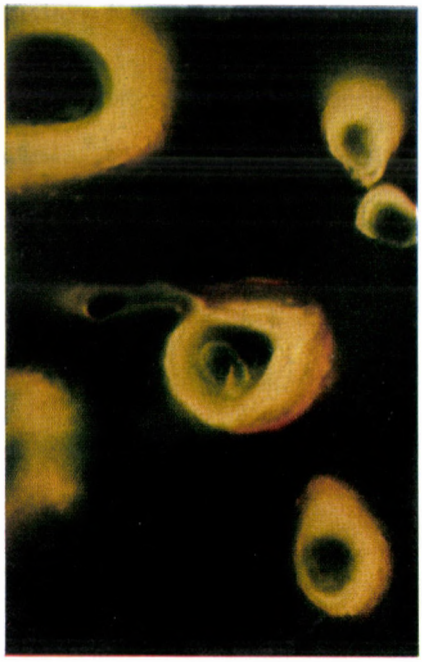

Fig. 5. Tibia, middle third, transverse section; alizarin red S + tetracycline + fluorexon fluorescence. ×100. Filters: BG + OG. Six-month-old dog. The animal received alizarin in the first, tetracycline in the second, and fluorexon in the third week. Outward shift of the fluorescent rings shows the direction in which the Haversian system is growing

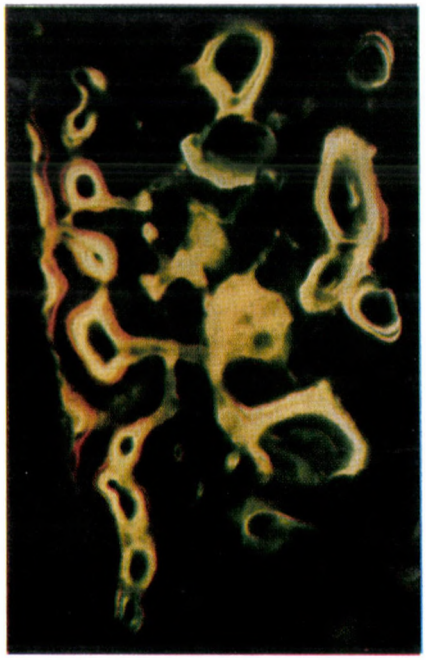

Fig. 6. Tibia, middle third, transverse section; alizarin red S + tetracycline + fluorexon fluorescence. ×40. Filters: BG + OG. Six-month-old dog. The Haversian canals of the periosteum and the cortical substance display fluorescence in three colours according to the injections administered at weekly intervals. The fluorescent stripes of the periosteum represent (in an outward sequence) alizarin, tetracycline, fluorexon

Fig. 7. Tibia, middle third, transverse section; alizarin red S + tetracycline + fluorexon fluorescence. ×100. Filters: BG + OG. Six-month-old dog. Endosteum. Treatment as before. Sequence of rings as in Fig. 6

3. *Fluorexon*

DIEHL [11] was the first to use calcein (fluorescein-iminodiacetic acid) as an indicator of calcium, and it was WALLACH et al. [71, 72] who produced its active principle, 2,4 bis [N,N'di (carbomethyl/aminomethyl), i.e. fluorescein (DCAF). SUZUKI and MATHEWS [65], combining the compound with tetracycline, obtained good diphasic fluorescence pictures of bones and teeth. It seems strange that we have found no further report on the application of this excellent combined method which produces more distinguishable annual rings than the other techniques and is moreover suitable for the determination of the direction of calcification.

4. *Morin*

FEIGL's [20] morin method allows the histochemical demonstration of calcium in vitro.

The aim of the present study was to combine the useful properties of all current in vivo methods and so to elaborate a simple procedure for the demonstration of mineralizing processes. The new methods for the qualitative and quantitative study of the development and mineralization of the Haversian system are described in the following.

Material and method

Dogs, about 6 months old, were intraperitoneally injected with the following fluorochromes.

1. Oxytetracycline (Chinoin Budapest), $C_{22}H_{24}O_9N_2 \cdot 2H_2O$.
2. Fluorexon (Chemapol, Prague). Chemical structure: condensation product of fluorescein iminodiacetic acid 2,4 bis(N,N')carbomethyl(aminomethyl)fluorescein. $C_{30}H_{26}N_2O_{13}$ (as acid).
3. Alizarin red S (Schuchardt, Munich). Chemical structure: sodium alizarin sulphonate, $C_{14}H_7O_7SNa$.
4. Morin (BDH-England). Chemical structure: 3,5,7,2'4'-pentahydroxyflavone.

Dosage. Oxytetracycline, 25 mg/kg, dissolved in its special solvent.
Alizarin red S: 20 mg/kg of a 1 : 100 solution in distilled water.
Fluorexon: 20 mg/kg dissolved in distilled water.
Morin: 100 mg/kg dissolved in distilled water.

Experiment I

One dose of each fluorescent agent was administered to different animals which were sacrificed a week after the injection.

Experiment II

The dogs received fluorexon, a week later oxytetracycline and were killed after another week.

Experiment III

The dogs received first alizarin, a week later oxytetracycline, after another week fluorexon, and were killed in the fourth week after the first treatment.

Experiment IV

The dogs received one dose of oxytetracycline (weekly) over a period of 5 weeks, and were killed in the 6th week.

Microscopic examination

Transverse sections were manually prepared from the cortical substance of the tibia and mounted in dammar. The sections were examined under a fluorescence microscope by means of a Zeiss HBO-50 type mercury-vapour lamp, with combined filters BG 3/4-OG 1/1 and UG 1/3.5—GG 9/1. Agfacolor films CT 18 and Orwocolor films UT 16 were used for photography.

Statistical evaluation

The longest and shortest diameter of the fluorescent rings around the Haversian canals was measured by means of an ocular micrometer at tenfold magnification; the data so obtained were evaluated according to PALKOVITS [59] and the results converted to microns by means of an objective micrometer.

Results

Tetracycline produced well circumscribed rings of yellow fluorescence in blue, and of yellowish-green fluorescence in ultraviolet light; rings obtained with fluorexon in green light, those with alizarin red S in blue light were brick-red, while in U.V. light the rings were purplish red in colour. Morin in blue and U.V. light produced hardly distinguishable green rings of weak fluorescence; the compound moreover proved to be toxic so that we abstained from its further use. The results of micrometric measurements are illustrated by columnar and circular diagrams in which each unit is equal to 162.162 μ. Development of the Haversian system can be followed well in Figs 8, 9 and 10. Prolonged tetracycline treatment inhibited calcification; the distance between the rings became gradually smaller in the dogs treated for 5 weeks (Fig. 11). The best results were obtained with a combined treatment with alizarin red S, tetracycline and fluorexon. A green fluorexon ring in the inner zone of the Haversian system was surrounded by a yellow oxytetracycline ring, while the outer zone displayed a red alizarin ring in the areas freshly calcifying after the injections. A red band was observed in the periosteum and the periosteal zone of calcification towards the cortical substance in the innermost part; there was a yellow stripe more outward, and a green one in the outer zone.

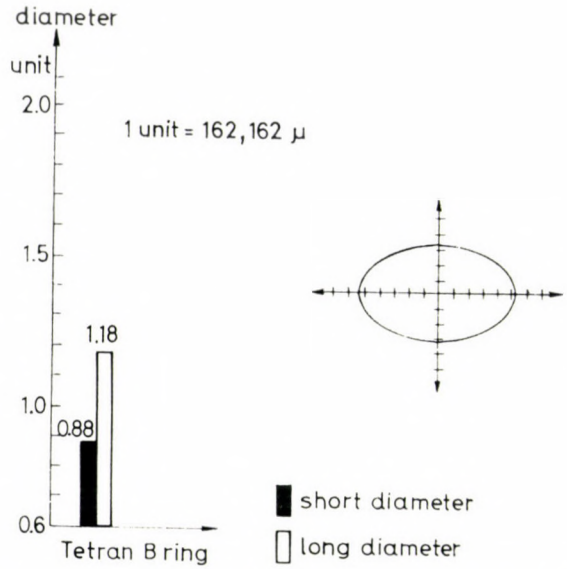

Fig. 8. Columnar and circular diagrams illustrating fluorescence in the Haversian system of dog treated with one dose of tetracycline

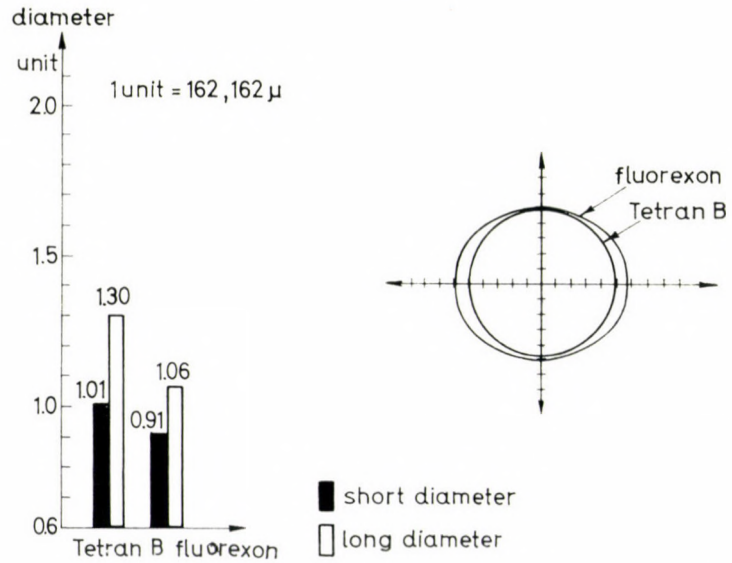

Fig. 9. Columnar and circular diagrams illustrating fluorescence in the Haversian system of dog treated with fluorexon and tetracycline

It is evident that the combined treatment producing trichromatic fluorescence is eminently suitable for following the trends of ossification and mineralization. Results are presented in Figs 1 to 11.

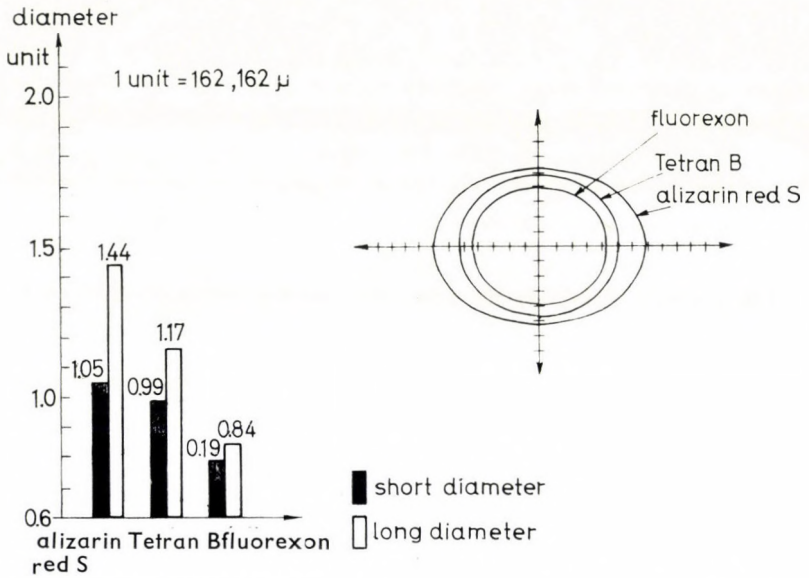

Fig. 10. Columnar and circular diagrams illustrating fluorescence in the Haversian system after the combined administration of alizarin, tetracycline and fluorexon. The inner ring is narrower than in Fig. 9

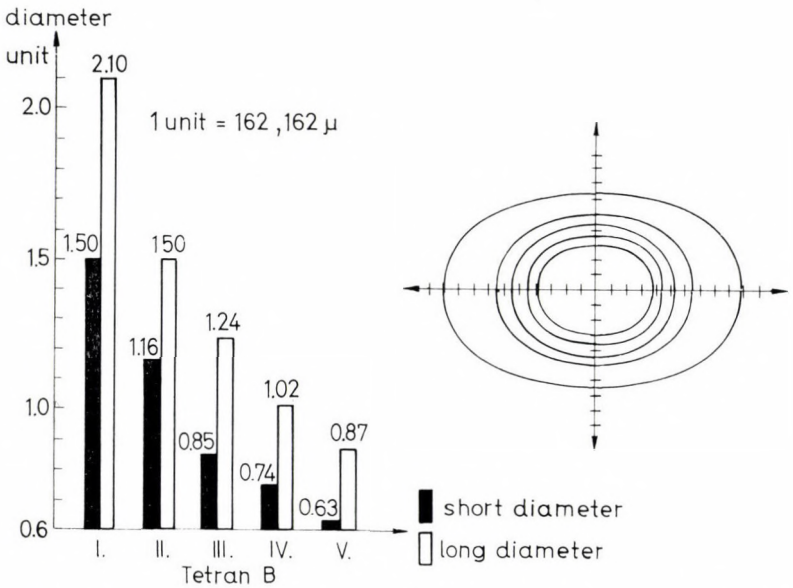

Fig. 11. Columnar and circular diagrams illustrating fluorescence in the Haversian system of animal treated with a total of five tetracycline doses administered at weekly intervals. Distances between the inner rings are narrower, and their diameters are shorter

Discussion

The observations have shown that the combined fluorescence obtained with alizarin red S, tetracycline and fluorexon is particularly useful for a prolonged observation of ossification dynamics, bone reorganization and mineralization. It is presumably by one and the same mechanism that these fluorochromes are bound to freshly calcifying tissue components. URIST and MCLEAN [69] postulate three possibilities of binding in this respect:

a) collagen-polysaccharide-apatite-fluorochrome;

b) collagen-fluorochrome-polysaccharide-apatite;

c) collagen-polysaccharide-fluorochrome-apatite.

No matter to which component fluorochromes are attached in the freshly mineralized tissue, their presence requires in any case that of the other cardinal constituents as well.

Our method of employing three different fluorochromes represents a perfection of the combined treatment employed by SUZUKI and MATHEWS [65]. Our procedure has the following advantages: better visual distinction (three essentially different colours); consequent facilitation of quantitative measurements; unequivocal demonstration of otherwise hardly interpretable trends of ossification. This last one was clearly displayed by the demonstration of the development of the Haversian lamellae and the periosteal apposition. The combined method might be used with advantage in experiments where the process of mineralization is morphologically not as simple as in the case of lamellar bone.

Experiment IV has shown that a prolonged tetracycline administration inhibits calcification. Although such inhibition has been observed with alizarin red S as well, it is evident from the results that, if the in vivo indicators are alternated, their inhibitory actions do not sum up as effectively as those of repeated doses of tetracycline.

Micrometric measurement of the rings offers simple and reliable possibility of statistical evaluation, of following the dynamics of the growth of the Haversian system and its changes. PALKOVITS's method for the computation of limits of error [59], elaborated for the measurement of thyroid acinus diameters, is well adaptable for our purposes. A minimum of 500 Haversian systems have to be measured in order not to exceed a 10% limit of error.

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FLUORESZENZ-HISTOCHEMISCHE UNTERSUCHUNG VON STÜTZGEWEBE

II. Untersuchungsmöglichkeit der Mineralisationsdanynamik des lamellösen Knochengewebes mittels Fluorochrom-Verbindungen

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Verfasser beschreiben das von ihnen für die Untersuchung der Dynamik des Wachstums, der Umbildung sowie der Mineralisationsprozesse von Knochen erarbeitete Markierungsverfahren. Die Vorteile des kombinierten Alizarin Red S–Tetracyclin–Fluorexon in vivo Fluoreszenzverfahrens sind: leicht und zuverlässig bewertbare und meßbare Ergebnisse, geringere Kalzifikation-hemmende Wirkung und Eignung für die Aufklärung der Richtung der obigen biologischen Prozesse. Für die statistische Auswertung empfehlen die Verfasser das Messen des Diameters der Havers-Lamellen.

ФЛЮОРЕСЦЕНТНО-ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ СОЕДИНИТЕЛЬНОЙ ТКАНИ

II. Возможности изучения динамизма минерализации пластинчатой костной ткани при помощи соединений флюорохрома

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Авторы сообщают разработанную ими методику для изучения динамизма процессов роста, перестраивания и минерализации костей. Преимущества комбинированного флюоресцентного метода исследования с помощью ализарин-красный-S-тетрациклин-флюорексона in vivo следующие: получаются легко и надежно расцениваемые данные, его торможающее действие на кальцификацию меньше, и он пригоден для выявления направления вышеуказанных биологических процессов. Для статистической оценки авторы рекомендуют измерение диаметра Гаверсовых пластинок.

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EFFECT OF EXPERIMENTAL LYMPH CONGESTION ON CORONARY ARTERY PERMEABILITY IN THE DOG

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Disturbances of permeability accompanying coronary changes induced by experimental lymph congestion have been studied. Intravenously administered colloidal iron, used as indicator, was found to have passed into the vessel walls, a sign of increased permeability. A theory is advanced for the explanation of this phenomenon and the mechanism through which the observed vascular changes occur.

Myocardial lesions induced by insufficiency of lymph circulation have been known since the investigations of FÖLDI et al. [4] and RUSZNYÁK et al. [16]. Earlier experiments of our team [17] showed that in addition to myocardial necrosis, lymph congestion induces also coronary changes consisting in vasoconstrictive subendothelial plasmatic inhibition (plasmatic vasculosis) and a necrosis of the smooth muscles of the media. Disturbances affecting the permeability of the vessel walls are a decisive factor in the development of vascular disorders [1, 6, 7, 8, 10, 12], and it was in this connection that the question arose as to whether changes in permeability are involved in the pathomechanism of coronary lesions induced by lymph congestion.

Material and method

Eleven dogs of both sexes with body weights of 15 to 25 kg were used, two of which served as controls. Mechanical lymph congestion was induced by the slightly modified [17] method of FÖLDI et al. [4]. Under hexobarbital and ether anaesthesia the chest was opened in the fourth intercostal space; the regional lymphatics and lymph nodes were made visible by ingesting Evans blue. These organs were ligated and the thoracic duct before its entry into the subclavian vein. On the third postoperative day, all animals were given 3 doses of 10 ml of Ferrlecit* at 30 min. intervals, and killed three hours after the last dose by an overdose of hexobarbital. From the heart several specimens were excised, fixed in formalin, embedded in paraffin and stained by PAS and Prussian blue, haematoxylin-eosin, azan and Mallory's stain.

Results

Light-microscopic inspection following the administration of Ferrlecit revealed no iron in the coronary wall of the controls, whereas the Prussian blue reaction was positive in all the vessels in which lymph congestion had resulted

* Organic-complex compound of Fe(III).

in the subendothelial accumulation of plasma displaying PAS-positivity (Fig. 1) and staining red with azan. These areas were well visible not only subendothelially but appeared at certain points to extend into the media (Figs 2, 3).

Discussion

Few authors have been concerned with the normal lymphatic drainage of the arterial wall and the effect produced on the arterial wall by lymphatic insufficiency. Neither YOFFEY and COURTICE [18] nor RUSZNYÁK et al. [16] have touched upon this subject in their monographs. RÉNYI-VÁMOS [15] introduced Indian ink to the boundary between the media and adventitia of the dog aorta, and observed its passage to the regional lymph nodes. PAPP and JELLINEK [14] demonstrated the lymphatic drainage of the wall of veins. However, in addition to lymphatic drainage the permeability of vessel walls is determined also by the pinocytotic activity of the endothelial cells, the condition of intercellular junctions [2, 11] and the filtering effect of the ground substance which regulates inflow [3, 9, 13].

The disturbance of vascular permeability is especially conspicuous in the early phase of damage induced by mechanical lymph congestion in the minor coronary branches. The subendothelial accumulation of plasma substances is greatly due to a pathological increase of permeability as proved by the inversion of Ferrlecit into the vessel walls. It was shown earlier [8, 12] that this colloidal iron preparation is bound by certain plasma fractions, and its appearance in the vessel walls points to a pathological increase of permeability.

Table 1

Lymph congestion →	disturbance of transport of the coronary wall
	↓
	pH changes, shift of the ionic milieu
	↓
	physico-chemical changes of the ground substance
	↓
	disturbance of the permeability
	↓
	→ intima oedema

As regards now the mechanism through which permeability is increased by lymph congestion, we suggest the following theory concerning the subendothelial plasma accumulation and the enhanced influx (Table 1). The lymph congestion interferes with the transporting activity of the arterial wall and thus with the removal of metabolites arising in the course of the cells' normal metabolism. Their gradual accumulation induces pH changes and a shift of the ionic milieu. This phenomenon presumably results in physico-chemical

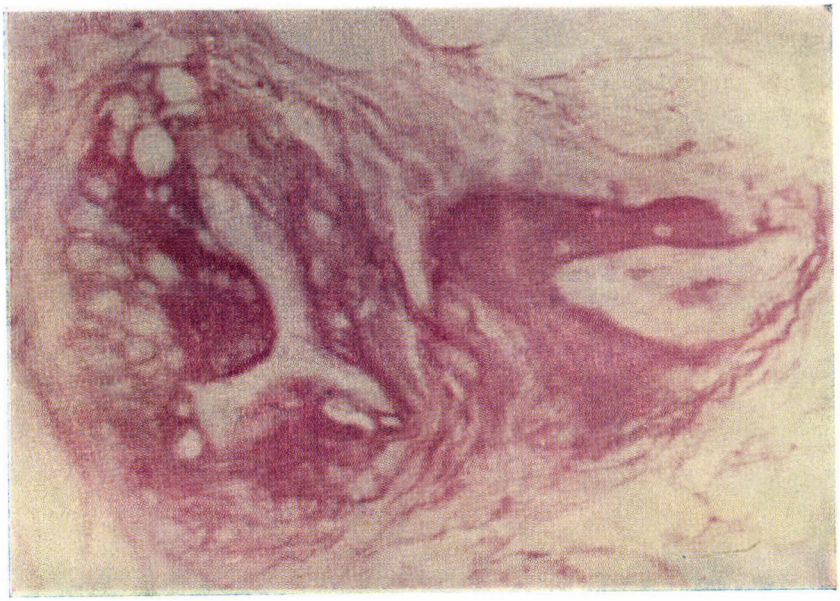

Fig. 1. Extensive vasoconstrictive subintimal plasmatic vasculosis involving the media of a minor coronary branch after three days mechanical lymph congestion. PAS. ×800

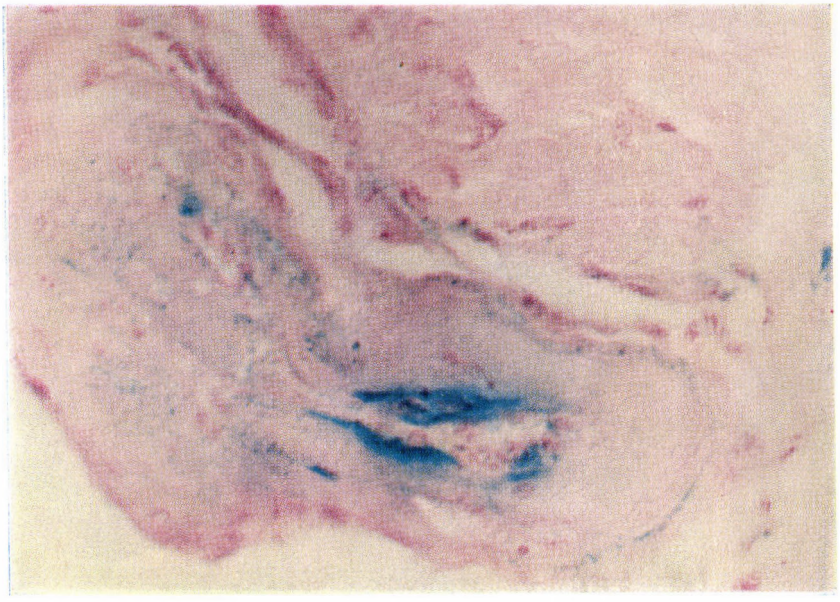

Fig. 2. Continued section of the coronary branch shown in Fig. 1. Corresponding to the PAS-positive areas, iron-positive areas subintimally to a slighter extent, also in the media. Prussian-blue. ×800

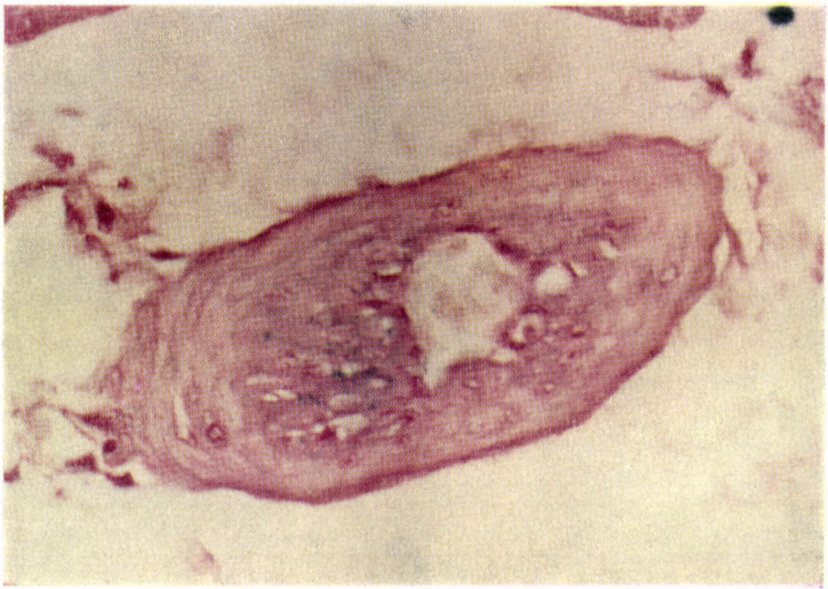

Fig. 3

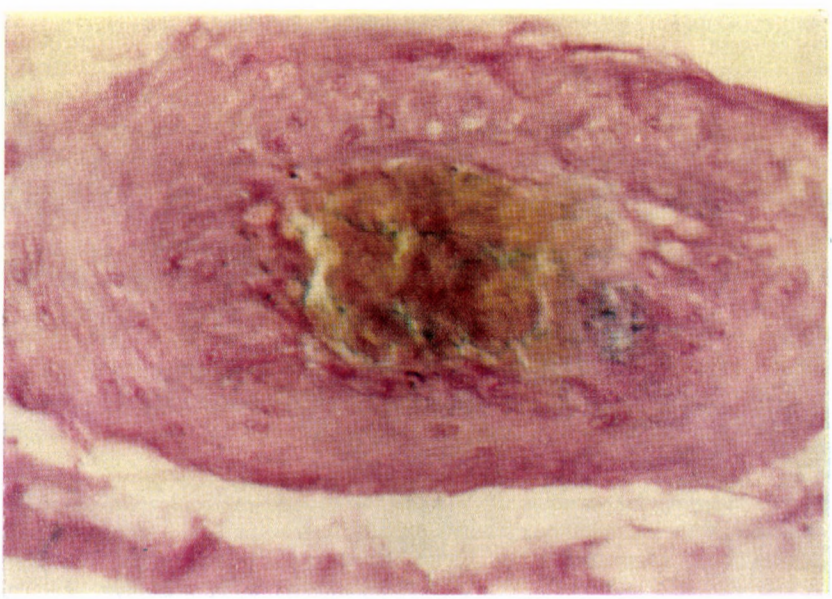

Fig. 4

Figs 3 and *4*. Prussian-blue iron positive-granules partly subintimally and partly in the media of minor coronary branches after three days mechanical lymph congestion. Prussian-blue. ×600 and ×800

changes of the mucopolysaccharides of the endothelial basal membrane which tend to increase the permeability of the vessel wall.

The inadequate transportation of substances passing through the vessel wall leads, thus, shortly to a pathologic change of permeability so that both factors should be regarded as responsible for the observed vascular disorders.

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DIE WIRKUNG DER EXPERIMENTELLEN LYMPHSTAUUNG AUF DIE PERMEABILITÄT DER ARTERIA CORONARIA IM HUND

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Verfasser untersuchten die sich an die unter experimentell herbeigeführter Lymphstauung entstehende Koronarveränderung anschließende Permeabilitätsstörung. Entsprechend der geschädigten Koronarwand konnte das Eindringen des intravenös als Markiersubstanz eingeführten kolloidalen Eisens beobachtet werden, was als Zeichen der gesteigerten Permeabilität gewertet wird. Schließlich besprechen die Verfasser eine mögliche Erklärung für den im Verlauf der nach Lymphstauung entstehenden Gefäßveränderungen beobachteten Permeabilitätsanstieg bzw. den Pathomechanismus der Gefäßveränderungen.

ДЕЙСТВИЕ ЭКСПЕРИМЕНТАЛЬНОГО ЗАСТОЯ ЛИМФЫ НА ПРОНИЦАЕМОСТЬ ВЕНЕЧНОЙ АРТЕРИИ У СОБАКИ

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Авторы изучали расстройство проницаемости, сопровождающее изменение венечной артерии, возникающее под влиянием экспериментально вызванного застоя лимфы. Соответственно поражению стенки коронарной артерии наблюдалось проникновение внутривенно введенного коллоидального железа (мечущего-вещества) в стенку сосуда. Это наблюдение расценивается как знак повышения проницаемости. В заключение сообщается возможное объяснение для повышения проницаемости, развивающегося под влиянием изменений сосудов при застое лимфы и патомеханизма изменений сосуда.

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CORNEAL VASCULARIZATION

ROLE OF LACTIC ACID

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The intracorneal injection of L-lactate induced more marked vascularization of the cornea than the injection of D-lactate which does not occur in the organism. This confirms the fact that endogenous lactic acid plays a specific role in vascularization. The vascularizing effect of racemic lactic acid is considerably stronger than that of racemic lactate. Considering that the pH value is indifferent in this respect, it is probable that the vessel-producing cells are stimulated by the non-dissociated lactic-acid molecules.

The racemic form of lactic acid, if injected intravitreally or intracorneally into animals, induces significantly earlier and more pronounced vascularization than other substances do [4, 5]. Although experimentally induced avascular corneal swelling and human bullous keratopathy are accompanied by swelling, an important condition of corneal vascularization [1, 2], they do nor give rise to a vascularization of the cornea, and its lactic acid concentration is significantly below the normal level [6]. This concentration is significantly elevated for several weeks after alkali burnt corneal injury [3], and corneal vascularization is known to be especially severe in such cases.

Although lactic acid constitutes an important factor in the formation of new vessels, which is evident from these experimental and clinical observations, its specific effect requires further elucidation. This purpose is served by the present study in which the vascularizing action of L-lactic acid, contained in the organism, is compared with that of D-lactic acid which does not occur in the organism.

Material and method

A total of 16 adult rabbits with an average body weight of 2.5 kg was used. The technique of intracorneal injections was described earlier [5].

In the first series of experiments, the substances injected were of 0.1% solution of D-lactic acid lithium salt (Calbiochem) and L-lactic acid lithium salt (Calbiochem), while in the second series an 0.1% racemic solution of D- and L-lactic acid lithium salt and DL-lactic acid (Schidamsche) were intracorneally administered.

The length of the corneal vessels was measured daily under high power, and the difference of the two sides was examined for statistical significance by Student's t test.

Table I

No. of experiment	No. of animals	Side	Intracorneal injections (0.1%, 0.05 ml)			Days of incipient vascularization
			Compound	pH	No.	
1.	11	r.	D-lactate	4.5	3	4—6
		l.	L-lactate	4.5	3	3—6
2.	5	r.	DL-lactic acid	3.0	3	2—3
		l.	DL-lactate	4.5	3	4

* = $p < 0.01$, ** = $p < 0.02$

Results

Detailed results are shown in Table 1.

First series. The intracorneal injection of L-lactate induced earlier and more pronounced vascularization than that of D-lactate. Corneas treated with L-lactate exhibited longer vessels even on the fourth and the sixth day. The difference was significant ($p < 0.02$) on the fourth day. Vascularization began to disappear on both sides after the sixth day.

Second series. The intracorneal injection of racemic lactic acid produced significantly more vigorous vascularization than that of racemic lactate ($p < 0.01$ on the 4th, 8th and 10th days, $p < 0.02$ on the 6th day). The vascularizing action of racemic lactate was even inferior to that of L-lactate.

Discussion

The fact that the vascularizing potency of L-lactate is significantly higher than that of D-lactate shows that lactic acid produced by the organism plays a specific role in vascularization. The minimum vascularizing effect of D-lactate must be due to the corneal swelling caused by the injection, as well as to the effect of the physiologically high lactic acid concentration of the cornea, brought about in this way [7, 8]. The concentration of L-lactate is low in racemic lactate, the reason why its vascularizing capacity must be inferior to that of L-lactate.

In the second series of experiments, the vascularizing effect of racemic lactic acid proved to be significantly stronger than that of racemic lactate, a phenomenon that cannot be explained with the different pH-values of the injected solutions, since earlier experiments showed this factor to play no role in vascularization [4, 5], where lactic acid of pH 3.0 had the strongest vascularizing effect, while that of acetic acid of pH 3.5 and hydrochloric acid of pH 1.5 was considerably weaker in this respect. Although the organism's well buffered media contain lactate in the first place, it would appear from the present results that the vessel producing cells are stimulated by the non-dissociated

No. of experiment	No. of animals	Side	Distance (mm) between vascularized area and limbus (mean value ± standard deviation)			
			4th day	6th day	8th day	10th day
1.	11	r.	0.05±0.11	0.23±0.40	—	—
		l.	0.14±0.15**	0.47±0.38	—	—
2.	5	r.	0.74±0.16	1.46±0.31	1.98±0.35	2.76±0.43
		l.	0.18±0.29*	0.24±0.53**	0.22±0.41*	0*

lactic-acid molecules. Elevated concentration or damages of the physiological buffer system may produce this phenomenon under non-experimental conditions.

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ÜBER DIE ROLLE IN DER HORNHAUTVASKULARISATION

GY. IMRE

Die intrakorneale Injektion von L-Lactat bringt eine intensivere Vaskularisation der Kaninchenhornhaut zustande, als die intrakorneale Injektion des im Organismus nicht vorkommenden D-Lactats. Diese Beobachtung spricht für die spezifische Bedeutung der im Organismus entstehenden Milchsäure für die Vaskularisation. Die Wirkung der razemischen Milchsäure übertrifft erheblich den Vaskularisationseffekt des razemischen Lactats, was, angesichts dessen, daß die Rolle des pH-Wertes ausgeschlossen werden kann, wahrscheinlich macht, daß die nichtdissoziierten Milchsäuremoleküle die gefäßbildenden Zellen stimulieren.

О РОЛИ МОЛОЧНОЙ КИСЛОТЫ В ВАСКУЛЯРИЗАЦИИ РОГОВИЦЫ

Д. ИМРЕ

Интракорнеальное введение L-лактата вызывает достоверно более выраженную васкуляризацию роговицы кроликов, чем интракорнеальные инъекции D-лактата, не имеющегося в организме. Это наблюдение указывает на специфическое значение молочной кислоты, образуемой в организме, в васкуляризации. Рацемическая форма молочной кислоты в значительной мере превосходит действие рацемического лактата на васкуляризацию. Ввиду того, что роль величины pH можно исключить, это наблюдение позволяет делать вывод, что недиссоциированные молекулы молочной кислоты стимулируют сосудообразующие клетки.

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LYMPHADENOPATHIE, VERURSACHT DURCH ARZNEIMITTEL

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Anhand von 10 Fällen werden die histologischen Kennzeichen und die wichtigsten klinischen Daten der durch Arzneimittel verursachten Lymphadenopathie beschrieben. Die Veränderung ist reversibel und bedarf keiner Behandlung; nach Entzug des Medikamentes erfolgt Spontanheilung.

In allen Fällen sind der Nachweis allergischer Komplikationen anläßlich einer medikamentösen Provokation und der Ausfall der Antikörperprobe von diagnostischer Bedeutung.

Histologisch charakteristisch sind eine Mikroangiopathie, die sich nebst einem tumorartigen Bild in allergisch-entzündlicher Form manifestiert, eine Vasculitis mit hämorrhagischer Nekrose und Thrombose. Fibrose und Sternberg-Reed-Zellen sind nicht vorhanden. Gegenüber der kortiko-medullären Veränderung ist die Struktur der Hilusregion erhalten.

Zur richtigen Diagnosestellung ist die Kenntnis der klinischen Daten unerläßlich.

Bei arzneimittelüberempfindlichen Patienten kann sich im Zusammenhang mit der Behandlung eine Lymphknotenschwellung — "drug-induced lymphadenopathy" — (im weiteren DLP) ausbilden, die sich aufgrund der klinischen Symptome von anderen reaktiven Lymphknotenveränderungen unterscheidet, so daß das einem malignen Lymphom ähnliche histologische Bild Anlaß zum Irrtum gibt.

Die Frage liegt nahe, inwiefern man die DLP als präneoplastischen Zustand betrachten kann, und inwiefern eine direkte Verbindung zwischen der tumorartigen DLP und den malignen Lymphknotenveränderungen anzunehmen ist. Die Frage wurde in dieser Form zuerst von SALTZSTEIN an einer Klinikopathologischen Konferenz 1962 angeworfen, dann berichteten 1966 HYMAN und SOMMERS über einen Morbus Hodgkin bzw. ein Lymphosarkom, das im Laufe einer antikonvulsiven Behandlung aufgetreten war. Sie nahmen einen Zusammenhang zwischen der antiepileptischen Behandlung und den malignen Lymphknotengeschwülsten an, wobei sie einen direkten blastomogenen Effekt der Hydantoin-Abkömmlinge vermuteten. Die Frage wird sicherlich zu ausgedehnteren Untersuchungen Anlaß geben und zwar vor allem im Hinblick auf die hauptsächlich im Kindesalter verbreitete Anwendung antikonvulsiver Mittel.

Die Mehrzahl der Mitteilungen beschäftigt sich mit der DLP als differentialdiagnostisches Problem (CHIARI 1951, BODART 1953, SALTZSTEIN und Mit-

arb. 1958, SALTZSTEIN und ACKERMAN 1959, ROSENFELD und Mitarb. 1961, DOYLE und HELLSTROM 1963, DÉVÉNYI 1964, SCHWARTZ 1966, KRASZNAI und SZEGEDI 1967, KRASZNAI und GYŐRY 1968). JUHÁSZ und Mitarb (1968). konnten nach Diphenylhydantoin-Behandlung bei Mäusen Lymphoreticulosarkom und Leukämie beobachten. Demnach ist die Erkennung der DLP, die Feststellung der richtigen Diagnose nicht leicht, weder am Krankenbett noch bei der histologischen Untersuchung. Aufgrund unserer eigenen Erfahrungen ist die DLP eine reversible Veränderung, die keiner Behandlung bedarf. Dem Entzug des Arzneimittels folgt eine Spontanremission, die man mit immunosuppressiven Mitteln beschleunigen kann. Demgegenüber bedeutet für den Kranken eine falsche Diagnose eine nicht gleichgültige cytostatische Behandlung.

In den letzten 7 Jahren haben wir 10 DLP-Fälle beobachtet. Durch die Mitteilung der histologischen Befunde und der klinischen Daten möchten wir zur Lösung der diesbezüglichen differentialdiagnostischen Probleme beitragen.

Klinische Daten. In 7 Fällen hat sich nach Behandlung mit Hydantoin-Abkömmlingen (Mephenytoin, 3-Methyl-5-phenyl-5-aethyl-hydantoin), in 2 Fällen nach Sulfanylamiden (Sulfathiourea, Sulfadimidin, Sulfamethoxypyridazin, Sulfadimethylpyrimidin, p-Aminobenzolsulfonthiocarbamid) und in einem Fall nach Phenylbutazon eine Lymphknotenschwellung ausgebildet (s. Tabelle 1). Nach ein- bis zweiwöchiger Latenzzeit entwickelt sich eine im allgemeinen symmetrische zervikale, axillare oder generalisierte Lymphknotenschwellung, meist mit schmerzlosen, beweglichen bohnen- bis haselnußgroßen Lymphknoten. Der eigentlichen DLP gehen die mit dem allergischen Immunstatus verbundenen Symptome voran, wie hohes Fieber, hohe BSG, Dermatitis, Leukozytose, Eosinophilie und abhängig vom Medikament spezifische Komplikationen. Bei Hydantoin-Abkömmlingen sieht man Panzytopenie, Blutungsbereitschaft, und eine positive Rumpel-Leede-Probe; bei Sulfonamiden dagegen Thrombopenie, und Photosensibilisierung, und bei Phenylbutazon Stomatitis und Vulvitis.

Die Hautveränderungen sind durch makulo-papulöse (Erythema multiforme-artige), skarlatiniforme oder morbilliforme Ausschläge gekennzeichnet. Zwei unserer Fälle wurden mit der Diagnose Scharlach (Fall Nr. 6) bzw. Masern (Fall Nr. 4) in die Infektionsabteilung eingewiesen.

In drei der Fälle bildeten sich die Symptome nach mehrmonatiger, in 7 der Fälle nach kurzer, 2—3wöchiger Behandlung aus. Nach Entzug der Arzneimittel wurden die Patienten rasch fieberfrei, und die Hautveränderungen heilten. Im allgemeinen folgte der schnellen Besserung der allgemeinen Symptome eine Spontanremission der Lymphknotenschwellungen.

Bei der Mehrheit unserer Fälle war die Lymphadenopathie das vorherrschende klinische Symptom, der Kliniker dachte an einen malignen Prozess, und eine Biopsie wurde vorgenommen. Wegen des Mangels an entsprechender

Tabelle 1

Fall Nr.	Alter, Geschl.	Medikament	Lymph-adenopathie	Histologisches Bild	Symptome	Beobachtungszeit	Verlauf
1.	13 J. weibl.	Mephenytoin 3 Jahre	Generalisiert	Foll.-medull. Retikulose	Dermatitis, Fieber	3 Jahre	Beschwerdefrei
2.	8 J. männl.	Mephenytoin 6 Monate	Zervikal Axillar	Foll. Lympho-blastomartig	Hepato-Splenomegalie, Fieber	3 1/2 Jahre	Beschwerdefrei
3.	35 J. weibl.	Mephenytoin 3 Wochen	Generalisiert	Herdförmige Retikulose (H)*	Dermatitis, Panzytopenie	1 Monat	Tumor cerebri op. Haematocephalus int. Exitus
4.	21 J. männl.	Mephenytoin 3 Wochen	Zervikal Axillar	Herdförmige Retikulose (H)*	Dermatitis, Antikörper-Probe positiv	4 1/2 Jahre	Exazerbation nach Suizidversuch mit Mephenytoin, Spontanremission
5.	35 J. weibl.	Mephenytoin 3 Wochen	Generalisiert	Retikulosarkomartig	Dermatitis, Fieber	2 1/2 Jahre	Beschwerdefrei
6.	24 J. männl.	Mephenytoin 2 Wochen	Generalisiert	Herdförmige Retikulose (H)*	Dermatitis, Fieber	2 Jahre	Beschwerdefrei
7.	16 J. männl.	Mephenytoin 3 Monate	Generalisiert	Herdförmige Retikulose (H)*	Dermatitis	4 Jahre	Beschwerdefrei
8.	67 J. männl.	Sulfonamid 2 Wochen	Generalisiert	Retikulosarkomartig	Thrombozytopenie, Fieber	8 Jahre	Beschwerdefrei
9.	34 J. weibl.	Phenylbutazon 2 Wochen	Zervikal Axillar	Retikulosarkomartig	Stomatitis, Dermatitis, Vulvitis	3 1/2 Jahre	Beschwerdefrei
10.	60 J. weibl.	Sulfonamid 3 Wochen	Zervikal Axillar	Retikulosarkomartig	Dermatitis, Fieber, Photosensibilisation	2 Jahre	Provokation nach wiederholter Selbstmedikation mit Sulfonamid

* Morbus Hodgkin-ähnliches histologisches Bild. Im Sinne der Aufteilung von LUKES [10].

Zusammenarbeit ist es nicht selten, daß der Pathologe nur von der Lymphknotenschwellung weiß und die zur Diagnose führenden anamnestischen Angaben nicht kennt. Ein gutes Beispiel dafür stellt der Fall eines unserer männlichen Patienten dar (Fall Nr. 4). Nach dreiwöchiger antiepileptischen Behandlung nahm ihn die Infektionsabteilung mit Masern-Verdacht auf, in der Annahme eines Morbus Hodgkin wurde dann die Biopsie vorgenommen. Von der mit Hautausschlägen einhergehenden akuten fieberhaften Erkrankung wußte der Pathologe nichts. Histologisch wurde die Veränderung als Morbus Hodgkin gedeutet, worauf der Patient Cytostatica erhielt. Vier Monate später unternahm er mit 40 Tabletten Mephenytoin einen Suicidversuch, wonach sich eine ausgeprägte Lymphknotenschwellung ausbildete. Die Antikörperprobe mit Mephenytoin fiel positiv aus. Im Grunde genommen hat diese Arzneimittelprovokation geholfen, die richtige Diagnose zu stellen. Zur Zeit, drei Jahre später, ist Patient beschwerdefrei, es bestehen keine auf Tumor hinweisende Veränderungen.

Eine unserer Kranken (Fall Nr. 3) starb an hämatologischen Komplikationen. Wegen eines Hirntumors (Astrocytoma diffusum) wurde sie operiert, nach der Operation begann man eine präventive antikonvulsive Behandlung. Nach drei Wochen bildeten sich eine schwere Knochenmarksschädigung — Hydantoin-Pancytopenie — und Symptome intrakranieller Drucksteigerung aus. Todesursache war Haematocephalus internus.

Neun Patienten sind nach einer durchschnittlich 3.5jährigen Beobachtungszeit beschwerdefrei, Lymphknoten sind nicht tastbar.

Histologie

Die histologischen Veränderungen sind im wesentlichen in zwei Gruppen einteilbar: 1. allergisch-entzündliche Erscheinungen, mit Blutung, Nekrose, Gefäßveränderungen; 2. tumorartige Veränderungen.

Mit Ausnahme von zwei Fällen (Fall Nr. 1 und 2) fanden wir stets entzündlich-nekrotisierende Veränderungen. Die Lymphknotenkapsel, das periglanduläre Binde- und Fettgewebe zeigen entzündliche Zellinfiltrationen in wechselndem Ausmaße. Bei der durch Hydantoin und durch Sulfonamide verursachten Lymphadenopathie bildete sich eine mäßige, nach Phenylbutazon-Behandlung eine ausgeprägte allergisch-granulomatöse Entzündung mit zentraler hämorrhagischer Nekrose sowie mit leukozytärer, epitheloidzelliger Reaktion aus. Die Veränderungen ähneln den Läsionen der allergischen Granulomatose oder der Wegenerschen Granulomatose. Bei der durch Arzneimittel verursachten Lymphadenopathie hingegen ist immer eine hämorrhagische Nekrose (Abb. 1, 2) zu beobachten. Subkapsulär war die Rinde blutreich, das Lumen einiger erweiterten Kapillaren wurde durch einen frischen Thrombus

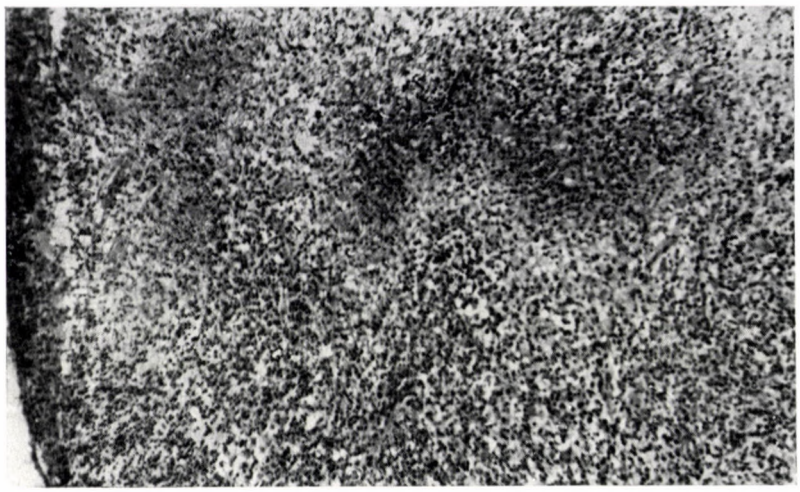

Abb. 1. Fall Nr. 4. Allergisch-granulomatöse Entzündung in der Rindensubstanz eines Lymphknotens (am linken Rande des Bildes). Die Lymphdrüsenstruktur ist nicht erkennbar. H.E. $\times 100$

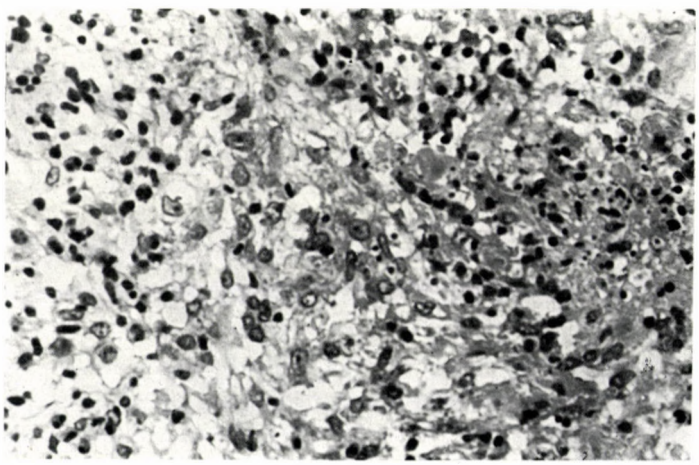

Abb. 2. Fall Nr. 6. Hämorrhagisch-nekrotischer Bezirk auf der rechten Seite des Bildes. Das nekrotische Zentrum enthält Kerntrümmer, wenig Leukozyten. Um die Nekrose eine aus Histiozyten und Fibroblasten bestehende Reaktion. H.E. $\times 200$

verschlossen (Abb. 3, 4). Um die oben genannten Gefäße bildeten sich in kleinen Herden hämorrhagische Nekrosen aus, mit gemischtzelliger Reaktion am Rande der Nekrose, vielen eosinophilen Leukozyten, Plasmazellen, Epitheloidzellen und wenigeren segmentkernigen Leukozyten. Im Zentrum der Nekrose waren Blutungen und Kerntrümmer zu finden, ohne Neigung zu eiterzelliger Einschmelzung.

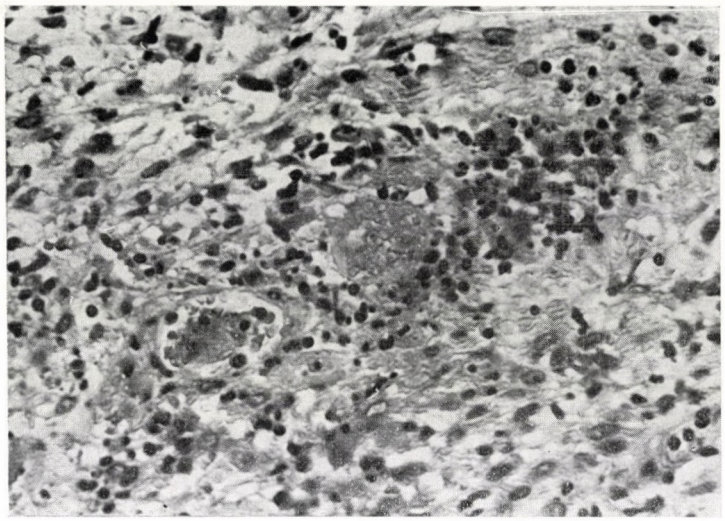

Abb. 3. Fall Nr. 9. Thrombotische Mikroangiopathie mit Gefäßwandnekrose und gemischter perivasaler Zellreaktion. H.E. ×250

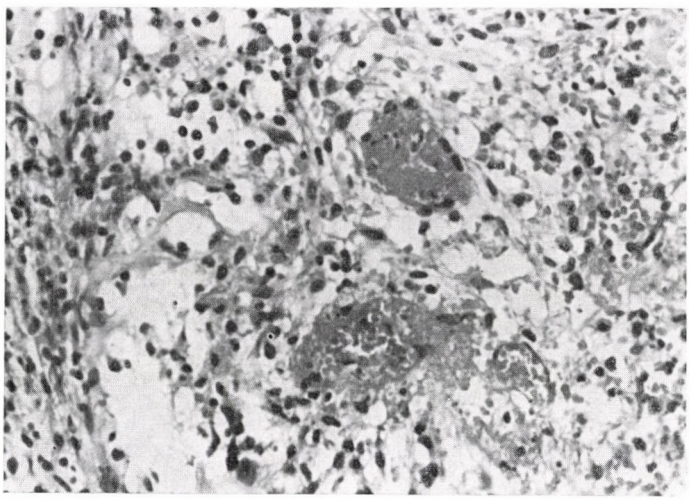

Abb. 4. Fall Nr. 7. Thrombotische thrombozytopenische Purpura ähnliche Veränderung. Um die Gefäße eine aus Fibroblasten, Histiozyten, Erythrozyten bestehende Reaktion. H.E. ×200

In der Rinden-Marksubstanz kann, abhängig von der jeweiligen Arzneimittelüberempfindlichkeit, neben der erhaltenen Struktur eine follikulär-medulläre retikuloseartige Veränderung (Fall Nr. 2) oder ein mit schwerer Strukturschädigung einhergehendes, einem Retikulosarkom ähnliches Bild erscheinen (Abb. 5, 6). Die follikulär-medulläre Retikulose war dabei die mildeste

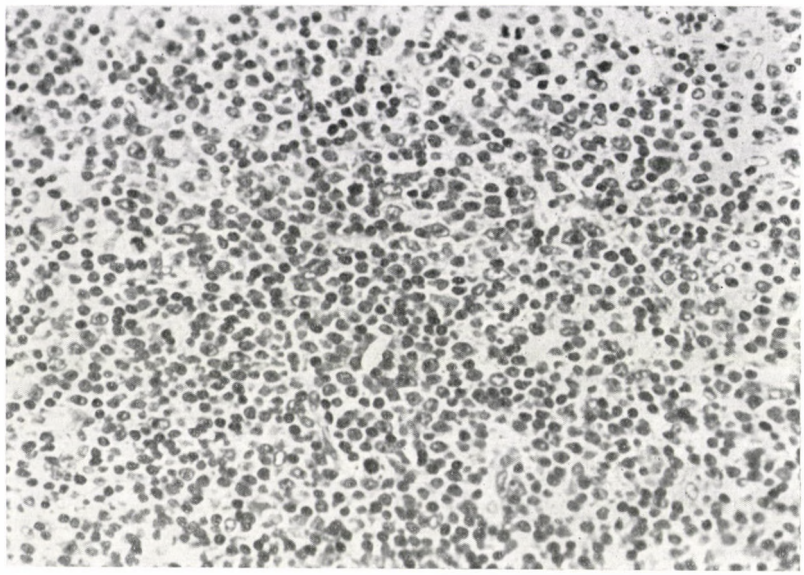

Abb. 5. Fall Nr. 10. Aus gleichförmigen Zellen bestehende zellreiche, tumorartige Veränderung. Die Lymphdrüsenstruktur ist nicht erkennbar. H.E. ×200

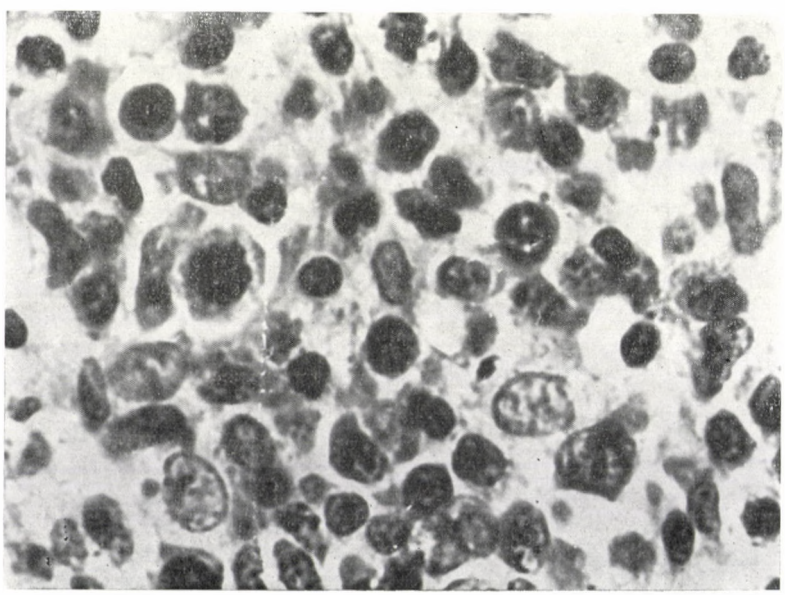

Abb. 6. Fall Nr. 7. Tumorartige, pleomorphe Retikulumzell-Proliferation mit 2 Mitosen im linken oberen Quadranten des Bildes. Zwischen den Retikulumzellen sind einige Plasmazellen und Lymphozyten sichtbar. H.E. ×450

Veränderung. Die Follikel sind in solchen Fällen groß, ihre Grenzen verschwommen, die Markstränge breit, zellreich. Sie enthalten neben zahlreichen Retikulumzellen viele Plasmazellen und Mitosen. Die Sinus sind erweitert, die Lymphknotenstruktur ist noch überall deutlich. In Fällen tumorartiger Veränderungen ist die Lymphknotenstruktur nicht erkennbar. Man sieht keine Follikel; in großen, zusammenfließenden, zellreichen Herden sind atypische Retikulumzell-Proliferate sichtbar.

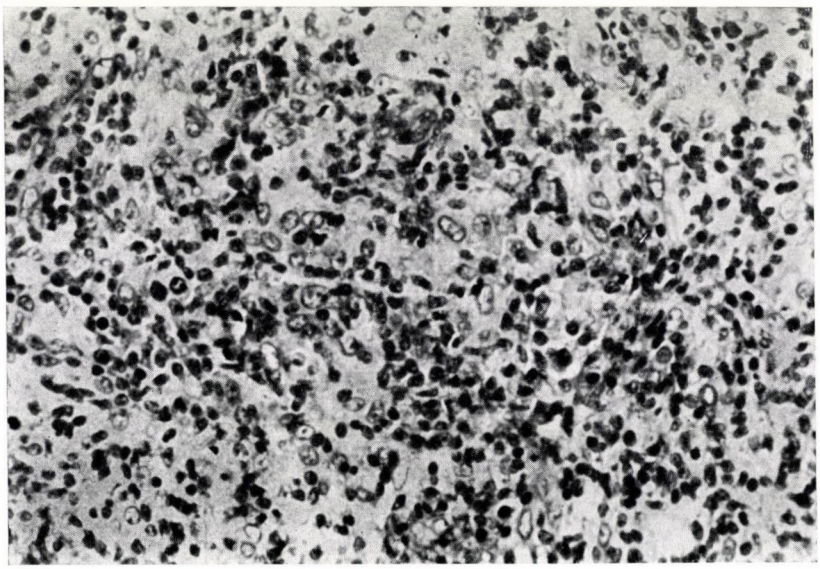

Abb. 7. Fall Nr. 6. Morbus Hodgkin ähnliches histologisches Bild. Die Reaktion enthält Retikulumzellen, Fibroblasten, Plasmazellen und Lymphozyten. H.E. ×250

In anderen Fällen bestanden die tumorartigen Gebiete aus gleichförmigen Zellen mit zahlreichen Mitosen (Abb. 5, 6). Ein andermal fanden sich unter den Retikulumzellen viele Eosinophilen, so daß das Bild einer Lymphogranulomatose ähnlich war (Abb. 7). Die Nekrose hingegen war hämorrhagischen Charakters, Fibrose und typische Sternberg-Reed-Zellen waren nicht zu sehen.

Dem tumorverdächtigen kortico-medullären Prozeß gegenüber war in der Hilusregion die Struktur im wesentlichen erhalten. Hier ließen sich Sinus- und Faserstruktur gut erkennen, die Markstränge waren zellreich und enthielten viele Plasmazellen.

Diskussion

Bei unseren Fällen war die Lymphadenopathie das prominente Zeichen der Arzneimittel-Allergie. Die Medikamente bzw. deren einzelne Komponenten

spielen als Allergene oder als Haptene eine Rolle. Die Symptome sind Indizien eines allergischen Immunstatus.

Klinisch-diagnostisch wertvoll ist der positive Ausfall der Antikörperprobe gegenüber dem Medikament und der Hautprobe. Negative Proben hingegen sind kein Beweis gegen das Vorliegen einer Arzneimittelallergie. Die medikamentöse Provokation könnte mit überzeugenden Beweisen dienen, aber im Interesse der Kranken haben wir davon abgesehen. In zwei Fällen, bei wiederholter Einnahme des Pharmakons ohne ärztlichen Rat, erschienen die allergischen Symptome erneut (Fälle 4 und 10).

Für die richtige Diagnosestellung leisten die speziellen Arzneimittelallergiesymptome Hilfe, wobei sie sich zu den allgemeinen allergischen Symptomen gesellen. Von diesen sind die Dermatitis und die hämatologischen Zeichen bedeutungsvoll. Mit anfänglicher Leukozytose und Eosinophilie wechseln Leukopenie, Thrombopenie, schließlich Panzytopenie ab. Maligne Geschwulsterkrankungen der Lymphknoten grenzen sich durch die entsprechenden Knochenmark- und Blutbildbefunde ab.

Für die Erkennung der DLP sind die histologischen Zeichen der Allergie wichtig. In der hyperämischen subkapsulären Rindensubstanz können allergische Vasculitis, thrombotische Mikroangiopathie, und um die Gefäße herum frische hämorrhagische Nekrosen, in manchen Fällen allergisch-granulomatöse Perilymphadenitis wahrgenommen werden. Trotzdem standen in allen Fällen die tumorartigen Veränderungen im Vordergrund. Die entzündliche nekrotische Veränderung liegt dabei nur in kleinen Herden vor, und wir fanden sie, mit Ausnahme von nur 2 Fällen, bei der Aufarbeitung zahlreicher Schnitte aus mehreren Blöcken.

Die DLP kann zahlreichen Krankheitsbildern ähneln, teils wegen der allergisch-granulomatösen Entzündungszeichen, teils wegen der tumorartigen Veränderung mit Strukturverlusten. Verwechslungsmöglichkeiten bestehen für folgende Krankheitsbilder: Wegenersche Granulomatose, allergische Granulomatose, thrombotische thrombozytopenische Purpura, Polyarteriitis nodosa bzw. maligne Lymphknotentumoren und Lymphogranulomatose. Im Hinblick auf die Differentialdiagnose machte LENNERT (1961) auf zwei weitere Krankheitsbilder aufmerksam, und zwar auf die Mononukleose und die Piringersche Lymphadenitis.

Im Gegensatz zu den oben erwähnten granulomatösen Veränderungen finden sich bei der DLP durch frische Fibrin- und Plättchenthromben verschlossene, kleine Gefäße, ferner eine aus Leukozyten, Erythrozyten und Kerntrümmern bestehende perivaskuläre entzündliche Reaktion. Außerdem liegt eine hämorrhagische Nekrose vor. Palissadenhaft geordnete Fibroblasten, Histiozyten und Langhanssche Riesenzellen sind nicht vorhanden.

Bei Polyarteriitis nodosa sind die charakteristischen Läsionen extranodal, sie bilden sich an den Hilusgefäßen aus (LENNERT, 1961). Bei thrombo-

tischer thrombopenischer Purpura findet sich um das mit homogenem Thrombus verschlossene Gefäß keine entzündliche Reaktion, die Lymphknotenstruktur ist erhalten.

Sternberg-Reed-Zellen und fibrotische Herde sind nicht sichtbar. Bei der infektiösen Mononukleose und der Piringerschen Lymphadenitis fehlen die für die DLP kennzeichnenden Nekrosen, ferner die thrombotische Mikroangiopathie und Hyperämie.

Die durch Arzneimittel verursachte tumorartige Lymphknoten-Retikulose ist keine seltene Veränderung, mit der man neben Hydantoin-Abkömmlingen, Sulfonamiden, Phenylbutazon auch bei anderen Mitteln rechnen soll.

Es kann festgestellt werden, daß die allergische Vasculitis verursachenden Pharmaka in den Lymphknoten neben den typischen Gefäßveränderungen auch tumorartige Zellproliferation mit einem strukturellen Umbau der Lymphknoten hervorrufen.

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DRUG-INDUCED LYMPHADENOPATHY

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The histological characteristics and pertinent clinical data of drug-induced tumour-like lymphadenopathy are reported on the basis of 10 own cases. Drug-induced lymphadenopathy is a reversible lesion which does not call for specific treatment. Drug withdrawal is invariably followed by complete remission. Allergic manifestations complicating all cases,

positive results of provocative drug administration and that of antidrug-antibody tests ensure the correct diagnosis.

In addition to tumour-like features, characteristic of the histological picture allergic-inflammatory micro-angiopathy and vasculitis with thrombosis and haemorrhagic necrosis were. Fibrosis and Sternberg-Reed cells were absent. In contrast to the cortico-medullary involvement, the hilar region is well preserved.

For the correct diagnosis, a knowledge of the clinical data is indispensable.

ЛИМФАДЕНОПАТИЯ, ВЫЗВАННАЯ ЛЕКАРСТВАМИ

Г. КРАСНАИ и Д. СЕГЕДИ

В связи с 10 наблюдениями обсуждаются гистологические показатели и более важные клинические данные опухолеобразной лимфаденопатии, вызванной лекарствами. Это обратимое изменение, не нуждающееся в лечении. После отмены лекарства наступает самопроизвольное улучшение. При постановке правильного диагноза во всех случаях большую помощь оказали аллергические осложнения, в некоторых случаях медикаментозная провокация и положительный результат пробы на антитела.

Для гистологической картины характерны, наряду с опухолеподобным процессом, аллергически-воспалительные явления, тромботическая микроангиопатия, аллергический васкулит, геморрагический некроз, плеоморфноклеточная картина. Фиброз и клетки Штернберг—Рида отсутствуют. В противоположность кортикомедуллярному изменению структура области гилюсов сохранена.

Существенным для постановки правильного диагноза является выяснение клинических данных.

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RELATIONSHIP OF ELASTIC FIBRE PRODUCTION WITH SMOOTH MUSCLE CELLS AND PULSATION EFFECT IN LARGE VESSELS

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(Received May 3, 1969)

The development of the elementary units of elastic fibres and the process of elastic fibre and lamella formation have been studied in experimentally induced intimal thickenings and in the embryonic rat aorta. The results were compared to findings made during the organization process of arterial grafts and surgical meshes.

It appears that vascular smooth muscle cells play a decisive role in the synthesis of elastic granules which, made up of elastin, appear to be the elementary units of elastic fibres. The smooth muscle cells apparently originate from multipotent mesenchymal cells specialized for fibre synthetizing function.

The absence of elastic lamella formation during the organization of arterial grafts and surgical meshes has confirmed the decisive role of the pulsation effect in elastic fibre formation.

The elastoblast theory is revised by a functional interpretation of elastic fibre formation.

Elastic fibre formation is an unresolved problem of connective tissue research and vascular pathology, though it has been extensively studied in various tissues [4—9] and vessel walls [2, 3, 13, 20, 21, 22].

Recently, views on the morphology of elementary units composing the elastic fibre seem to have come closer (Table 1) [24], but the mechanism of the formation of elementary units and their junction to elastic fibres or lamellae as well as the role of the factors involved are still far from clear.

In the present study, the process of elastic fibre synthesis was approached from the cellular angle, i.e. with respect to the site and initiation of elastic fibre and lamella formation.

Material and methods

The examinations were carried out on experimental intimal thickenings of the rat and embryonic rat aorta, and the results were compared to the organization of arterial grafts and surgical meshes. In part of the studies on arterial grafts we have been aided by the cooperation of G. BARTOS (Department of Surgery, Pécs), whereas in our studies on surgical meshes by L. SIN (Department of Surgery, Municipal Hospital, Székesfehérvár).

The test material, processing and methods have been described in detail earlier [10, 11, 12, 25].

Results

a) In the intimal thickenings arising after injuring the rat's aortic wall the cells making appearance beneath the superficial row of endothelial cells

(Fig. 1) showed a positive birefringence reminiscent of smooth muscle cells. Simultaneously, delicate fibre structures showing a negative birefringence appeared between the former cells, often in close junction with them (Fig. 2). Electron microscopically, all cells of the thickening proved to be smooth muscle cells, with the ultrastructural properties typical of these. The hyaloplasm was filled with parallel myofilaments, the cell organelles aggregated around the nucleus, in the cytoplasm glycogen granules were apparent, along the cytoplasmic membrane pinocytotic vesicles and attachment bodies were seen and along the cells there was a basement membrane (Figs 3, 4). In the dilated ergastoplasmic ducts a delicate granular substance 60—80 Å in diameter was present. In the extracellular space round electron dense particles or granules of 60—80 Å diameter, considered to represent the elementary units of elastic fibres, and fibrils of 70 Å diameter and 140 Å periodicity composed of the elementary units were seen. These units merged to form elastic clumps of irregular shape, 500—2000 Å in diameter and binding PTA. In every case, elementary elastic granules in close junction with the periphery of the clumps, and elastic fibrils practically radiating into the clumps, were clearly apparent (Figs 5, 6). In the extracellular space, microfilaments 30—50 Å in diameter and collagenous fibres were also present. The elastic clumps joined to form intercellular elastic fibres (Figs 7a, b, c) and in the intimal thickening the elastic lamina (Fig. 8). Thus, the proliferation above the impaired vascular area was composed of 3 layers, viz. (1) the new endothelium; (2) beneath it, a newly formed undulating internal elastic lamina; and (3) the media composed of the smooth muscle cells of the thickening, with newly formed elastic fibres between the cells (Fig. 9). The newly formed vessel wall above the impaired straightened internal elastic lamina may take over the role of the original vessel wall, inclusive of its elasticity function.

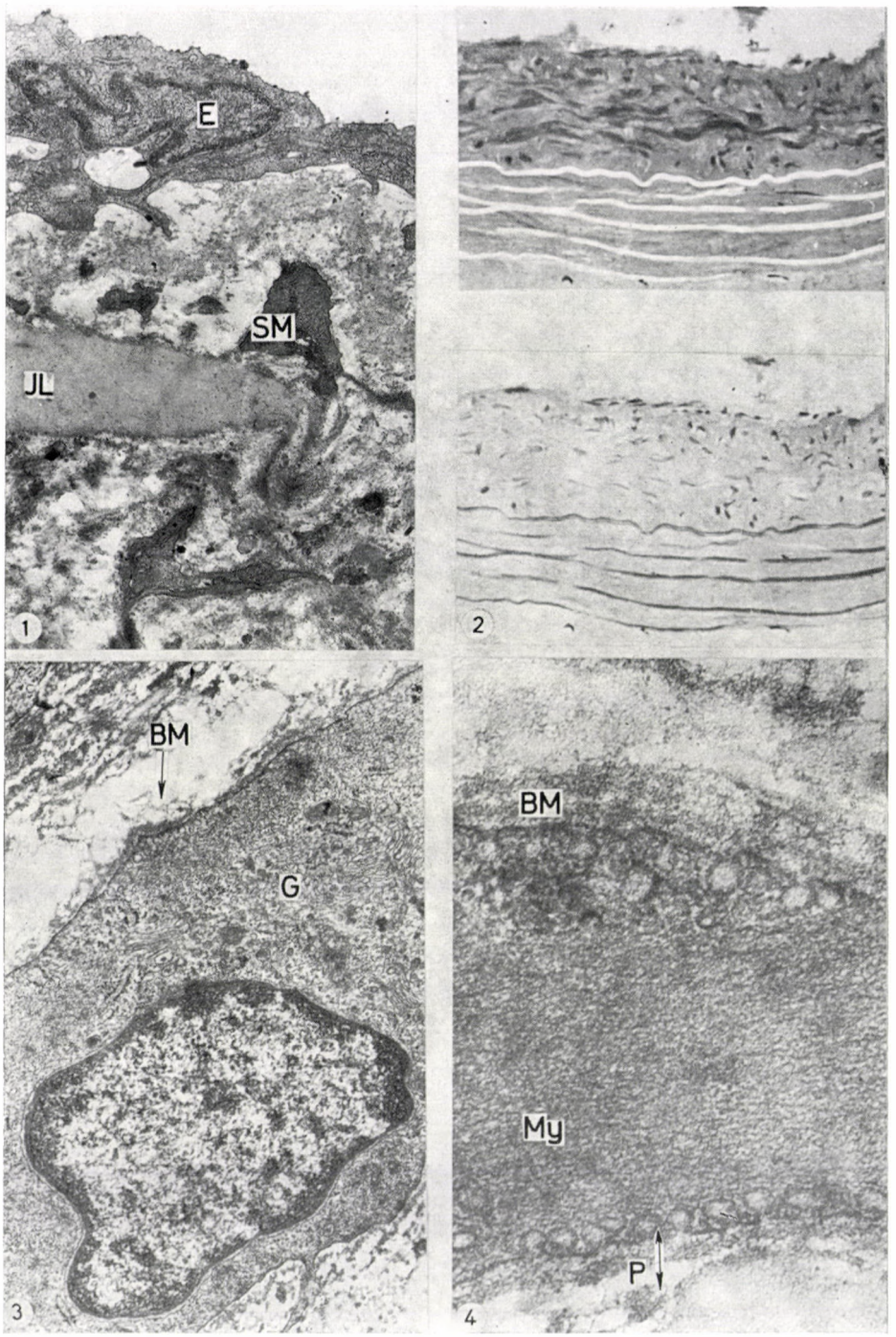
b) In the embryonic rat aorta elastic fibre formation was found to take place in the same way. Beneath the endothelial cells and between the smooth muscle cells, elastic clumps intensively binding PTA were apparent (Fig. 10); at their margins, but also independently, elastic granules, elastic fibrils, micro-

Fig. 1. Newly formed endothelial cell. Below it the granular substance, and underneath a fragment of the impaired original elastic membrane. Above there are processes of smooth muscle cells. Uranyl acetate—lead hydroxide stain. ×2400

Fig. 2. Polarisation microscopical picture. Note positive birefringence of smooth muscle cells as contrasted to the negative birefringence of the surrounding fibre structures. Phenol-Canada cover. ×120

Fig. 3. Smooth muscle cell in a multi-layered intimal thickening. Note the basal membrane along the cells. The cell organelles are in perinuclear location. Uranyl acetate—lead hydroxide stain. ×7600

Fig. 4. Part of smooth muscle cell from intimal thickening. The hyaloplasm is filled by parallel myofilaments. Note vigorous pinocytotic activity along the cytoplasmic membrane. Uranyl acetate—lead hydroxide stain. ×60 000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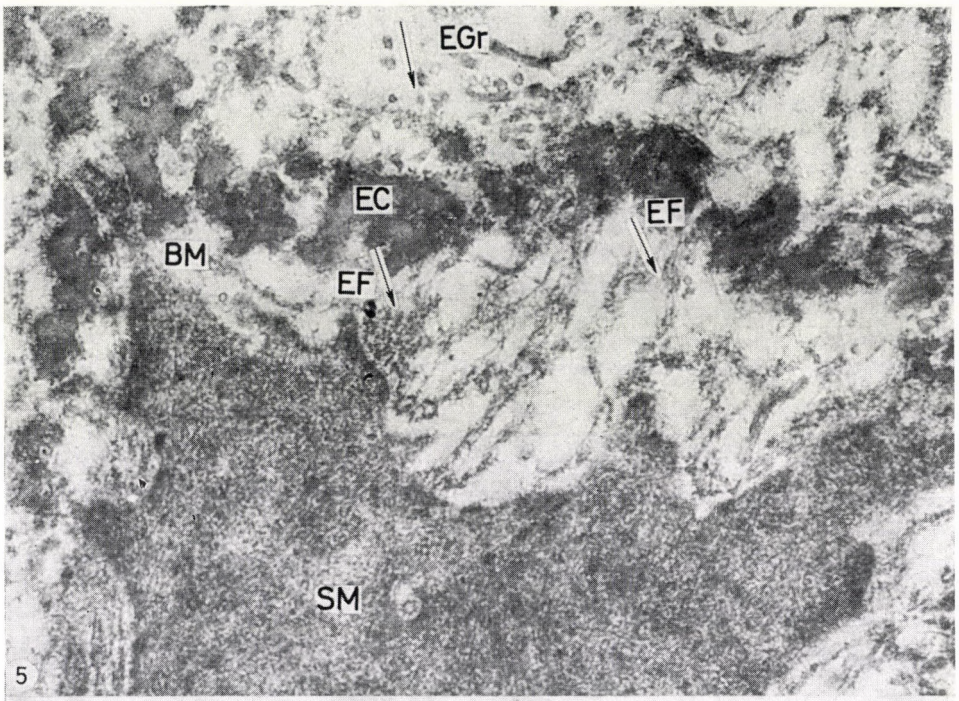

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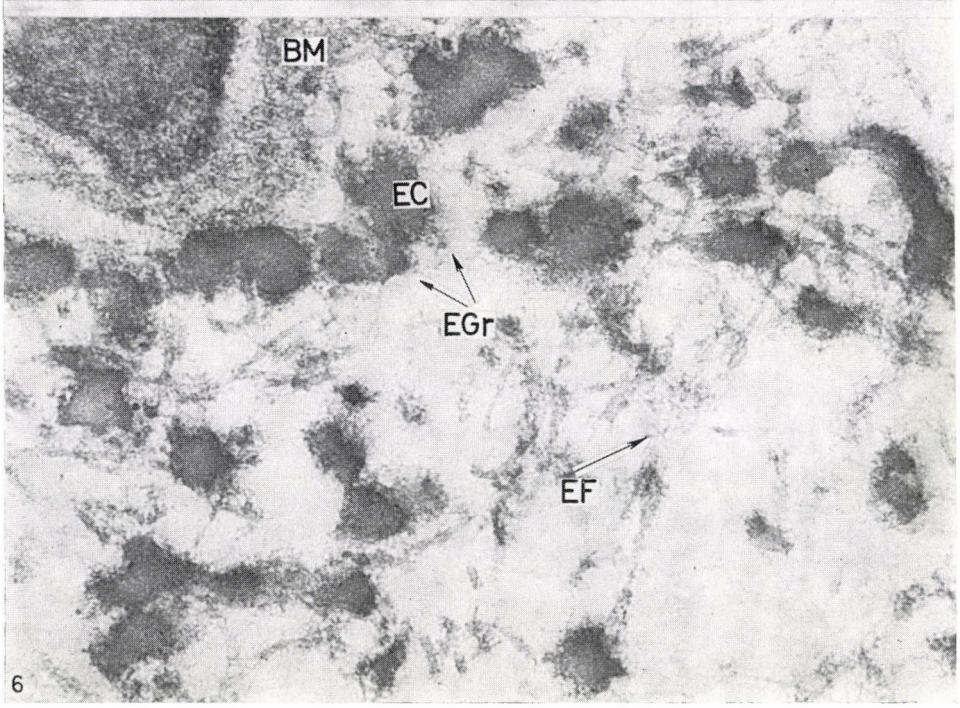

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filaments and collagenous fibres were seen (Fig. 11). The medial smooth muscle cells resembled those composing the intimal thickening (Fig. 12). Inside the vessel wall the degree of merging of the elastic clumps seemed to be variable; while in its inner third nearly fully developed lamellae, still binding PTA, were present, in the outer part of the media only sporadic small elastic granules were seen, showing a greater affinity to PTA than the fibres in the inner third.

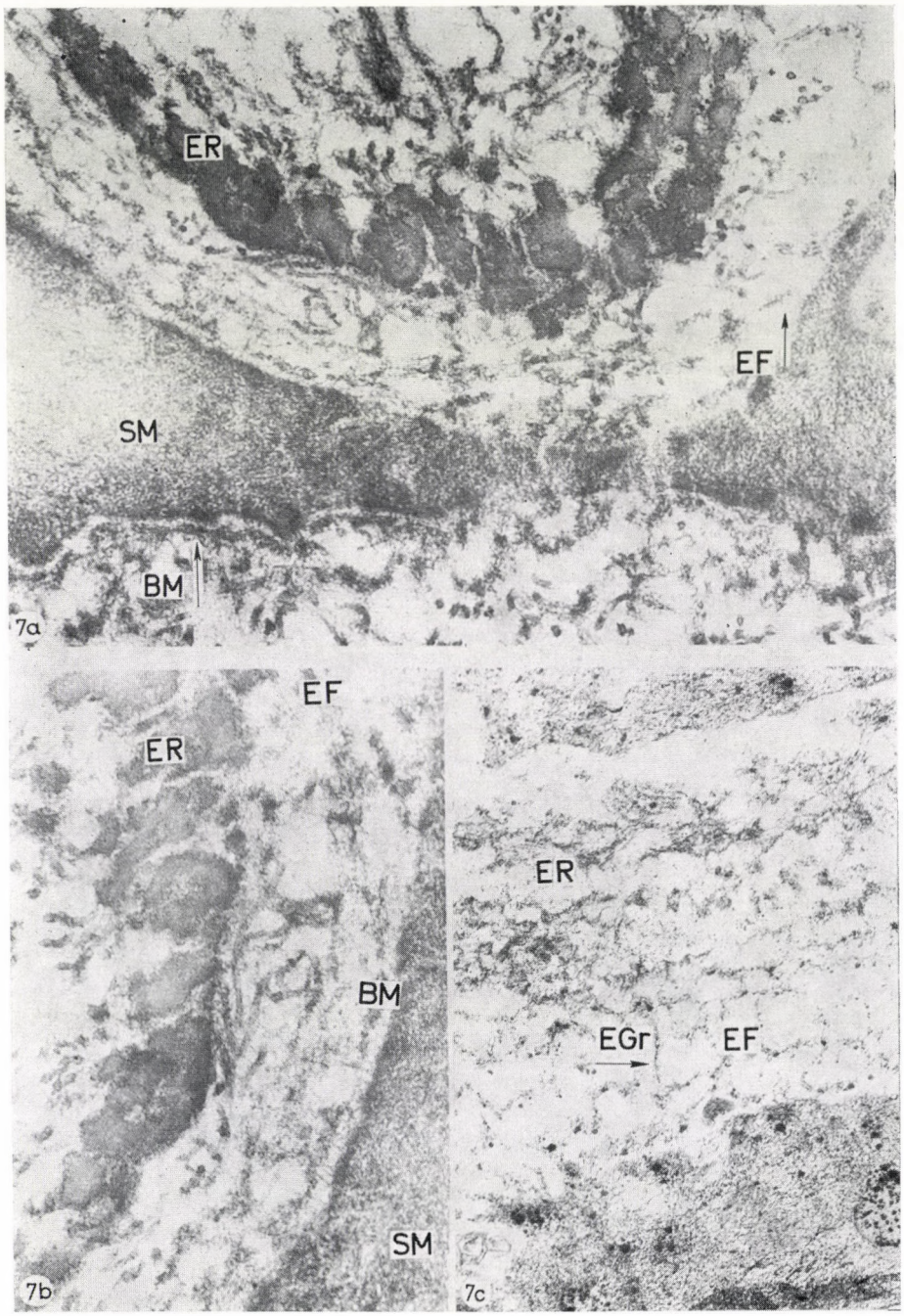
Both in the intimal thickenings (Figs 13b, c, d) and in the embryonic aorta (Fig. 13a) a close relationship was found between elementary units, elastic fibrils and the basement membrane of the smooth muscle cells. Inside the basement membrane, the elastic fibrils were clearly distinguishable and also their connection with elastic clumps and fibres located at some distance to the basement membrane was conspicuous (Figs 13a, b, c, d).

c) The above findings were compared to observations made during the organization of arterial grafts. In the latter, elastic fibres or fibre parts occurred only in the superficial areas of the thickening brought about by the process of organization or in areas close to the border between the host aorta and the prosthesis. In no case did they form a mature, continuous elastic lamella, but many collagenous fibres developed in the thickening, imparting to it the appearance of a scar tissue (Fig. 14) According to the fibre structure, part of the cells of the younger proliferation were smooth muscle cells occurring in very low numbers (Fig. 15), whereas the aged proliferation consisted exclusively of fibroblasts or fibrocytes (Fig. 16) and cell forms which seemed to be cartilage-cells with large glycogen-like areas and 100—150 Å thick filaments forming a fingerprint-like pattern.

d) In the organization of surgical meshes used for the repair of abdominal hernia, no elastic fibres were demonstrable in the granulating tissue. Initially, the synthetic grafts were surrounded by many foreign body giant cells, macrophages and fibroblasts, while later only collagenous fibrous tissue, poor in cells, was found (Fig. 17).

Fig. 5. In the extracellular space of the intimal proliferation, PTA-binding elastic granules are apparent at the marginal parts of the elastic clumps. The basement membrane and the elastic fibrils radiating into the clumps show a periodicity of 140 Å, being composed of elastic granules. PTA stain. ×3800

Fig. 6. Intimal thickening. At the marginal parts as well as inside the elastic clumps, the elastic granules and elastic fibrils are clearly apparent. PTA stain. ×45 000



Figs 7a, b. Formation of elastic fibres from elastic clumps in intimal thickening. PTA stain. *a*: ×60 000; *b*: ×80 000

Fig. 7c. Intimal thickening. Part of a mature elastic fibre, which has lost affinity to PTA. PTA stain. ×27 000

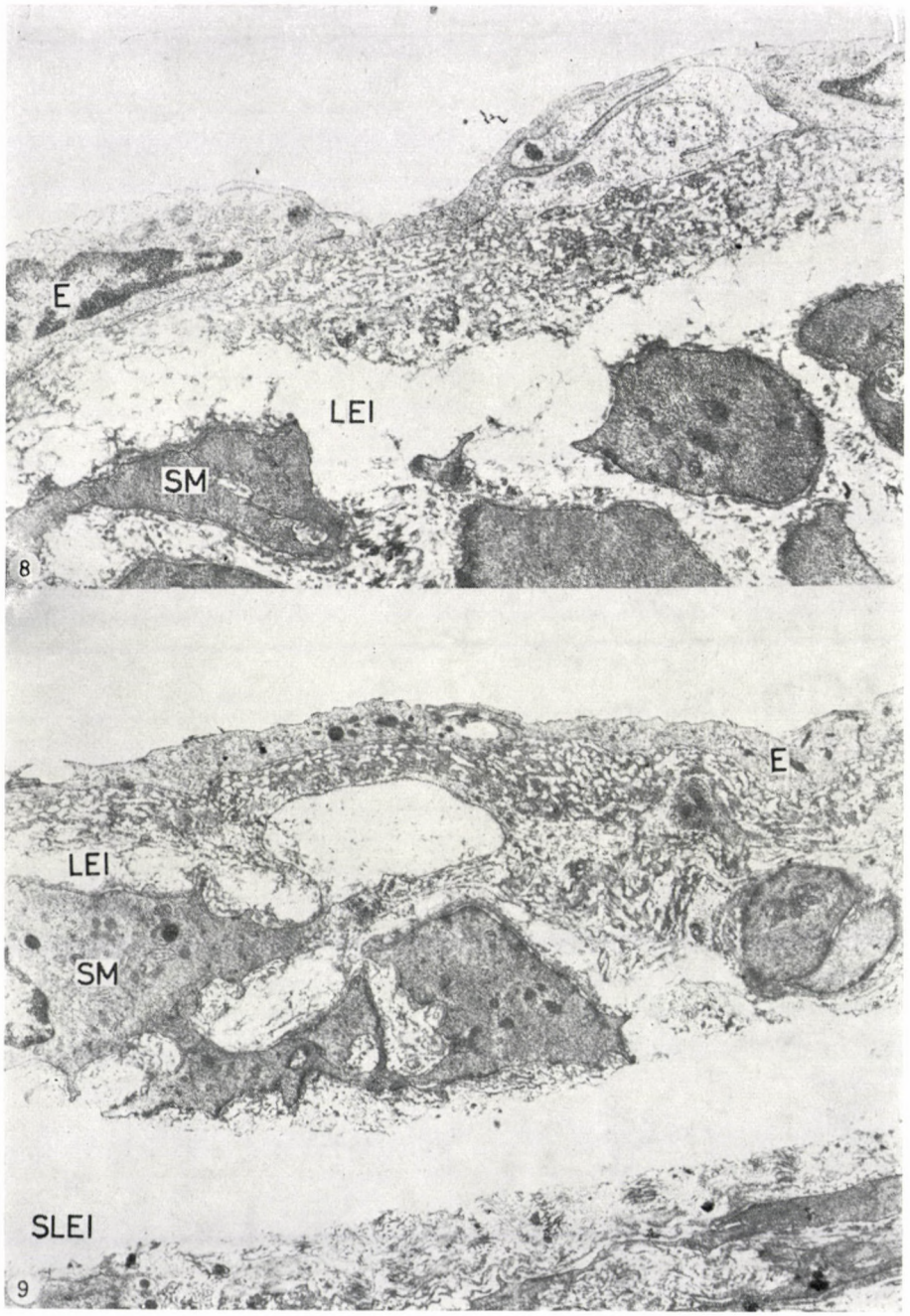


Fig. 8. Newly formed internal elastic lamina. Note the clumps and fibres between this and the newly formed endothelial cells. Beneath the new internal elastic lamina there are smooth muscle cells surrounded by a basement membrane. Uranyl acetate–lead hydroxide stain. × 10 400
Fig. 9. The proliferation formed above the straightened injured internal elastic lamina appears to consist of three layers: an upper endothelial cell row, a nearly electron-lucent new internal elastic lamina in the middle, and typical smooth muscle cells below. Uranyl acetate–lead hydroxide. × 6400

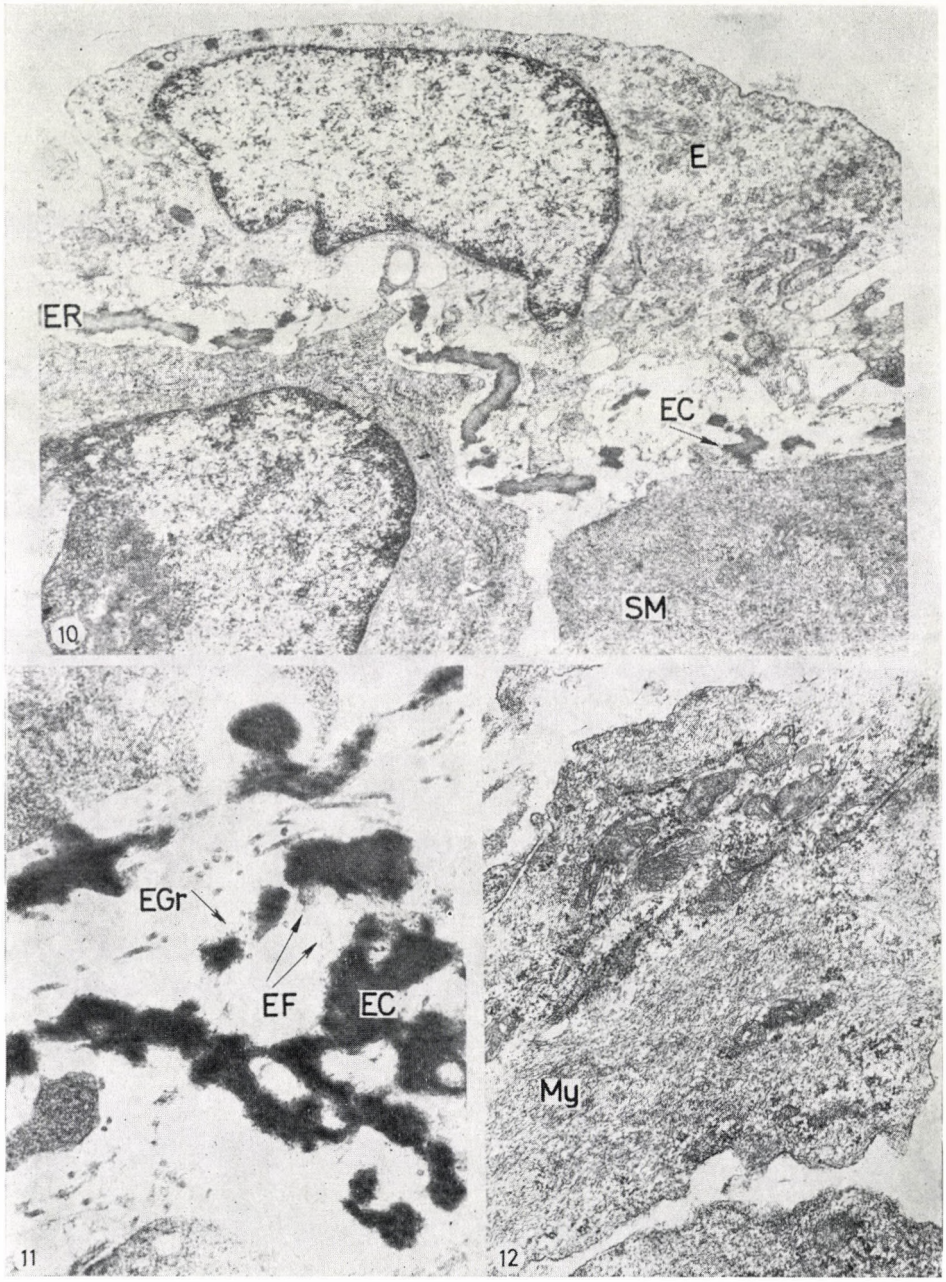


Fig. 10. Part of elastic fibre — or lamella — with medium PTA-binding, between the endothelial cells and smooth muscle cells in the aorta of a 15-day rat embryo. PTA stain. ×9440
Fig. 11. Elastic clumps showing intensive binding of PTA in the wall of embryonic rat aorta. Note elastic granules and elastic fibrils at the clumps' marginal parts. PTA stain. ×28 000
Fig. 12. Smooth muscle cells from the media of embryonic rat aorta. Note their marked resemblance to smooth muscle cells in intimal thickening. Uranyl acetate–lead hydroxide stain. ×23 400

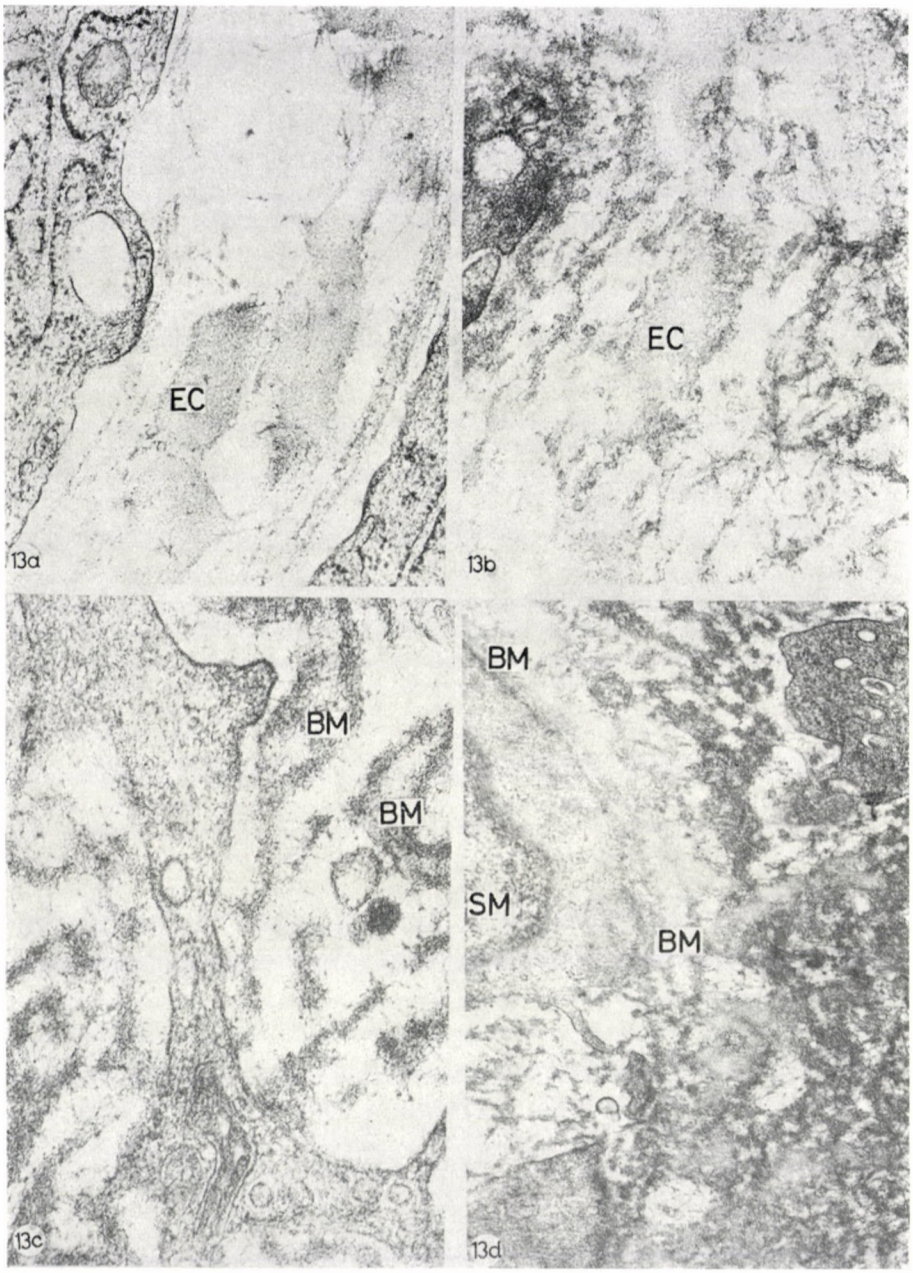
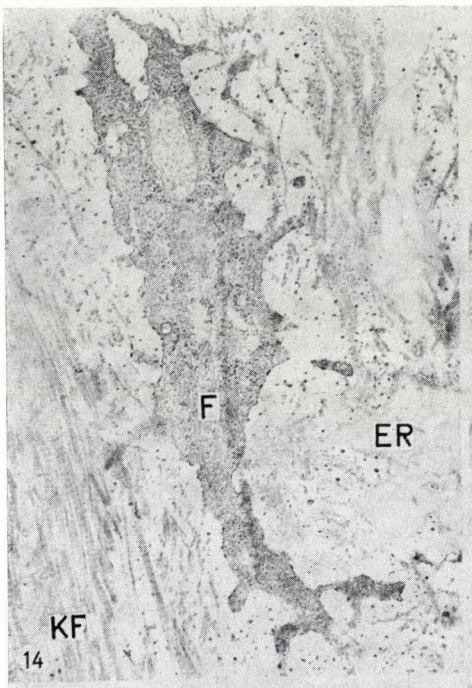


Fig. 13a. In the basement membrane of the smooth muscle cells of an embryonic rat aorta there are clearly distinguishable elastic granules and elastic fibrils in close connection with the marginal parts of elastic clumps and fibres. Uranyl acetate–lead hydroxide stain. × 36 000
Fig. 13b. Note the close relationship between the basement membrane of smooth muscle cells of an intimal thikcening and the elastic clumps or fibres. Uranyl acetate–lead hydroxide stain. × 39 000
Figs 13c, d. In the basement membrane of the intimal thickening's smooth muscle cells there are elastic granules and fibrils whose connection with elastic clumps located at a distance to the basement membrane is clearly apparent. *a:* Uranyl acetate–lead hydroxide stain, × 48 000. *b:* PTA stain, × 21 000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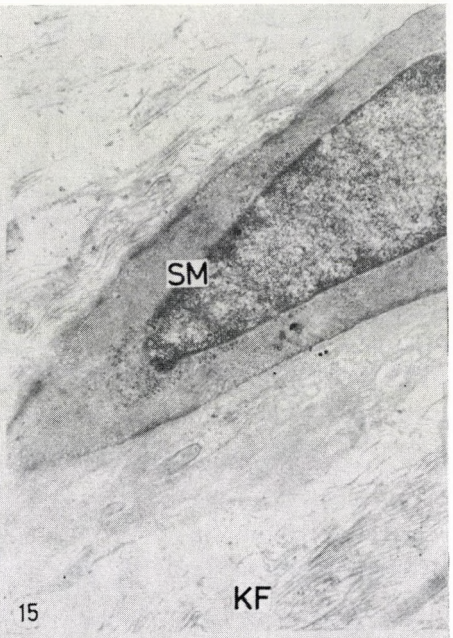
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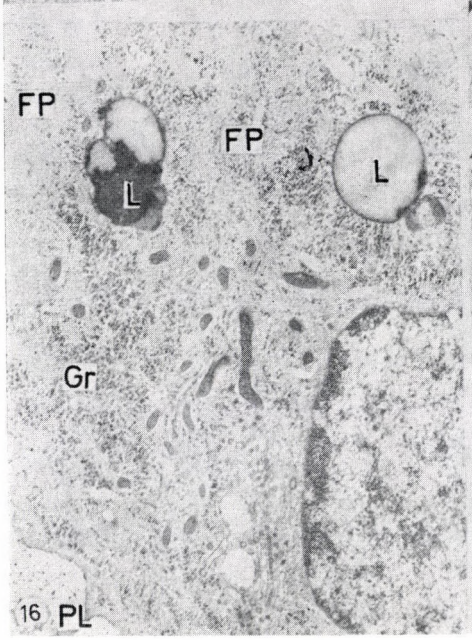
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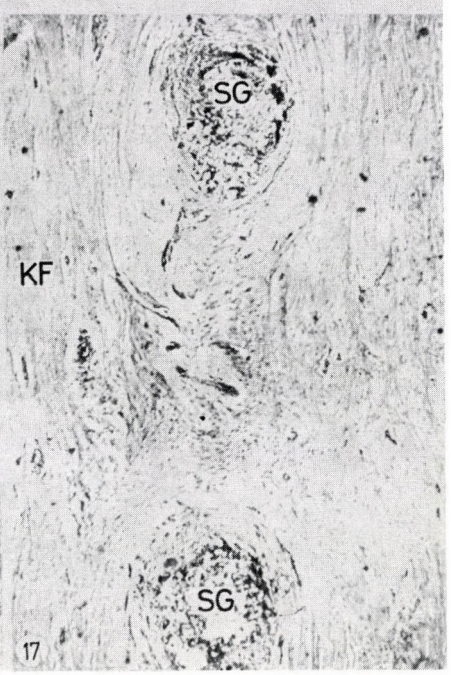
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Discussion

Our results suggest that the elementary units of elastic fibres, the elastic granules composed of elastin, are produced by smooth muscle cells. It appears that according to functional requirements, in given sites, as e.g. in the embryonic rat aorta and in intimal thickenings, the multipotent mesenchymal cells and the vascular smooth muscle cells, respectively, may transform to modified smooth muscle cells specialized for fibre production by being capable of, or at least participating in the synthesis of elementary units or ground substance required for elastic fibre formation.

The elastic granules synthetized by the smooth muscle cells form elastic fibrils in the extracellular space, where through aggregation, junction and embedding into the acid mucopolysaccharide matrix they give rise to elastic clumps which again unite to form the elastic fibres (KÁDÁR et al. in press).

As shown by ROMHÁNYI's [23] polarisation optical studies, inside the elastic fibre the fibrils do not run parallel to the longitudinal axis, but are wound around it like a spiral ascending at 45° in young, and at 25° in mature fibres. This spiral structure as well as the acid mucopolysaccharide coat encasing the fibres (BANGA [1]) seem to bear the primary responsibility for the elastic nature of the fibres.

It appears that the extracellular aggregation of elastic elements and lamina formation are either preconditioned or brought about by the mechanical pulsation effect. This effect seems to be responsible for the merging of elastic clumps to elastic fibres and for the junction of the latter to a new elastic lamina, in intimal thickenings and normal embryonic rat aorta alike. In the areas where the pulsation effect is diminished or absent, as e.g. in the organization process of abdominal surgical meshes, or where elasticity is provided artificially, as e.g. in the case of arterial grafts, modified smooth muscle cells and newly formed elastic fibres appear hardly or not at all and no elastic lamellae whatever develop.

Similar conclusions have been drawn by LELKES and KARMAZSIN [19] who studied explants of chick embryo heart and aorta. In the pulsating myo-

Fig. 14. Specimen from arterial graft implanted 4 years ago into the abdominal artery of a dog. Between collagenous fibres a process of a fibrocyte is seen; at the margin of the picture part of an elastic clump or fibre is apparent. Uranyl acetate–lead hydroxide stain. × 13 650

Fig. 15. Part of a smooth muscle cell formed in an early proliferation during the organization of an arterial graft. Note myofilaments and glycogen granule in the cytoplasm and pinocytotic vesicles and attachment bodies along the cytoplasmic membrane. Along the cell there is a basement membrane, with collagenous fibres in its surroundings. Uranyl acetate–lead hydroxide stain. × 12 500

Fig. 16. Cartilage-cell from aged proliferation formed during the organization of an arterial graft. On the cell, surface processes, while in the cytoplasm lipid droplets, electron dense granules and thick fingerprint-like filaments are seen. Uranyl acetate–lead hydroxide stain. × 14 350

Fig. 17. Organization of surgical mesh. The synthetic filaments are surrounded by an oligocellular scar tissue containing many collagenous fibres. Light microscopic picture. H and E. stain. × 60

cardial explant, elastic fibre formation started on the addition of embryonic extract, while no such phenomenon took place in the absence of pulsation. In aortic explants, synthesis of elastic fibres occurred exclusively in the aortic specimen connected with the pulsating heart explant. In aortic explants pulsation seems to represent the adequate stimulus of elastic fibre formation. According to KROMPECHER [15], pulsation is the specific stimulus of elastic membrane formation as undifferentiated mesenchymal cells respond to the intermittent mechanical stimulus by elastin production. In examinations carried out on the ligated rat ureter, LADÁNYI and LELKES [17] found that the smooth muscle cells of the pulsating muscular tunica above the ligature played a role in elastic fibre formation. This function of the smooth muscle cells was considered a qualitative adaptation which represents the activation by mechanical factors of the "primordial fibro-elastoblast forming ability". In this context, reference should be made to the much disputed existence or nonexistence of the "elastoblast", a cell supposed to be specialized for elastic fibre synthesis. Its existence was postulated by KROMPECHER [14] in 1928, but recent literary data including our own findings and KROMPECHER's revised view (personal communication [16]) seem to exclude the existence of such specialized cells in the vessel wall. It appears that vascular smooth muscle cells are capable of synthetizing either the precursor, or certain components, of the elastic fibre. According to KROMPECHER (personal communication) there would exist a cellular function specialized for elastic formation.

In our view, under certain conditions, as e.g. under functional influences or in a given stage of ontogenesis, vascular smooth muscle cells may specialize for the elastic fibre synthetizing function. These young, multipotent mesenchymal cells whose smooth muscle cell nature could only recently be confirmed by electron microscopy, seem to be the counterparts of the "elastoblasts" described by Krompecher.

*

The electron micrographs were prepared in the Central Electron Microscopy Laboratory, Institute of Histology and Embryology, University Medical School, Budapest.

Symbols in the figures

BM = Basement membrane; E = Endothelial cell; EC = Elastic clump; EF = Elastic fibril, EGr = Elastic granule; ER = Elastic fibre; F = Fibrocyte; Fp = Fingerprint pattern; G = Golgi apparatus; Gr = Granule; IL = Internal elastic lamina; KF = Collagen fibre; L = Lipid; LEI = Internal elastic lamina (newly formed); My = Myofilament; P = Pynocytotic vesicle; PL = Plasma process; SLEI = Smoothened internal elastic lamina (old); SG = Part of Teflon graft

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ZUSAMMENHANG ZWISCHEN DER BILDUNG VON ELASTISCHEN FASERN, DEN GLATTEN MUSKELZELLEN SOWIE DEM PULSATIONSEFFEKT IN GROSSEN GEFÄSSEN

Anna KÁDÁR, B. VERESS und H. JELLINEK

Die Entwicklung der elementaren Einheiten von elastischen Fasern und der Prozeß der elastischen Fasern- und Lamellenbildung wurden in experimentell herbeigeführten Intimaverdickungen und in der Aorta von Rattenembryonen untersucht. Die Ergebnisse wurden den bei dem Organisationsvorgang von Arterientransplantaten und chirurgischen Netzen gemachten Erfahrungen gegenübergestellt.

Es hat den Anschein, daß die glatten Muskelzellen der Gefäße eine entscheidende Rolle in der Synthese von elastischen Granuli innehaben, welche aus Elastin bestehen und vermutlich die elementaren Einheiten der elastischen Fasern darstellen. Die glatten Muskelzellen stammen scheinbar aus den multipotenten Mesenchymzellen, die für die Fasersynthese spezialisiert sind.

Das Fehlen der Entstehung von elastischen Lamellen während der Organisation von Arterientransplantaten und chirurgischen Netzen bestätigt die entscheidende Bedeutung des Pulsationseffekts in der Bildung von elastischen Fasern.

Die Elastoblastentheorie wird revidiert und durch eine funktionelle Interpretation der Bildung von elastischen Fasern ersetzt.

СВЯЗЬ МЕЖДУ ОБРАЗОВАНИЕМ ЭЛАСТИЧНЫХ ВОЛОКОН, КЛЕТКАМИ ГЛАДКИХ МЫШЦ И ПУЛЬСАЦИОННЫМ ЭФФЕКТОМ В КРУПНЫХ СОСУДАХ

А. КАДАР, Б. ВЕРЕШ и Х. ЙЕЛЛИНЕК

Развитие элементарных единиц эластичных волокон и процесс образования эластичных волокон и пластинок изучались в экспериментально вызванных утолщениях интимы и на аорте эмбрионов крыс. Результаты были сопоставлены с данными, полученными во время организации артериальных трансплантатов и хирургических сетей.

Полагается, что клетки гладких мышц сосудов играют решающую роль в синтезе эластичных зернышек, состоящих из эластина и, по-видимому, представляющих элементарные единицы эластичных волокон. Клетки гладких мышц, по-видимому, происходят от мультипотентных мезенхимальных клеток, специализированных для синтеза волокон.

Отсутствие продукции эластичных пластинок при организации артериальных трансплантатов и хирургических сетей доказывает решающее значение пульсационного эффекта при образовании эластичных волокон.

Теория эластобластов подвергается ревизии и выдвигается функциональная интерпретация образования эластичных волокон.

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ESTERASE ACTIVITY IN THE HYPOTHALAMUS

T. MÉSZÁROS, I. J. CSURI, J. HÁZAS and M. PALKOVITS

(Received August 12, 1968)

AChE activity of varying intensity and cellular localization may be demonstrated in the different areas of the hypothalamus. No neuropile reaction may be observed. Non-specific esterase activity occurs in all the nuclei and areas.

In the different hypothalamic areas, there is no direct relationship between AChE activity and the functional state, or at least no such relationship can be revealed with the present histological methods. A change in the reaction may be observed at sites where no adequate functional changes can be assumed to occur and, conversely, AChE is lacking in areas where it might be expected to be present. The AChE content of the hypothalamus seems to be connected to general cellular activity and not to humoral regulation. Removal of some endocrine organs is usually followed by a decrease in activity, independently of the kind of the organ and of the hypothalamic localisation.

Introduction

The role in neuro-humoral regulation of the morphologically distinct areas of the hypothalamus has considerably been clarified in the last two decades. Within the main connexions the kind of cellular activity with which the neurons and neuron groups participate in the above mechanism is, however, still discussed. The question arises whether this functional difference is due to the different metabolism of the cells of functionally different areas, or to the specificities of neuronal connexions, or to both.

The exact morphology of the neurons of certain areas is known from the descriptions of KRIEG [28] and SZENTÁGOTHAI [45], the synaptic conditions and the intrahypothalamic organisation from those of SZENTÁGOTHAI [45]. The anatomical concept of the small cellular neurosecretory system has been introduced by SZENTÁGOTHAI [45].

The biochemical and histochemical examinations of the enzymes of cellular metabolism were confined to a few selected hypothalamic areas. Esterase activity of some hypothalamic nuclei was described by PEPLER and PEARSE [37] and PEARSE [36], while BERNSOHN et al. [4] reported on the subcellular localization of these enzymes in the different parts of the brain.

Among the esterases, acetylcholinesterase (AChE) is of outstanding importance. The first histochemical method for the morphological localization of AChE was developed by KOELLE and FRIEDENWALD [26]. The electron-

microscopical histochemical examinations of the last years have shown that AChE is found not only in the active cellular membrane but is also located in great quantities in the endoplasmic reticulum of some neurones [6, 11, 19, 25, 32, 33, 47].

Earlier reports were mainly concerned with the AChE and pseudocholinesterase (pseudo-ChE) activity of the hypothalamic magnocellular nuclei, parvicellular areas were mentioned but sporadically. Data referring to non-specific esterase ("B" esterase, aliesterase) activity are even more scarce [2, 36, 37, 40, 44].

The aim of our examinations was *(i)* to study AChE and non-specific esterase activity in every hypothalamic area in normal rats; *(ii)* to establish whether AChE activity changed under the effect of changes of the hormonal activity in those areas which have been found to play a regulating role in the production of the given hormone.

Material and methods

Fifty white adult rats of both sexes (weighing 150—180 g) and 16 infantile females (weighing 40 g) were used; they were divided into an intact group and an experimental group. The latter animals were examined 12 days after uni- and bilateral adrenalectomy and 10 days after uni- and bilateral ovariectomy. In the second group sham-operated animals were used as controls.

Examination of AChE activity

Under hexobarbital anaesthesia, the animals were perfused through the aorta first with 50 ml physiological NaCl solution, then for half an hour with 150 ml of 10 % formalin, pH 7.2. The removed brain was fixed in 10% formalin for 22 hours at 2—4°C. Series of frozen 40 μ thick sections were made from the hypothalamus. Applying the method of KOELLE and FRIEDENWALD [26] modified by us, the preparation was incubated in acetyl-thiocholine-iodide (Fluka). By decreasing the Na_2SO_4 concentration of the incubation solution to 5% we succeeded in inhibiting the crystallization of the reaction product. Control sections were inhibited for 30 minutes with 10^{-6} *M* nitrogen mustard, or 10^{-6} *M* DFP and 10^{-6} *M* neostigmine.

Examination of non-specific esterase

The material was fixed with the same perfusion technique for 2 hours. The substrates applied were 1. α-naphthyl-acetate, and 2. naphthol-AS-acetate. 1. The cuts were incubated with α-naphthyl-acetate for 30 seconds, then with pararosaniline hexazonium chloride according to Davis and Ornstein [10] for 2 minutes. 2. The naphthol-AS-acetate method of Gomori [14] was applied with the following modification: 0.1 g naphthol-AS-acetate was mixed with 10 ml 0.1 *M* Sörensen buffer pH 7.2, then the mixture was shaken in a funnel. Ten mg of Garnet GBC diazonium salt was added to 5 ml Sörensen buffer and mixed thoroughly. The two solutions were mixed and shaken again in the funnel for 10 minutes, then clarified. The sections were incubated in the filtrate for 2 hours, at 37°C, then the preparation was rinsed in tap water for 5 minutes and covered with gelatin.

After unilateral and bilateral adrenalectomy it was examined karyometrically whether the hypothalamic changes induced by corticoid deficiency had brought about an increase of nuclear volume in the ventromedial nucleus. In the nucl. ventromedialis of both sides (uniformly in the pars medialis) in 3000× projection magnification, the diameters of 140—250 cell nuclei were measured and their volume calculated. The group average represents values for 6 animals.

Results

1. Intact group

A) *AChE activity in the hypothalamus*

A cellular reaction of varying intensity may be observed in the different areas of the hypothalamus. A significant neuropile reaction, like that in the striatum, is not seen in any region of the hypothalamus.

Pars optica. A rather intensive activity may be seen in the magnocellular neurosecretory nuclei, in the nucleus supraopticus (NSO) and in the nucleus paraventricularis (NPV). There is no appreciable difference in intensity between the part of the NPV containing small and that containing large cells (Fig. 1, a—d). The small fusiform cells of the nucleus periventricularis anterior (NPVA), arranged parallel to the ventricular surface give an average reaction (Fig. 1, e—f). The area hypothalamica lateralis (AHL) exhibits a strong activity. In its anterior part, fusiform nerve cells and also some of irregular shape occur. The thick dendrites of a few irregular nerve cells ramifying near the cell are also discernible (Fig. 2, a). Regular, mostly ovoid, cells are found in the posterior part of the area, the initial portion of the thick dendrite is mostly well seen (Fig. 2, b). The intensity of the reaction agrees in the two areas.

The reaction is negative in the nucleus hypothalamicus anterior (NAH) and in the nucleus suprachiasmaticus (NSCH). In the area retrochiasmatis (RCA) cells with two-three thin dendrites exhibit a slight AChE activity. Some larger round cells are also observable among them.

Pars tuberalis. The intensity of the AChE reaction, as compared with the optic region is considerably slighter and less circumscript. In the nucleus ventromedialis (NVM) and in the nucleus arcuatus (NARC), the reaction is usually negative (Fig. 2, c, e). At the border of NVM and NARC, along the median-posterior third of the two nuclei, 20—30 cells manifesting a very intensive AChE activity are arranged in orocaudal direction (Fig. 2, f). The cells of the nucleus dorsomedialis (NDM) give reactions of different intensity among the cells of characteristic medium intensity, some exhibit a considerable activity (Fig. 2, d). Behind the NDM and partly ventrally, in the dorsal part of the area hypothalamica posterior (AHP), from the 3rd ventricle to the fornix, as well as in the region of the area perifornicalis (APF) an intensive reaction may be observed, especially in the cells which embrace the fornix from below. These are small, round or oval cells displaying a reaction of varying intensity (Fig. 3, a—d).

Pars mamillaris. AChE activity of the mamillary area is generally slight. Some cells of the nucleus supramamillaris (NSUM) and the large cells of the nucleus praelateralis mamillaris (NPL) give medium reaction, while the

nucleus praemamillaris ventralis (NPMV) and dorsalis (NPMD) are practically AChE negative. In the area between the ventro-medial edge of the fornix and the capsula interna, the cell masses arranged at the level of the premamillary nuclei display a slight reaction, occasionally some cells give a medium reaction (area hypothalamica latero-dorsalis (AHLD)).

The above described AChE reactions were completely inhibited by 10^{-6} *M* nitrogen mustard.

B) *Non-specific esterase (NSE) activity in the hypothalamus*

α-Naphthyl-acetate method. In the hypothalamus of the rat, NSE activity of different intensity occurs in every area. In the magnocellular area (NSO, magnocellular part of the NPV, NPL) the reaction is usually of medium intensity, (Fig. 4, a, c). In the parvicellular nuclei and areas only some cells give reactions of variable intensity, while others are negative. NSE positivity could be observed in the NDM (Fig. 5, a) and NVM (Fig. 5, c); it was intense in the NSUM and in the premamillary nuclei. A strong positivity was observed in the posterior lateral part of the hypothalamus, around the fornix (Fig. 5, b), where AChE activity is also fairly intensive. A slight perikaryonal reaction (Fig. 5, d) is found occasionally in the NARC. The most intensive cellular reaction manifests itself in the cells arranged at the medial side of the fornix, not far behind the level of the anterior commissure (Fig. 5, e).

A granular reaction is found in the ependymal cells of the 3rd ventricle (Fig. 4, g) and a rather intensive one in the so-called tanycyte-ependyma (Fig. 4, e, f) and in the vascular organ of the lamina terminalis.

Naphthol-AS-acetate method. NSE activity is rather intensive in the magnocellular neurosecretory nuclei (NSO, magnocellular part of the NPV) (Fig. 4, b, d). Cells of different intensity are found within the nuclei. Apart from these two nuclei, the reaction which may be observed in every hypothalamic area, appears only sporadically in a few cells, and in the form of coarser granules than with the α-naphthyl-acetate method. The reaction of medium intensity in the glia cells occurs in larger granules. In contrast to the α-naphthyl-acetate reaction, average activity is found in the NSCH (Fig. 6, d) and the NDM-cells (Fig. 6, c). Many cells are positive in the mamillary region (Fig. 6, e, f) and in the preoptic area (Fig. 6, a, b).

II. Experimental group.

A) Effect of adrenalectomy on the AChE activity of the hypothalamus and on nuclear volume in the nucleus ventromedialis

Following adrenalectomy a general decrease of AChE activity may be observed in the hypothalamus, which becomes significant after bilateral

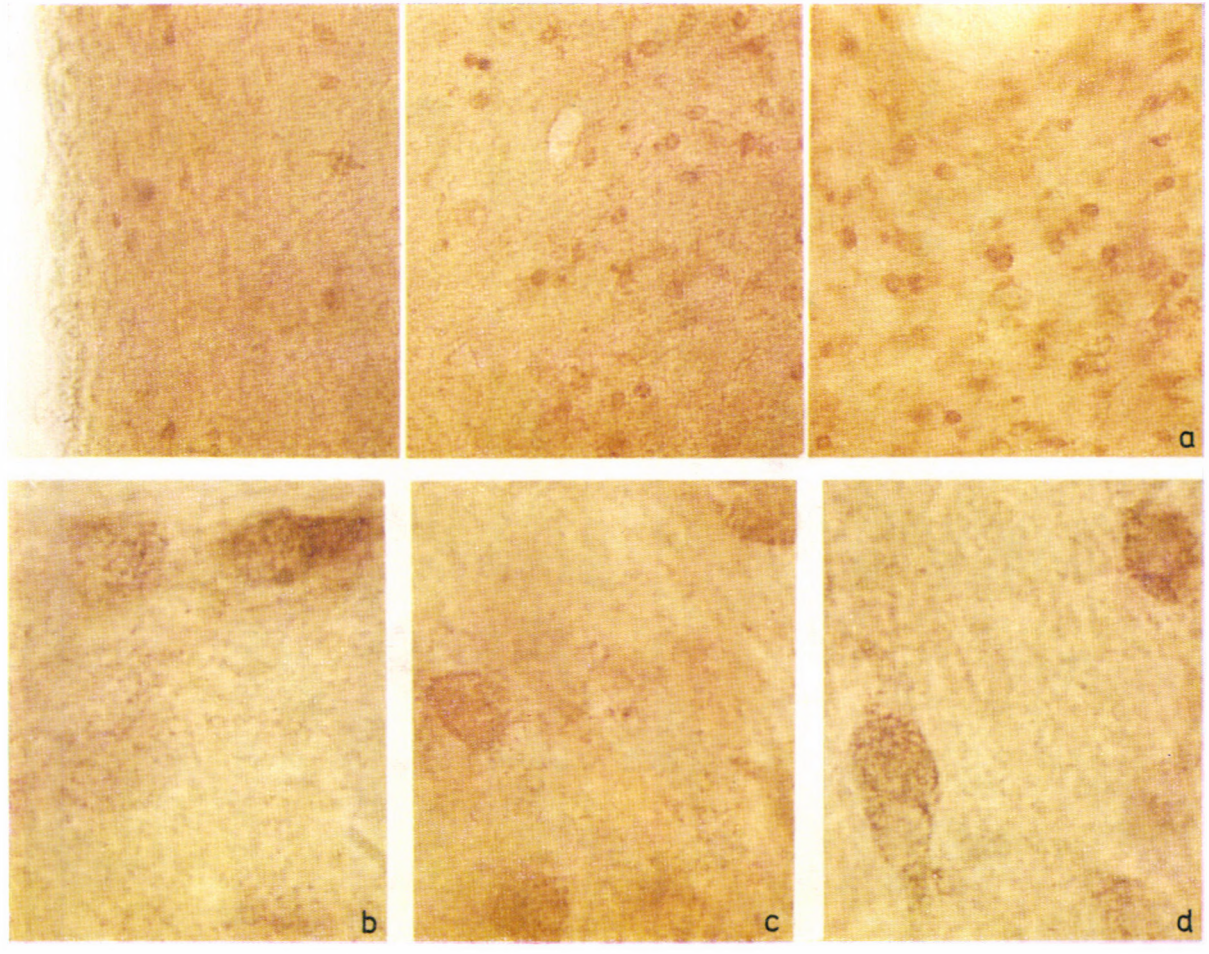


Fig. 3. AChE reaction of AHP and APF *(a—d)*. Modified Koelle—Friedenwald's method ($a = \times 100$; $b—d = \times 500$)

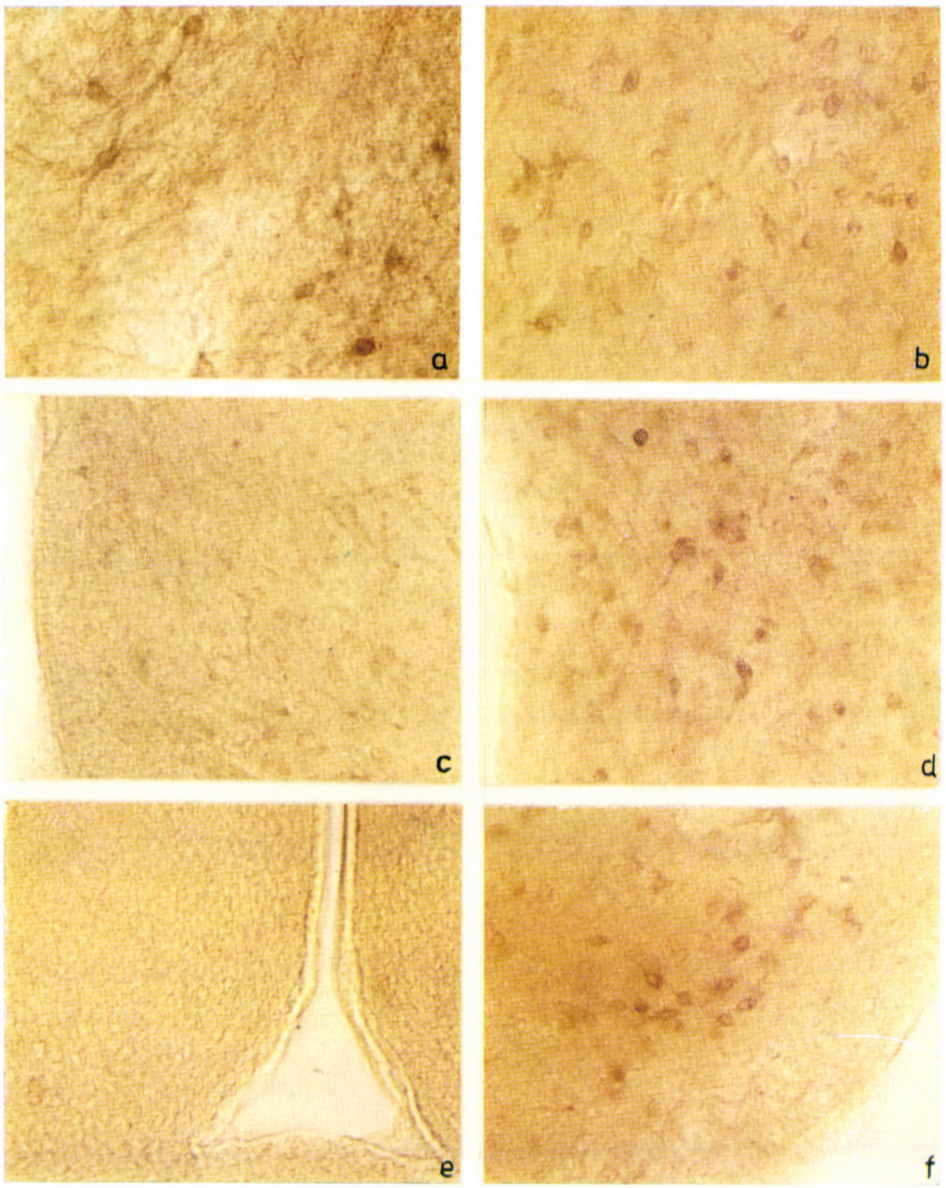


Fig. 2. AChE reaction in the oral (*a*) and caudal part (*b*) of the AHL. Negative AChE reaction in the NVM (*c*), NARC (*e*). Cellular reaction in the NDM (*d*) and at the border of NVM—NARC (*f*). Modified Koelle—Friedenwald's method (*a*, *b*, *c*, *e* = ×50; *d*, *f* = ×500)

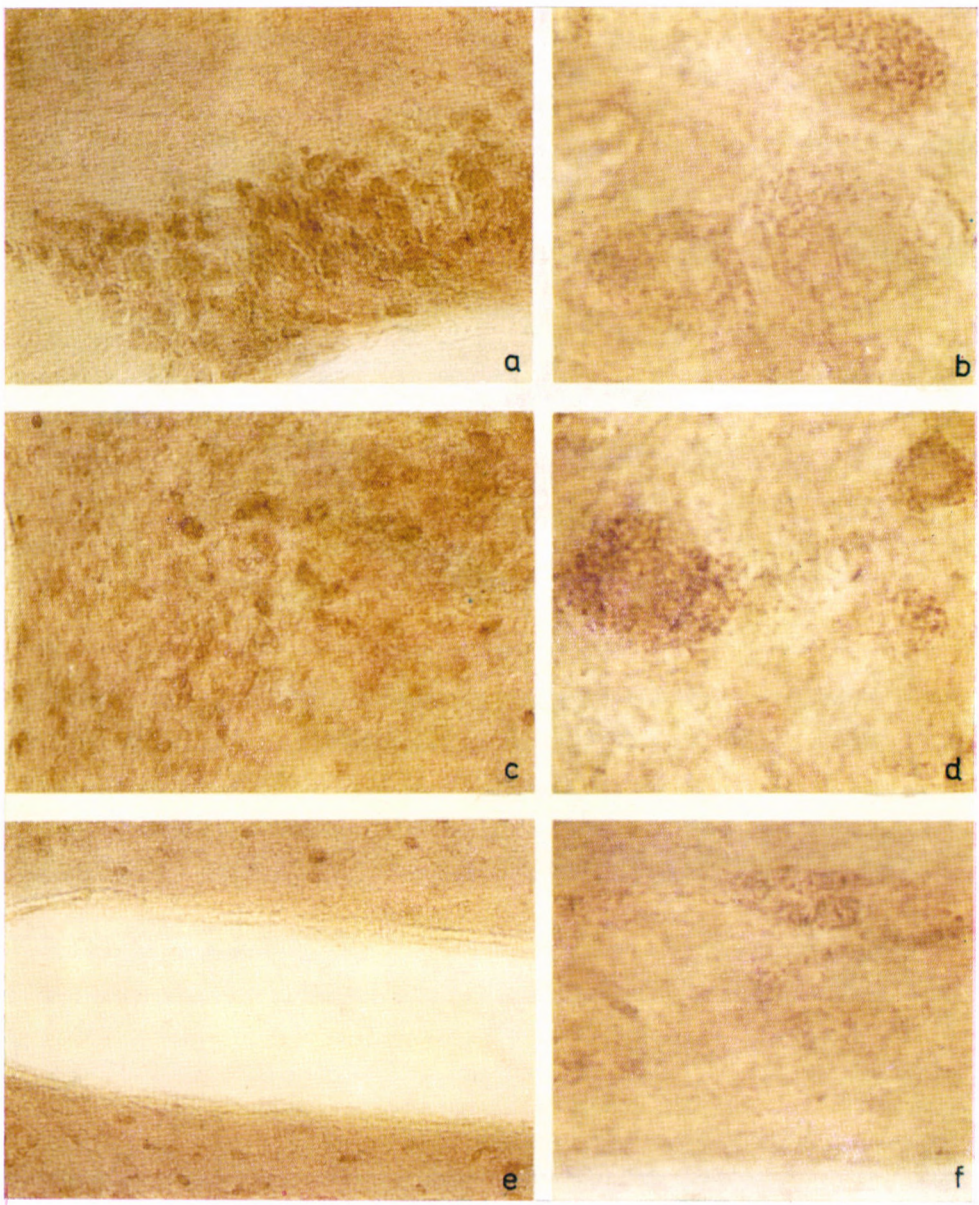


Fig. 1. AChE reaction in NSO *(a, b)*, NPV *(c, d)* and NPVA *(e, f)*. Modified Koelle—Friedenwald's method (*a, c, e* = ×50; *b, d, f* = ×500)

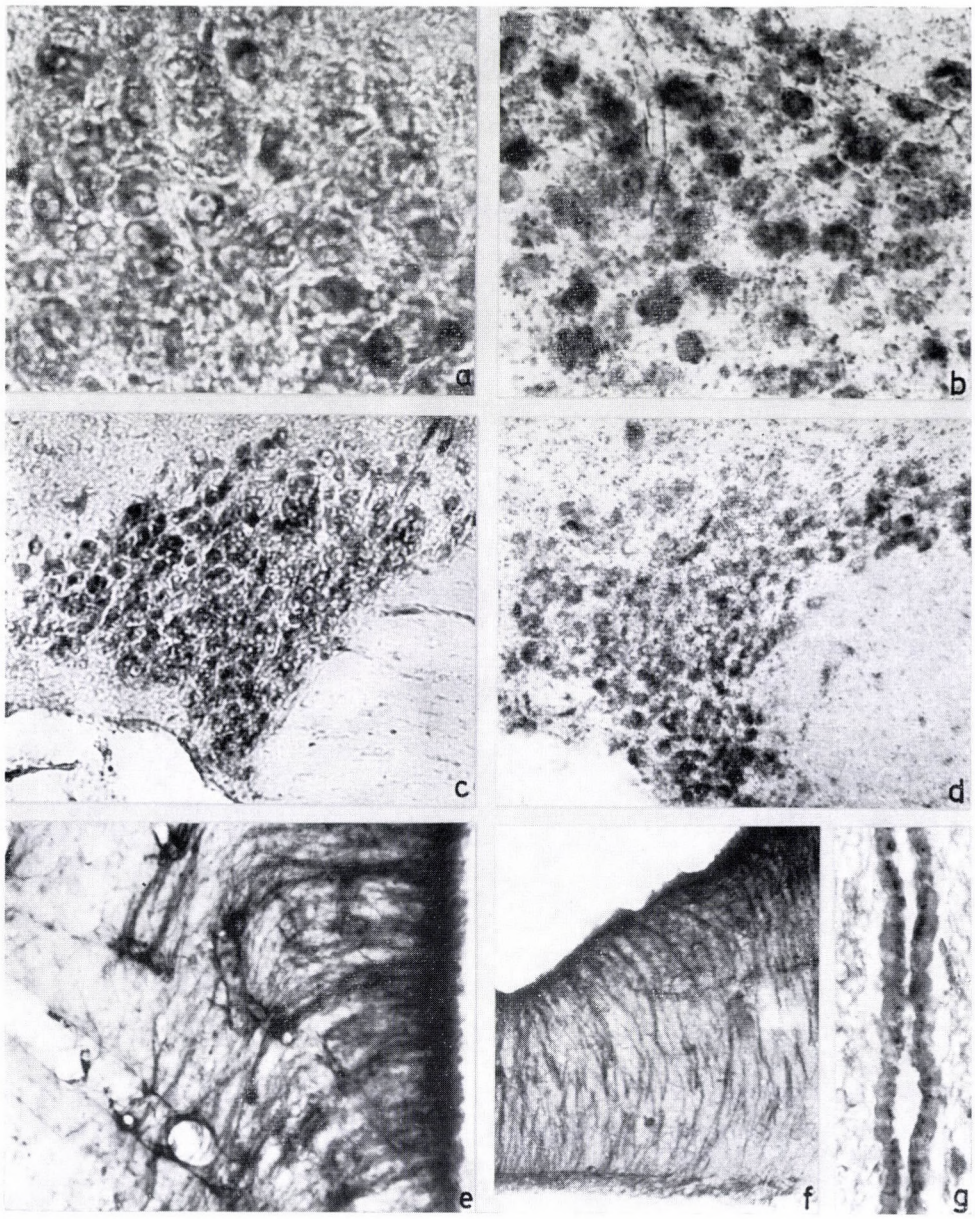


Fig. 4. Non-specific esterase reaction in NPV *(a, b)*, NSO *(c, d)*. α-naphthyl-acetate method — *a, c.* Naphthol-AS-acetate method — *b, d.* Non-specific esterase reaction in the ependyma-cells "tanycyte-ependyma" of the recessus infundibuli *(e)* and the recessus inframamillaris *(f)* in the ependyma of the median area of the 3rd ventricle *(g)*. α-naphthyl-acetate method. (*a, g* = ×420; *b* = ×350; *c, d* = ×140; *e, f* = ×220)

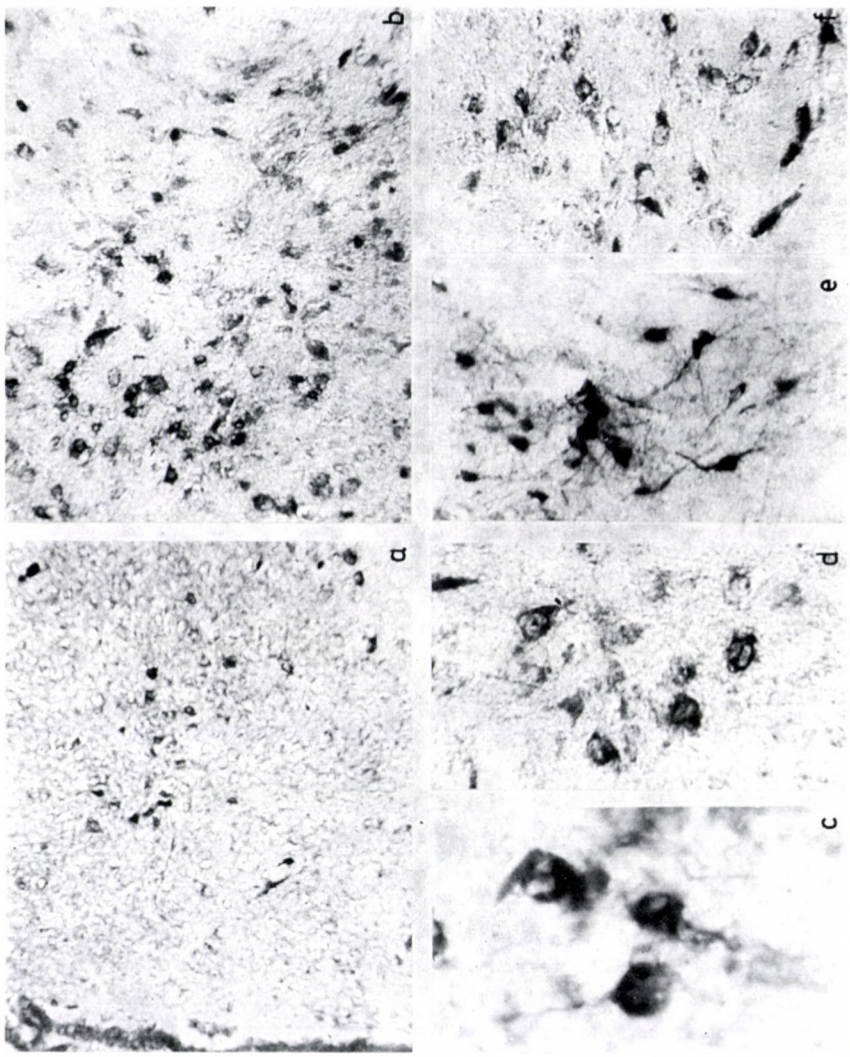


Fig. 5. Non-specific esterase reaction in NDM *(a)*, periformically *(b, e)*, in NVM *(c)*, NARC *(d)*, NAH *(f)*. α-naphthyl-acetate method *(a, b, e, f* = ×220; *c* = ×875; *d* = ×420)

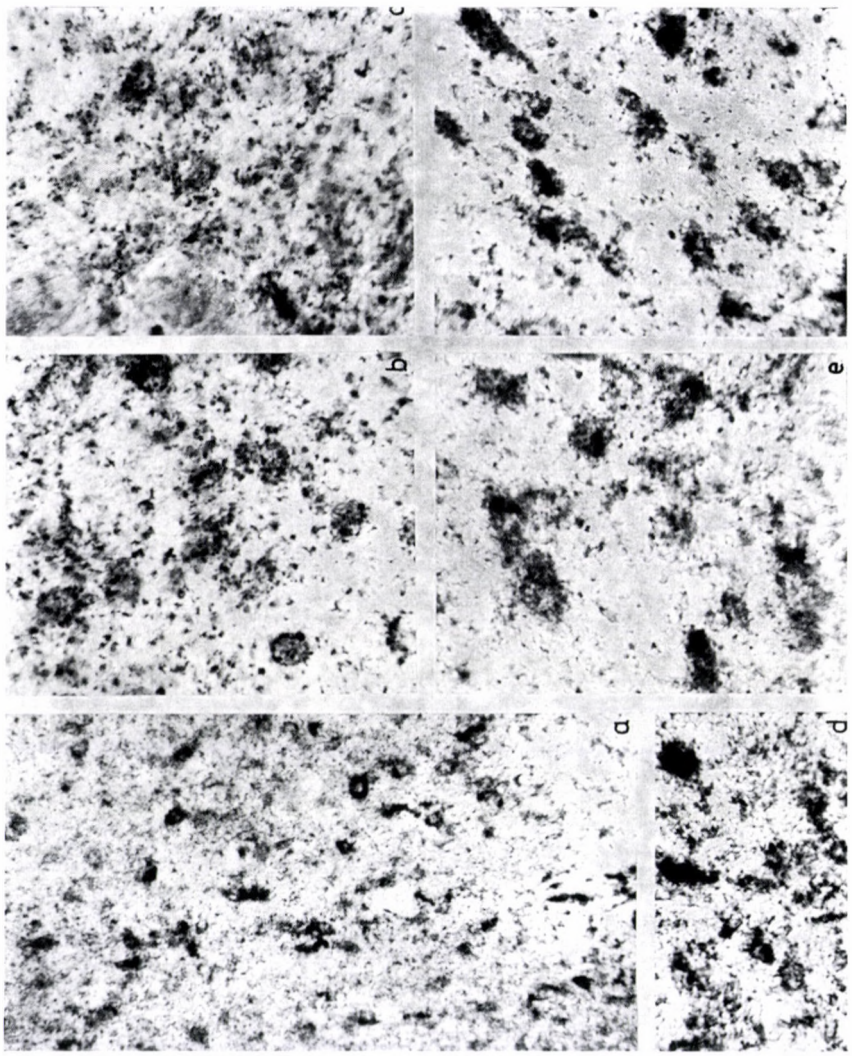


Fig. 6. Non-specific esterase reaction in the area praeoptica (*a*, *b*), NDM (*c*), NSCH (*d*), nucleus mamillaris medialis (*e*), nucleus mamillaris lateralis (*f*). Naphthol-AS-acetate method ($a = \times 140$, $b-f = \times 350$)

adrenalectomy. (In order to avoid subjective errors, concerning the reaction's intensity, AChE activity in the lentiformis and reticular nuclei was considered normal in intensity, since this does not change under hormonal effects.)

After bilateral adrenalectomy, AChE activity disappeared in the RCA cells, and decreased in the cells arranged at the border of NVM and NARC. The result was the opposite after unilateral adrenalectomy; AChE activity vanished in the cells arranged at the border of NVM and NARC and persisted

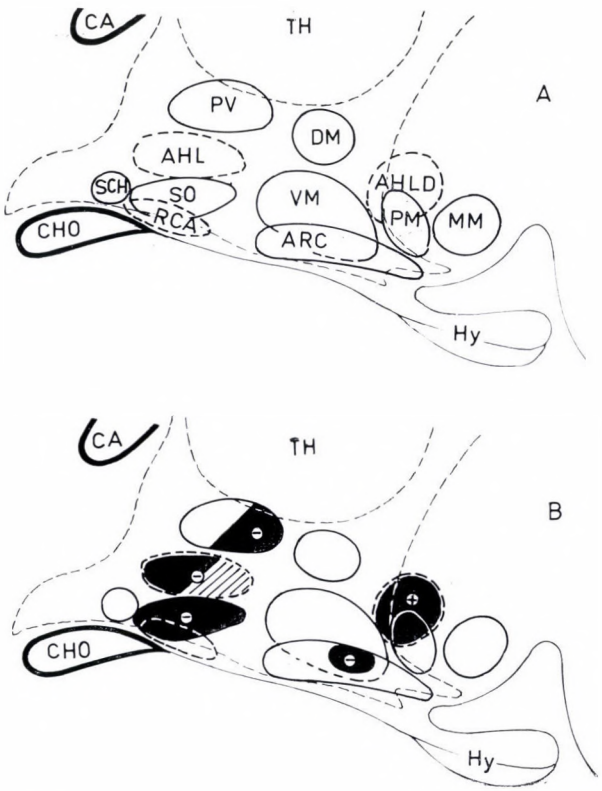
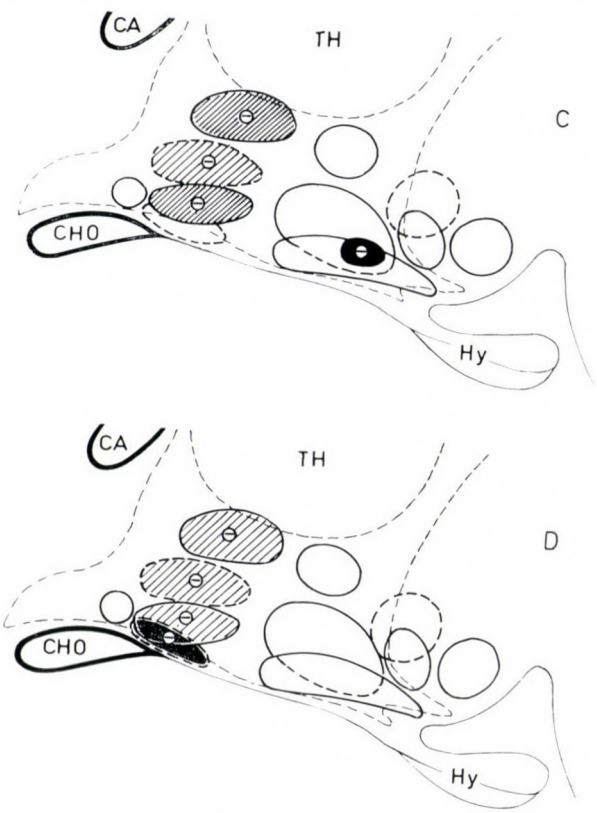


Fig. 7. Change of AChE activity of the hypothalamus after adrenalectomy and ovariectomy. Intensity of blackening represents the rate of the change; direction of the latter is labelled by + (increase), and — (decrease). *A* = examined areas (labelling see in the text); *B* = changes after ovariectomy;

in the RCA-cells (Fig. 7, *C*, *D*). After unilateral adrenalectomy, no difference in AChE activity was found in the identical areas of the two sides. Karyometric measurements revealed that unilateral adrenalectomy did not essentially influence nucleic volume in the NVM-cells, and the difference between the two sides exceeded the evaluable 7.5% only in two of the 6 examined animals (Table I). After bilateral adrenalectomy the cellular nuclei of the NVM increased in volume by 24.6%.

After unilateral adrenalectomy, AChE activity decreased in the NPV and NSO and this decrease was significant also when compared with the findings after bilateral adrenalectomy. Following both uni- and bilateral adrenalectomy, a few large cells displaying a medium activity appeared in the NAH.



C = changes after unilateral adrenalectomy; *D* = changes after bilateral adrenalectomy

B) Effect of ovariectomy on hypothalamic AChE activity

Unilateral and bilateral ovariectomy has essentially the same effect on the AChE activity of the hypothalamus, except for the intensity of the reaction which is less after total castration. In both cases AChE activity disappears almost completely, in the NSO it is less intensive in the magnocellular part of the NPV and negative in its parvicellular part. Simultaneously a capillary activity of average intensity becomes manifest in these two nuclei. (This cannot be considered a pseudo-ChE reaction, which is positive in the case of cerebral vessels, as the capillaries of other areas fail to produce a simul-

Table I

Nuclear volume in NVM after unilateral and bilateral adrenalectomy
I = intervention, II—III = values of the two sides, IV = difference, in per cent, between the two sides, V = group average, VI = difference, in per cent, between the group averages

I	II μ^3	III μ^3	IV %	V μ^3	VI %
	162.2	160.0	1.4		
	156.7	163.8	4.5		
Control	163.1	168.6	3.4	163.1	—
	186.5	175.0	6.6		
	153.4	159.8	4.2		
	154.1	154.1	0		
	149.3	150.1	0.5		
	151.5	155.0	2.3		
After	178.7	162.4	10.0	169.4	+ 3.8
hemiadrenalectomy	145.0	151.6	4.5		
	195.3	179.1	9.0		
	203.2	211.6	4.1		
	196.0	199.7	1.9		
	209.7	216,2	3.1		
After	205.3	211.9	3.2	203.2	+24.6
adrenalectomy	195.0	196.6	0.8		
	200.9	204.4	1.7		
	199.3	195.9	1.7		

taneous reaction.) The oral part of the AHL, the cells arranged at the border of NVM and NARC also become negative.

Conversely, AChE activity considerably increases in the premammillary area, in the cells arranged laterally from the fornix (AHLD) (Fig. 7, B).

Discussion

Both AChE and pseudo-ChE reactions are observed in the brain. Opinions differ as to the question whether one enzyme or a group of enzymes is responsible for the activity found in the hypothalamus. According to KOELLE [24], ABRAHAMS et al. [1], PEPLER and PEARSE [37] and PEARSE [36] cellular activity in the magnocellular nuclei is due to AChE. On the other hand, BURGEN and CHIPMAN [7] assume the presence of pseudo-ChE. Accord-

ing to ILJIMA et al. [18] the nerve cells give an AChE reaction of medium intensity, the neuropile and the vessels a moderate one, while the cell nuclei, the nucleoli and the ependyma show negative reaction. The pseudo-ChE reaction displayed by the glia, the neuropile, the ependyma and the vessels is medium in intensity, that given by the neurons is slight, the nuclei and nucleoli are negative.

SHUTE and LEWIS [43] found a strong positivity of the pseudo-ChE reaction only in the glia cells and in the vessels in the rat brain, — while every other intensive reaction points to the presence of AChE. In the evaluation of our own material we also adopted this view, since we succeeded in inhibiting the cellular reactions described above with 10^{-6} *M* nitrogen mustard. Thus, the topographical and functional aspects of the AChE content of the hypothalamus may be summarized as follows.

1. In agreement with numerous earlier authors [1, 2, 7, 16, 18, 21, 22, 24, 29, 30, 35, 36, 37, 39, 42] an AChE activity of medium intensity was found in the magnocellular neurosecretory nuclei. PICKFORD [38] verified the close connection between ADH production and acetylcholine. Numerous further examinations were performed in order to prove the parallelism of the special activity of the above nuclei and the AChE content [21, 22]. PEPLER and PEARSE [37], PEARSE [36], LEGAIT et al. [29] observed an increased intensity of AChE activity parallel to the neurosecretory activity consecutive to dehydration.

In our material an activity of average intensity was observed in the magnocellular nuclei. After adrenalectomy or ovariectomy a change occurred. The intensity of the reaction decreased after adrenalectomy while after ovariectomy, the reaction decreased in the large cells of the NPV and became negative in the small cells and the NSO. In this area the vanishing of the cellular reaction was associated with the appearance of a capillary activity of average intensity.

Following adrenalectomy the volume of the cellular nuclei of the NSO and NPV increases [46, 7] and AChE activity of the cells decreases, without any change in neurosecretory content [20]. After castration, the decrease of cellular activity [46, 12] is associated with the decrease or disappearance of AChE activity. BARRY and LEONARDELLI [3] also observed a decrease of AChE content after castration. These data favour the assumption that after the decrease in the blood adrenal and ovarian hormone level, there is no parallelism between the quantitative histological picture of NSO and NPV and the change of AChE activity.

2. The connexion between AChE and cellular activity is similarly inconsistent in the parvicellular area of the hypothalamus. There is considerable uncertainty in the evaluation of the normal picture. According to some authors, the reaction is negative in the parvicellular area. COHN and RICHTER [9]

observed an increase of AChE activity until the 15th day after birth. BURGEN and CHIPMAN [7], ABRAHAMS et al. [1] found a positive reaction in the eminentia mediana, while KOBAYASHI and FARNER [23], FOLLET et al. [13] described a positive reaction in several species. In our normal material, the cellular reaction was negative in the NSCH and positive in RCA. The eminentia medialis was practically negative, apart from the cells arranged at the border of the NVM and NARC, where PORQUET and LEONARDELLI [39] also found a positive reaction in newborn cats. This cell group of strong intensity is most likely identical with cells described by SZENTÁGOTHAI [45] as the small cells of the NVM. Thus, these cells differ not only in size from the larger cells forming the main mass of the NVM, but also in their properties described above. Some cells of the NDM and NPVA gave a positive reaction.

A considerable activity was observed in the lateral area of the hypothalamus, from the preoptic to the mamillary region. Activity was especially intensive in the perifornical area.

The changes in AChE activity observed in the parvicellular nuclei after adrenalectomy and ovariectomy are also inconsistent. In the cells situated at the border of the NVM and NARC, AChE activity disappeared after unilateral adrenalectomy, while the cellular reaction of the RCA persisted. On the other hand, after bilateral total adrenalectomy the RCA-cells became negative but activity in the former cells was unchanged or decreased slightly. Both uni- and bilateral adrenalectomy resulted in the appearance of a few cells of average activity in the NAH.

Our observations do not support the view according to which a parallelism would exist between humoral regulatory activity and AChE activity, since this hypothalamic area is involved in the regulation of corticoid production. Following adrenalectomy quantitative changes may be observed in the NVM [15, 46] and, according to IFFT [17], in all the tuberal nuclei.

After unilateral adrenalectomy the reaction usually decreased in intensity and the cells between the NVM and NARC gave a negative reaction, though in the NVM no change could be revealed by means of karyometric measurements. HALÁSZ and SZENTÁGOTHAI [15], SAJONSKI et al. [41] using the karyometric method, observed changes in the NVM after denervation or extirpation of the contralateral adrenal. Surmising a contralateral connexion between the adrenal gland and the hypothalamus, we have expected that, if AChE activity is in connexion with humoral regulation, a difference in the reaction of the two sides will manifest itself following unilateral adrenalectomy. However, we did not succeed in substantiating this view nor the above mentioned contralateral effect.

After bilateral adrenalectomy, apart from a 24.6% increase in nuclear volume, a slight decrease of AChE activity was found in the NVM. The negative

reaction of the RCA might have been due to the special synaptic connexions [46] of these cells.

3. LEONARDELLI [30, 31], studying the posterior hypothalamic area of the guinea pig, observed cyclical changes in AChE activity, which were the most pronounced in prooestrus. BARRY and LEONARDELLI [3] also found the greatest change in the posterior hypothalamus after castration and on the basis of this observation assume the sexual centre to be located at this site (nucleus hypothalamicus latero-dorsalis interstitialis).

We also found an increased AChE activity in the posterior hypothalamus after ovariectomy. In spite of these observations concerning the area between the ventro-medial edge of the capsula interna at the level of the premamillary nuclei, we have failed to establish that localization would be determined by function, mainly as changes of similar order, though of negative nature, may be observed also in areas that are certainly not involved in sexual activity. It may be assumed that intensity of AChE activity is determined by several factors the complex effect of which results in the above changes.

4. SLOPER [44], PEPLER and PEARSE [37], PEARSE [36], ARVY [2] examined the non-specific esterases in the hypothalamus. Different methods, such as α-naphthyl-acetate, naphthol-AS-acetate, indoxyl-acetate substrates, revealed activity in the NSO and NPV. In contrast, our own studies showed non-specific esterase activity in all the hypothalamic nuclei. The two methods applied by us yielded a practically identical result. Activity could be revealed in a few cells in the area preoptica and in the NSCH with the naphthol-AS-acetate procedure.

The role of non-specific esterases in cellular metabolism has not been elucidated. It is assumed that, as enzymes occurring in the microsomal membrane [4, 8], they are involved in the detoxication of the endoplasmic reticulum [34] and in intracellular transport [5]. As lysosomal enzymes [27], they might play an important role in anabolic processes. Some observations point to their possible peptidase nature [37, 36, 40]. According to the data of BERNSOHN et al. [4], non-specific esterase may also occur in the neuropile structures and in the postsynaptic membrane.

These observations as well as the fact that non-specific esterases may be found in the whole hypothalamus, would certainly favour the view that within the hypothalamic neurons there is no probability of any special relationship to specific activities related to neuro-hormonal control functions.

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DIE ESTERASENAKTIVITÄT IM HYPOTHALAMUS

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In den verschiedenen Hypothalamusfeldern kann eine AChE-Aktivität unterschiedlicher Intensität und cellulärer Lokalisation nachgewiesen werden. Eine Neuropilreaktion läßt sich nicht beobachten.

Zwischen der AChE-Aktivität und dem funktionellen Zustand besteht in den verschiedenen Hypothalamusbezirken keine direkte Beziehung, zumindest kann mit den gegenwärtig zur Verfügung stehenden histologischen Untersuchungsverfahren keine solche Beziehung aufgezeigt werden. An Abschnitten, in denen keine adequaten funktionellen Veränderungen angenommen werden können, lassen sich Reaktionsänderungen beobachten, und umgekehrt, die AChE fehlt in Bereichen, wo ihre Anwesenheit erwartet werden könnte. Der AChE-Gehalt des Hypothalamus scheint an eine allgemeine celluläre Aktivität gebunden zu sein, und steht nicht unter humoraler Steuerung. Die Entfernung bestimmter endokriner Organe wird in der Regel von einer Aktivitätsverringerung gefolgt, unabhängig von der Art des entfernten Organs oder der hypothalamischen Lokalisation.

ЭСТЕРАЗНАЯ АКТИВНОСТЬ В ГИПОТАЛАМУСЕ

Т. МЕСАРОШ, И. Й. ЧУРИ Й,. ХАЗАШ и М. ПАЛКОВИЧ

В различных областях гипоталамуса можно выявить активность ацетилхолинэстеразы различной интенсивности и клеточной локализации. Неуропильной реакции не наблюдается. Неспецифическая эстеразная активность выявляема во всех ядрах и полях.

В различных полях гипоталамуса между активностью ацетилхолинэстеразы и функциональным состоянием не существует непосредственной связи, или по крайней мере такой связи нельзя выявить с имеющимися в распоряжение гистологическими методами исследования. Изменения реакции наблюдаются на участках, где нельзя полагать адекватных функциональных изменений, и, наоборот, участках, где можно полагать наличие ацетилхолинэстеразы, она отсутствует. Содержание ацетилхолинэстеразы в гипоталамусе, повидимому, связано с общей клеточной активностью, и оно не подчиняется гуморальной регуляции. Удаление определенных эндокринных органов, как правило, вызывает уменьшение активности, независимо от вида удаленного органа или от гипоталамической локлизацаии.

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EXPERIMENTAL CEREBRAL HAEMORRHAGE IN THE DOG

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Led by the assumption that in cases of cerebral haemorrhage, systemic hypertension plays an important role in producing dysfunction of the intracranial arterial and venous circulation, a model dog experiment has been elaborated. After craniotomy and opening of the dura, a nylon tube coming from one of the common carotids was retrogradely introduced into a superficial cortical vein. Simultaneously with this anastomosis, blood pressure was increased by adrenaline. As a result, 20 of 33 dogs developed haemorrhage in the white matter on the side of the anastomosis.

Hypertension endangers intracerebral circulation when the possibilities of compensation are exhausted. Functional disorders of the arterial side due to hypertension (spasm, vasodilatation, stasis) are accompanied by impeded venous drainage or venous reflux. Under such conditions, blood flow is slowed down in the capillary-venous area since the wall of these vessels becomes functionally passive. With the slow blood flow, the vessels follow that hydrodynamic law according to which kinetic energy changes into pressure when circulation is arrested. The increased pressure induces sudden rupture of the vessels in the capillary and venous area, the most vulnerable part of the cerebrovascular system and the extravasated blood forms a haematoma. Site and nature of the observed haemorrhages corresponded to those seen in human hypertensive cerebral haemorrhage.

Although investigations into the aetiology and pathomechanism of hypertensive intracerebral haemorrhage have an almost century-old history, no definitive conclusions have yet been reached. The modernized versions of certain initial notions have still a number of champions.

ROUCHOUX (1914) attributed cerebral bleeding to prehaemorrhagic parenchymal malacia, whereas CHARCOT and BOUCHARD (1868) regarded the rupture of miliary aneurysms as the pathogenic factor. Again, LÖWENFELD (1887), STAEMMLER (1936), WESTPHAL (1937) and others thought that cerebral bleeding was due to local vascular necroses induced by diverse pathogenic influences, e.g. degenerative vasopathies, enzymatic actions, angiolytic substances. Some authors, as for instance BEITZKE (1937), claimed to have located the point of rupture in or near the bleeding. It was pointed out by several authors (SCHWARZ, 1926; NEUBÜRGER, 1928—1932; ALAJOUNINE et al., 1936; WOLFF, 1937) that no gross morphological changes but vasomotor disorders were the most essential prehaemorrhagic phenomena. Such phenomena were observed by PAL (1905, 1931) and RICKER (1919) in connection with hypertensive vascular crises and brain commotion.

All the current theories reflect some old notion. The works of ROSEN-

BLATH (1918, 1926), BÖHNE (1927, 1929), LEHOCZKY (1933), GLOBUS et al. (1927, 1937, 1949, 1952) and HICKS and WARREN (1950) are based on Rochoux's theory of prehaemorrhagic necrosis.

Although ELLIS (1909) and PICK (1950) refuted CHARCOT's and BOUCHARD's aneurysm theory, they actually developed it by attributing the haemorrhages to "supermiliary aneurysms". GREEN (1938) and RUSSEL (1963) also referred to the significance of anomalous aneurysms.

Specific vascular lesions as the cause of haemorrhage have been discussed by Japanese authors (OONEDA, 1959; KISHI, 1959; IKEDA, 1964); FEIGIN and PROSE (1959) also regard hypertensive fibrinoid arteritis as an important pathogenic factor of the haemorrhages.

The pathogenic role of dissecting aneurysms, suggested by Virchow, was later accepted by SHARAPOV (1938), SPATZ (1939), PAULIAN et al. (1939), ZIMMERMANN (1949) and QUANDT (1959, 1962).

The significance of prehaemorrhagic functional vascular disturbances continued to be recognized and it was pointed out by SCHWARZ (1926, 1961) that this was the most dynamic and most acceptable hypothesis of all. Functional disturbances associated with hypertension arise at some terminal arterial ramifications in the form of spasm, dilatation, paralysis of the vessels and stasis. Each subsequent disorder becomes graver and increases the possibility of bleeding. Prolonged spasm or vasomotor paralysis leads to diaeresis and diapedesis. This mechanism has been recognized by KÖRNYEY (1939) and SÁNTHA and HABERLAND (1951).

SCHEINKER (1940, 1945), too, described functional disturbances but observed their effect in the venous area and attributed the massive haemorrhages to venous rupture, a view shared by MURPHY (1954), ZIL'BERBERG and CHERKASSKII (1958), COURVILLE (1957), PAL (1931), HILLER (1936), DENNY-BROWN et al. (1956), COBB (1957), RAKONITZ (1961) and MUTLU et al. (1963).

This survey, although far from being complete, shows the divergence of views. Yet, two fundamental principles seem to be universally accepted. According to the first, hypertension is the chief pathogenic factor of haemorrhages: its double role in this respect consists in being one of the causes of morphological changes occurring in the cerebral veins and in being the source of haemodynamic disturbances directly responsible for the haemorrhage. While cerebrovascular lesions promote bleeding, they are less important in this respect than hypertension, a theory championed among others by MOLNÁR and BALAJTHY (1966).

The second almost unanimously accepted principle is that several mechanisms may be involved in the causation of massive haemorrhages. It is conceivable that — as has been instructively demonstrated by MUTLU et al. (1963) — bleedings may be due to rupture as well as to diapedesis. The present

author and his co-workers (1964) also recognized the distinction between diapedetic and rhexic bleedings and attempted to differentiate the two forms both clinically and pathologically. To support this view, experiments were made to imitate the beginning of bleeding and to discover its most essential and most frequent mechanism.

We relied on the assumption that generalized grave hypertension is accompanied by a slowing of venous flow in a given cerebral area and that venous reflux may even occur there. Hypertension, by causing fluctuating and variable changes in the vessels capable of contraction and dilatation, leads to a slowing down of circulation the effects whereof manifest themselves chiefly on the venous side of the capillary system. It is in this almost completely passive part of the cerebrovascular bed that conditions become favourable for the development of haemorrhages, this area being unable to compensate disturbances of arterial and venous circulation.

First, an attempt was made to induce cerebral haemorrhage by elevating blood pressure in the systemic circulation and by creating at the same time venous congestion in the head (Valsalva's manoeuvre), an attempt defeated by the abundant arterial and venous collaterals existing in the dog. Therefore, the method described in the following has been elaborated.

Method

Under chloralose anaesthesia, a mercury manometer was tied into the femoral artery. After isolating both common carotid arteries, a glass tube was introduced through the tracheotomy opening for mechanical ventilation. Then bilateral craniotomy involving the midline was performed. Anteriorly, the incision reached the frontal sinus, posteriorly the occipital protuberance, laterally the approximate level of the zygomatic arch (above the Sylvian sulcus). When opening the dura the superior sagittal sinus and the veins emptying into it were avoided. Bleedings from ruptured emissary veins were carefully controlled. The cortical surface behind the cruciate sulcus was exposed the posterior boundary whereof was usually constituted by a line drawn on the initial portion of the ectosylvian sulcus. The operation exposed furthermore the two upper (III and IV) gyri and the posterior part of gyrus II (see Fig. 1). Of the superficial cortical veins, the bilateral precentral, central and parietal vessels became well visible, together with their ramifications and connections. Following haemostasis NaCl-saturated gaze was applied to the cortical surface.

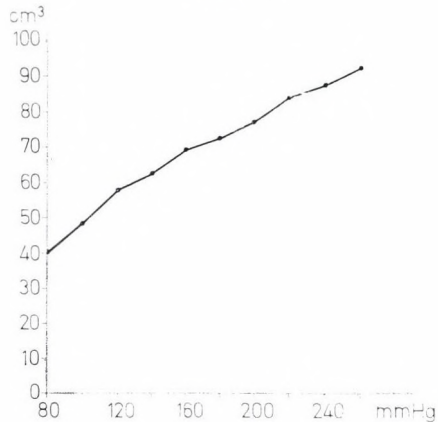
The next manipulation consisted in the distal ligature of one of the common carotid arteries into which a 55 cm long nylon catheter with a diameter gradually diminishing from 3 to 1 mm was inserted. The thin end of the heparinized catheter was connected to the largest contralateral precentral or central vein (Fig. 2), taking care to prevent reflux; we always tried to avoid fastening of the catheter since this manipulation inevitably injured the cortical surface.

The shunt between the common carotid and the cortical vein was clamped after a few seconds of preliminary trial necessary for the checking of adequate blood flow and avoid the possibility of coagulation. The shunt was reopened after 10 to 30 sec when after the administration of 0.5 mg of adrenaline arterial pressure had reached a peak; this usually amounted to 260—340 mm Hg. The brain invariably started to swell, first on the side of the shunt and then on the opposite side. The animals were killed by intravenous hexobarbital when the swelling had lasted 2 to 3 min. The shunt was closed for a short time whenever any disturbance in blood flow, blood pressure, or an injury of the cortical surface was observed.

Slight deviations from the above intervals occurred in some cases owing to the taking of photographs and arteriography.

The volume of arterial blood passing into the vein is given in ml, as determined manometrically from the flow. (Table I shows the amount of blood passing through the catheter

Table I



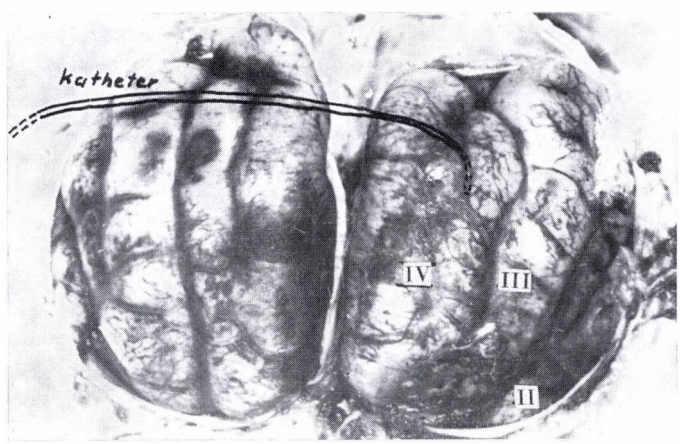


Fig. 1. The exposed area with a schematic illustration of the inserted catheter. Roman numerals indicate arcuate gyri

Fig. 2. Surgical intervention; swelling of brain (increase of mass) during free blood flow

per minute at the given pressures.) The values so obtained express the increase in blood flow into the superficial cortical veins in relation to the increase in blood pressure. Owing to the abundance of cerebrovenous anastomoses, jugular blood pressure would likewise have yielded relative values. It was impossible to determine the pressure in the affected hemisphere.

Some observations made in the course of the experiments have to be mentioned.

1. The arterio-venous shunt, even if it was left open for several minutes or sometimes half an hour, induced but slight swelling of the brain provided tension was normal; this was checked in several instances so as to observe the difference between the effects of normal blood pressure and hypertension. Abundant anastomoses between the superficial cortical veins enabled the venous circulation to bear such stress. Dissection after these manipulations revealed no changes, and histological examination disclosed but a few minute points of diapedesis in the affected cortex.

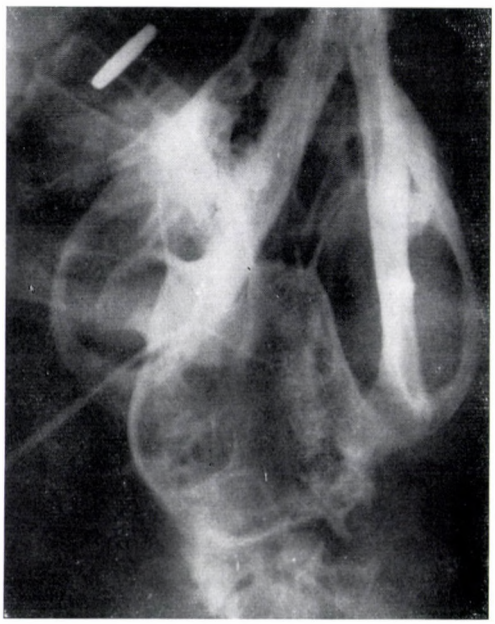


Fig. 3. Phlebography through catheter tied into right cortical vein. A few superficial veins are outlined, and the presence of a haematoma is indicated by the oval-shaped intracerebral accumulation of contrast material

2. The dark violet colour of blood promptly changed into a bright red immediately after the opening of the shunt, while the blood remained unchanged in the veins not involved in the shunt. This allowed to check the size of the area affected by the shunt.

3. In two cases the cortical surface grew livid and petechiae appeared immediately after opening the shunt. Venous drainage being impeded (by thrombosis or a malposition of the catheter) the arterial blood passed directly into the subcortical veins in these cases.

4. The short duration of the experiment, the arterial blood passing into the shunt and the abundant venous anastomoses prevented anoxia.

5. The pressure of blood flowing into the superficial cortical veins and the number of veins participating in the shunt were inversely related so that the amount of blood shown in Table I was dispersed in a few seconds.

6. When we did not succeed in inserting the catheter, it was introduced into the vein on the side of the catheterized carotid. This alternative made no change in either the course of the experiment or in the results obtained.

The experiment was too short to allow the registration of additional parameters, and so it was only after fixation of the brain that we were able to ascertain the result. Palpation of large haematomas (fluctuation!) provided information in some cases. Phlebography revealed considerable accumulation of contrast material in one or two cases. (Fig. 3.)

Material and results

To induce cerebrovascular changes in hypertension by means of perirenal fibrosis, both kidneys of 12 mongrel dogs were wrapped in cellophane (PAGE, 1931). Two dogs died during the manipulation; in the surviving 10 animals under chloralose anaesthesia blood pressure was measured in the femoral artery before the renal operation, then 3 to 4 months later and for the last time immediately before the cerebral operation, *i.e.* after 6 to 9 months, always under identical conditions. Values so obtained are listed in Table II. Blood pressure tended toward normal after six months, a well-known phenomenon in cases of experimental hypertension (KÖVÉR et al., 1960). Two of the hypertensive animals succumbed to the intracranial intervention so that only eight dogs of this group were left for the second part of the experiment.

Table II

Number of animals	Initial value, mm Hg	3—4 months mm Hg	6—9 months mm Hg
1	80	300	140
3	100	230	165
4	110	210	130
5	135	320	150
6	90	305	150
7	130	280	180
8	140	170	170
10	130	210	140
11	150	160	130
12	140	165	170

Only craniotomy was performed on 25 dogs; their blood pressure amounted (in chloralose anaesthesia) to 90—140 mm Hg at the outset of the experiment.

The two groups will not be treated separately in the following, as their members showed no difference as regards either the extent or the nature of the haemorrhages. The experiment with the 8 "hypertensive" and 25 "normal" dogs yielded positive results, namely more or less extensive subcortical bleeding, in 20 cases. Negative results were due mostly to our having failed to create an adequate shunt. The haemorrhage had to be neglected in some cases owing to superficial laceration.

The site of bleedings as also their nature and approximate extent are shown in Table III. Arabic numerals indicate members of the hypertensive,

Table III

Dog No.	Localization of haemorrhage	Nature	Size, cm
3	R. subcortical parietooccipital	Perisulcal linear	—
6	R. temporoparietal white matter	Massive	2.5 × 0.5
7	Bilateral subcortical parietal	R. destructive L. linear	2.0 × 0.5
8	L. subcortical central	Massive (ventricular)	2.0 × 1.0
III	R. subcortical parietooccipital	Destructive (ventricular)	4.5 × 0.5
IV	R. hemispheral white matter	Massive	4.5 × 2.0
V	L. subcortical parietal	Petechial	—
VI	R. subcortical hemisph.	Massive	5.0 × 2.0
VII	L. subinsular centroparietal	Massive	2.0 × 1.5
VIII	L. subinsular white matter	Massive	2.5 × 1.0
IX	L. subcortical central	Petechial	—
XII	R. subcortical parietooccipital	Linear	—
XIII	R. subcortical parietooccipital	Petechial linear	2.0 × 0.5
XIV	R. subcortical white matter	Linear	—
XVII	L. subcortical parietal	Linear (ventricular)	1.0 × 0.3
XVIII	L. temporoparietal white matter	Massive	3.0 × 1.0
XIX	R. frontocentral white matter	Massive	1.5 × 1.0
XX	L. subinsular centroparietal	Massive	3.5 × 1.5
XXIV	R. subinsular	Linear	—
XXV	R. subcortical-cortical parietooccipital	Haemorrhagic infarction	—

R. = right side
L. = left side

Roman numerals those of the normal, group. Figs 4 and 5 present pictures of haemorrhages.

According to localisation and general features, three types of bleeding were observed.

1. Subcortical linear haemorrhage limited in extension near the insertion of the catheter. It was usually parallel to the U-fibres and situated below one or two gyri. Small subcortical petechiae were also classified under this type (Fig. 6a).

2. More extensive, usually wedge-shaped massive haemorrhage beneath the parietal cortex reaching and often invading the upper and lateral border of the lateral ventricle (Fig. 6b).

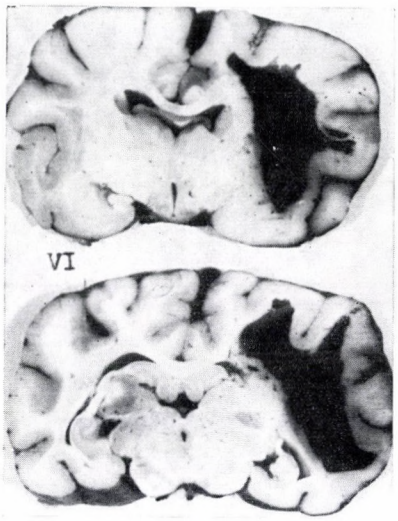


Fig. 4. Massive haemorrhage in the white matter of the right hemisphere which saves the cortex but destroys the basal ganglia

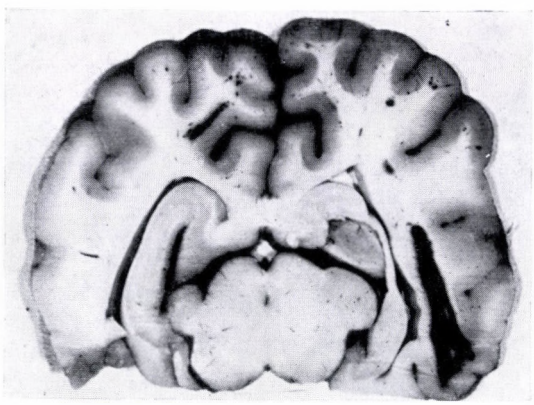


Fig. 5. Elongated haematoma in the temporal lobe. Bleeding on the opposite side is the result of a preceding unsuccessful experiment

3. Large massive bleeding which destroyed the major part of the hemisphere's white matter. Adjacent to the basal ganglia, it did not affect the cortex and was sometimes accompanied by ventricular haemorrhage (Fig. 6c).

Obstruction of venous drainage induced typical haemorrhagic infarction in one case (Fig. 7).

Two main factors seemed to determine the type of bleeding.

1. Successful catheterization, *i.e.* production of a blood flow of adequate kinetic energy. The extension of the venous system shunted with arterial

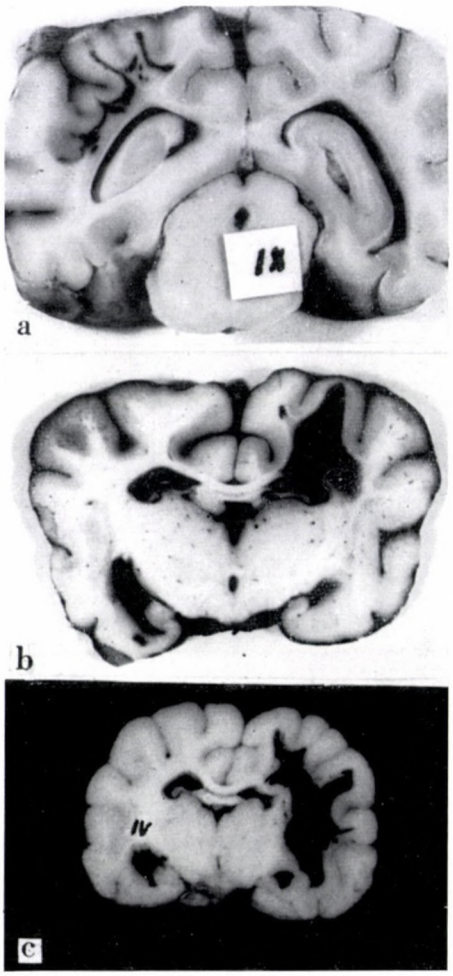


Fig. 6. Types of bleeding: *a* = subcortical elongate; *b* = wedge-shaped, invading the ventricle; *c* = massive, destructive in the larger hemisphere

flow was a decisive factor in this respect. If it was of limited extent, the resulting linear or petechial haemorrhage was subcortical.

Another factor was the degree of induced hypertension.

2. A further decisive factor was age. Massive hemispheral bleeding was observed in three old dogs whose dura was calcified and whose superficial pial arteries were tortuous and rigid. Age-conditioned vascular degeneration rendered the animals susceptible to bleeding.

Arteriograms made in some cases of open shunt revealed a considerable accumulation of contrast material in the subcortical medulla according to the site of the petechial or massive haemorrhage (Fig. 3).

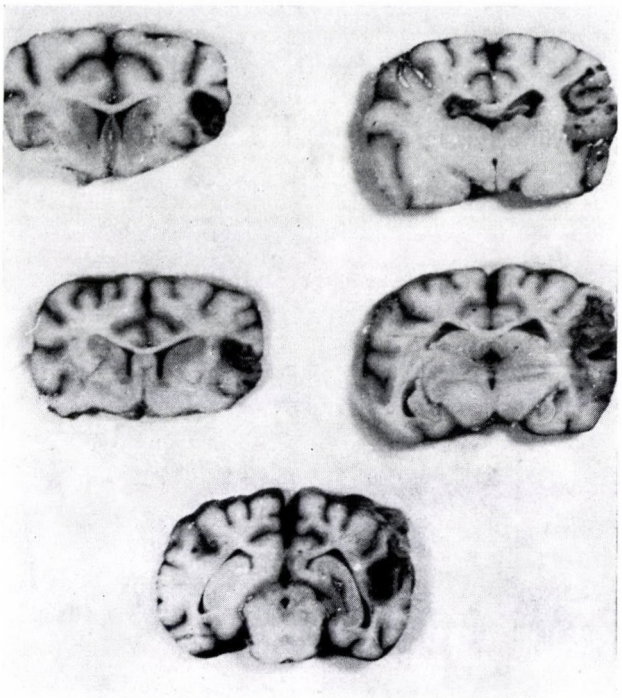


Fig. 7. Haemorrhagic infarction

Histological results. No changes were found in the basilar, pial and intracerebral vessels of the "hypertensive" dogs. The media in the arteries of older animals displayed fibrosis and elastolysis in both groups. Grave fibrosis and also hyalinosis of the basilar and pial vessels were registered in some members of the hypertensive group. This group was not large enough to admit of conclusions as to the vascular changes.

Bleedings were sharply circumscribed (Fig. 8). Petechiae, frequently accompanied by a loosening of tissues, inhibitions with plasma, and oedema, were observed in the marginal portions of the cerebral substance. Massive haemorrhages were structurally similar to those of human subjects. The blood flowed out sometimes at the removal of the brain leaving a narrow gap to show the destroyed tissue. The cross section of an occasional large vessel, suggestive of a vein, was dimly perceived in small haemorrhages (Fig. 9). The surrounding concurrent bleedings appeared — as in humans — to be of capillary or venous origin (Fig. 10).

Slight petechial and massive subcortical haemorrhages were often associated with minute cortical diapedetic bleeding which appeared in one or two foci. They were like the cortical bleedings seen in haemorrhagic infarction without, however, any sign of anoxia. No cortical lesions were observed

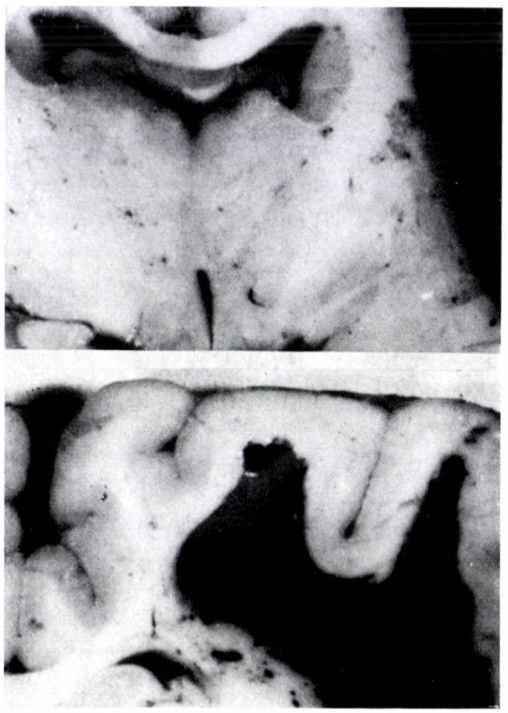


Fig. 8. The upper picture shows the sharp borders of bleedings, the lower one presents concurrent minute marginal bleedings

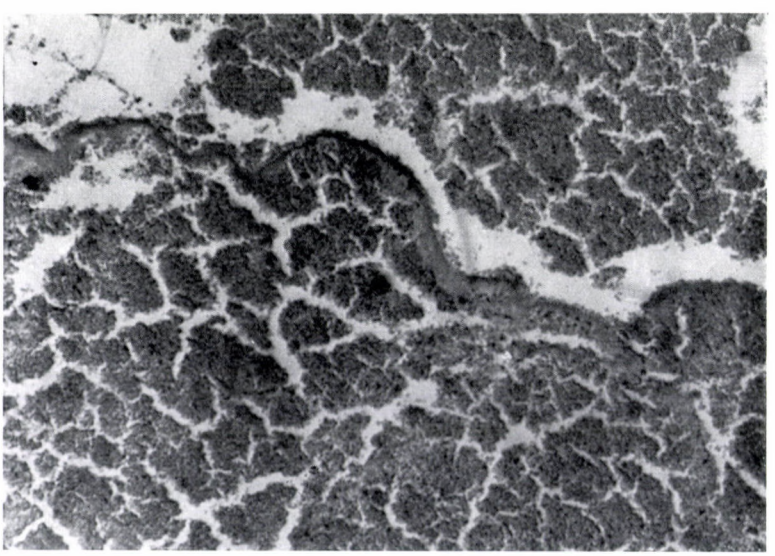


Fig. 9. Detail of vessel wall in haematoma

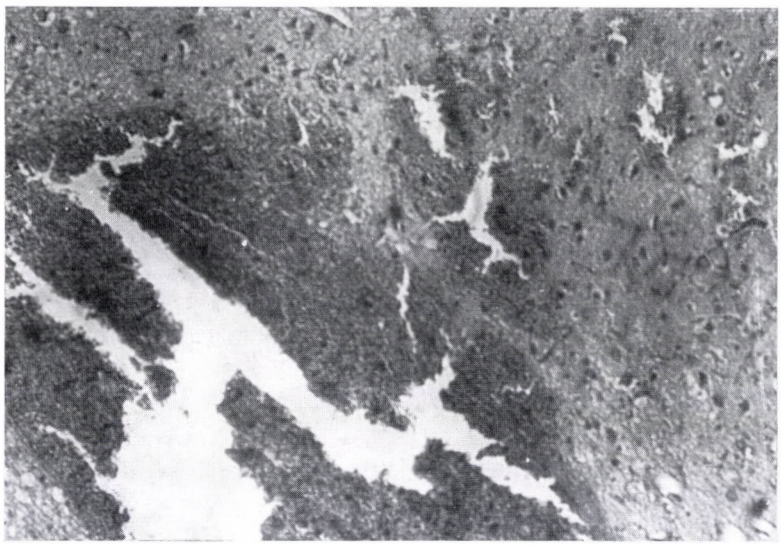


Fig. 10. Minor haemorrhages in subcortical white matter and cortex

in connection with massive haemorrhages; minute cortical lesions seen in cases of slight haemorrhage were often at some distance from and not immediately below the insertion of the catheter. These observations tend to show that our experiments did not consist in the rupture of one or two major vessels since the point of bleeding was in most cases remote from the maximum of inflow. The blood flowing in soon dispersed in the superficial venous apparatus.

Signs of passive hyperaemia were invariably more pronounced on the side of bleeding, a natural haemodynamic phenomenon.

The experiments have proved that the cortex is more, and the white matter in the subcortex and near the basal ganglion less, resistant to haemodynamic stress. The analogue of this fact is well-known from human hypertensive cerebral haemorrhage.

Discussion

Spontaneous cerebral haemorrhage imitating human apoplexy has not been observed in animals, nor have investigators succeeded in inducing it experimentally (STAMLER, 1958; WHISNANT, 1958; LUGINBÜHL, 1966). As to the attempts to induce cerebrovascular lesions in animals WERTHEIMER et al. (1933) caused bleeding by increasing and decreasing the pressure of cerebrospinal fluid. Since intracerebral venous tension depends on the pressure of the cerebrospinal fluid, their experiment showed that fluctuations of the venous pressure contribute to the development of cerebral haemorrhage. GLOBUS et al. (1949, 1952), after unilateral ligation of the middle cerebral artery, pro-

duced periodic hypertension by means of adrenaline and observed in some cases haemorrhages during the postoperative days as also at later dates. Following the ligature or embolism of the middle cerebral artery haemorrhages were after parenchymal damages with or without hypertension (VAN WULFFTEN, 1932; BROMAN, 1939; FAZIO and SACCHI, 1940—1943; KASE, 1959; KATO, 1964). These attempts seemed to support the theory of prehaemorrhagic malacia, but were — as stated by SCHEINKER (1946) and ADAMS (1953) — really imitations of haemorrhagic infarction.

We received the impulse to elaborate our method from the attempts of certain authors who tried to improve cerebroarterial circulation retrogradely via the venous channels (BECK, 1949; MCKHANN et al., 1950; GURDJIAN et al., 1950). OWENS (1960) increased cerebral flow by ligating of the internal jugular vein and demonstrated that a retrograde venous-capillary circle may be produced which improves the oxygen supply of a given area (1963). Another information concerning venous circulation was provided by DENNY-BROWN et al. (1956) who succeeded in provoking various functional disturbances and even haemorrhagic infarcts by retrogradely injecting saline solution into the cortical veins.

Relying on the evidence of the present results, we propose to explain our theory regarding the mechanism of cerebral bleeding.

Hypertension means a great stress for systemic circulation. Extracerebral and intracerebral regulatory mechanisms protect the brain from the harmful effects of the circulatory fluctuations. Prolonged hypertension and arteriosclerosis create a state in which the intracranial vessels too become involved in the circulatory disorder (AKSYANTEV, 1956) so that certain areas will be vulnerable by anoxia and stasis, a condition leading to spasms, vasodilatation, acute or prolonged vasoparalysis which may vary from one to the next vascular segment. The result — whatever the actual disorder — will be the slowing down of circulation. The compensatory mechanisms may counteract the disturbance for some time, but the situation on the arterial side may then become such as to exclude further possibilities of compensation. Venous and capillary dysfunction becomes still more pronounced since, lacking the power of compensation, the veins and capillaries will be passively involved in the arterial disturbance. According to hydrodynamic laws, extreme slowing or stoppage of blood flow in the arteries, capillaries and veins of a given area will cause these vessels to behave like rigid tubes. According to the most important biophysical law of blood flow, all kinetic energy changes into pressure when flow is arrested. Susceptibility of the vessels to rupture is greatly promoted by the pressure which acts on the vascular wall, its force depending on the caliber of the vessel.

Under such conditions it is the capillary and venous system which is most easily ruptured. This tendency is the more pronounced the longer the

vessel (e.g. the subcortical veins) and the poorer the anastomotic communications to divert the heavy pressure (regions of the basal ganglia) and, of course, the thinner the wall of the affected vascular segment.

Rupture may occur at any point. One is dealing in most cases with acute disturbances, and the development of a haemorrhage is a matter of seconds. Once a haematoma develops, different actions will become operative in and around the affected area. Extent, site and nature of the haemorrhage depend on the quality of the area, its size and the quality of its vessels in which blood flow is arrested to such an extent as to cause that amount of kinetic energy to change into pressure which suffices for inducing a sudden rupture of the vessel wall.

The last phase of massive hypertensive cerebral haemorrhage — irrespective of the nature of arterial dysfunction (spasm, dilatation) — is always accompanied by increased venous pressure which tends to aggravate the disturbance on the arterial side. Haemodynamic phenomena of this kind explain the development of haemorrhage.

If it is permissible to speak of a "haemorrhagic unit", it may be represented by the smallest capillary or postcapillary segment in which pressure becomes high enough to cause a sudden rupture and consequential bleeding at a given moment. Numerous such closely packed miniature units undergo rupture in massive hypertensive haemorrhage.

The pressure may be high enough to rupture a murally defective precapillary arteriole or venule, but the vessels of the "passive" (capillary venous) segment are more likely to cede to pressure.

Our model experiment, in addition to explaining the mechanism of the sudden development of massive bleedings, seems to prove that a lesion of the vascular wall is not an invariable precondition of haemorrhage. In the experiment the critical situation was produced by seriously impeding subcortical venous drainage over the entire hemisphere; this was achieved by means of a retrograde cortical venous-carotid anastomosis, while simultaneous hypertension increased the vascular resistance and so the slowing down of blood flow by way of the spasm of minor arteries, and of other circulatory disturbances. Dysfunction was mostly apparent in the area drained by the superficial veins. Our method seems to be suitable for producing haemorrhage also in other parts of the body by a retrograde shunt of some venous bed or sinus.

Similar "haemorrhagic units" are formed in connection with haemorrhagic infarcts, sinal and cortical venous thromboses, but anoxia, the essential feature of such lesions, makes these haemorrhages different; besides, also vascular factors and parenchymal damage have to be taken into account in haemorrhages of this kind.

It will be evident from the foregoing that a cerebral circulatory dysfunc-

tion is an essential feature of hypertensive haemorrhages. The functional disturbance is usually restricted to a circumscribed area. Another essential factor is the simultaneous or consequential increase of intracerebral venous pressure which aggravates the circulatory disorder to the point of stasis and may even give rise to backflow. The pathomechanism of cerebral haemorrhages acts in our opinion by way of haemodynamic factors as outlined above, and hypertensive bleeding is a result of their combined actions.

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EXPERIMENTELLES HERVORRUFEN EINER GEHIRNBLUTUNG AM HUND

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Unter der Annahme, daß bei der Gehirnblutung der allgemeinen Hypertension im Zustandebringen der funktionellen Störungen sowohl des intrakraniellen arteriellen, als auch des venösen Kreislaufs eine große Rolle zukommt, wurde an Hunden der nachstehend beschriebene Modellversuch angestellt. Nach Craniotomie und Eröffnung der Dura mater wurde aus der A. carotis communis der einen Seite in dem Blutstrom entgegengesetzter Richtung ein sich allmählich verjüngendes Nylonrohr in eine oberflächliche Corticalvene eingeführt. Gleichzeitig mit der Eröffnung dieser arterio-venösen Anastomose wurde den Tieren zwecks Blutdrucksteigerung Tonogen verabreicht. Im Ergebnis dieser Eingriffe waren bei 20 der 33 Hunde auf der Anastomosenseite Gehirnblutungen unterschiedlichen Ausmaßes in die weiße Substanz der Großhirnhemisphäre entstanden.

Die Hypertension wird für den intracerebralen Kreislauf dann zur Gefahr, wenn eine Lage entsteht, bei der das Einschalten der bekannten Kompensationsmechanismen unmöglich wird. Zu den funktionellen Störungen der arteriellen Seite (Spasmen, Vasodilatation, Stase), die in solchen Fällen durch die Hypertension herbeigeführt werden können, gesellt sich eine Behinderung des venösen Abflusses geringeren oder größeren Grades bzw. venöser Rückfluß hinzu. Durch diese Störung wird im Bereich der Venen und Kapillaren der Kreislauf erheblich verlangsamt, da die Wände dieser Gefäße funktionell passiv sind. Bei der bedeutenden Verlangsamung der Strömung gelangen diese Gefäße unter den Einfluß der Gesetze der Hydrodynamik, und es kommt die Regel zur Geltung, wonach bei der Einstellung der Zirkulation, in einem gegebenen Bereich, sich jede kinetische Energie in eine auf die Wände einwirkende Druckenergie umwandelt. Auf dem Gebiet der Kapillaren und Venen, das am verletzlichsten ist, entstehen in Sekundenschnelle in unterschiedlicher Ausbreitung Risse in der Gefäßwand, die zur Bildung eines zusammenhängenden Hämatoms führen. Das beschriebene Experiment ist eine Nachahmung dieser Lage, die gemäß dem Verfasser eine entscheidende Phase des Pathomechanismus der Gehirnblutung ist. Die Lokalisation und der Charakter der beobachteten Blutungen entsprachen der hypertensionsbedingten Gehirnblutung des Menschen.

СОЗДАНИЕ ЭКСПЕРИМЕНТАЛЬНЫХ КРОВОИЗЛИЯНИЙ В МОЗГ У СОБАК

Л. ЛЕЕЛ—ЭШШИ

Полагая, что при кровоизлиянии в мозг общее повышение кровяного давления имеет большое значение для возникновения функциональных расстройств внутричерепного артериального и венозного кровообращения, автором был проведен следующий эксперимент на модели. После краниотомии и вскрытия твердой мозговой оболочки в одну поверхностную кортикальную вену из общей сонной артерии одной стороны в направление против кровотока была введена постепенно утончающаяся найлоновая трубка. Одновременно с созданием этого артерио-венозного анастомоза кровяное давление было повышено дачей Тоногена. В результате этих вмешательств на стороне анастомоза у 20 из 33 собак возникли кровоизлияния в белое вещество полушария головного мозга.

Гипертония представляет опасность для внутримозгового кровообращения тогда, когда возникает такое положение, при котором включение известных компенсаторных механизмов становится невозможным. К функциональным расстройствам артериальной стороны (спазмы, расширение сосудов, стаз), вызываемым в таких случаях повышенным давлением, присоединяются более или менее значительная препятственность венозног

оттока или венозный обратный ток. Этим расстройством в существенной мере замедляется кровообращение в области капилляров и вен, так как стенки последних функционально пассивны. При значительном замедлении кровотока эти сосуды подчиняются управлению законов гидродинамики, и проявляется правило, согласно которому в случае приостановления циркуляции, на конкретной территории, вся кинетическая энергия преобразовываются в энергию давления, оказанного на стенки. В области капилляров и вен, в наиболее ранимой части, в течение мгновения возникают трещины стенки сосуда, что приводит к образованию слияющуейся гематомы. Вышеизложенный эксперимент подражал этому положению, которое, по мнению автора, является самой решающей фазой патомеханизма кровоизлияния в мозг. Локализация и характер наблюдаемых кровоизличний соответствовали кровоизлияниям в мозг у человека вследствие гипертонии.

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FLUORESCENCE HISTOCHEMICAL INVESTIGATIONS OF CONNECTIVE TISSUE

III. FLUORESCENCE HISTOCHEMISTRY OF THE HEPARIN CONTENT OF MAST CELLS*

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In an attempt to find a relation between the histochemical properties of the dyes and their chemical structure, staining of the heparin content of mast cells was investigated using 18 different fluorochromes, under various histotechnical conditions. Fluorochromes of strong cationic character and dyes showing metachromasy seemed to be most suitable for the histochemical demonstration of heparin and acid mucopolysaccharides. The group-reaction character of the staining processes is emphasized and the fact is stressed that the stains are heparin specific only.

Introduction

The mast cells occurring in most loose connective tissues have diverse functions. On account of their heparin, histamine and serotonin content they have a key position in several normal and pathological processes (blood coagulation, fibrillogenesis, allergy, chronic inflammation, oncopathological relations, etc.). They may rightly be called as unicellular endocrine glands of the organism. Numerous attempts were made to develop reliable techniques for the demonstration of mast cells. Their mono-amine content was demonstrated by means of fluorescence histochemical methods using paraformaldehyde *e.g.* for serotonin and o-phthalaldehydes for histamine. The heparin content of mast cells can be determined by any basophilic reaction under standard conditions, as in acid solution all cationic dyes or metal colloids of cationic nature are bound by electrostatic forces to the anionic sulphates of heparin. Considering that basophilia is a group reaction, the use of various staining techniques introduced by various authors as specific procedures may be misleading from the point of view of interpretation and evaluation of the results, all the more as no rational standardization based on uniform criteria of the staining reactions, has been achieved in histochemistry. Thus, the staining methods used for histochemical demonstration are rather unreliable.

For the estimation of heparin, numerous staining techniques and metachromasic reactions have been employed. On the basis of certain theoretical considerations [8] we have concluded that particularly sensitive and simple

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staining reactions for heparin and acid mucopolysaccharides can be obtained with cationic fluorochromes. Some fluorescent histochemical stains have been known to demonstrate acid mucopolysaccharides and heparin [2, 4, 11, 14] but the aim of the present study was to establish certain criteria for the right selection of the fluorochrome reaction using heparin as a chromotrope, by comparing the histochemical effects of various cationic fluorescent dyes of different chemical properties.

Material and method

a) *Preparation of mast cell samples*

1. Sterile coverslips were placed in the abdominal cavity of albino rats weighing 100—200 g, under ether anaesthesia. Four hours later the rats were killed, the abdominal cavity reopened and the coverslips removed and stained. On the coverslips a cellular layer rich in mast cells and lymphoid elements was found.

2. Albino rats of about 100—200 weight were killed under ether anaesthesia and 2×2 cm specimens were excised from the back skin and stretch preparations were made also from the panniculus carnosus.

b) *Staining*

Unfixed or prefixed preparations (prefixation in 4% neutral formol or absolute ethanol-formalin, 4 : 1, for two hours) were immersed in staining solutions of various concentrations (0.001; 0.01; 0, 1.0%) from 10 seconds to 30 minutes. Acetic acid in 5 and 10% concentration, distilled water or Walpole's buffer pH 1.4; 2.6; 3.5; 4.5; 5.2 were used as solvents.

The dyes employed were 1. Acridine Orange (Michrome); 2. Acridine Yellow (Michrome); 3. Acriflavine (Michrome); 4. Auramine O (Michrome); 5. Aurophosphine (Michrome); 6. Astra Violet 3R extra (Bayer); 7. Berberine (Fluka); 8. Coriphosphine O (Michrome); 9. Euchrysine 2GNX (Michrome); 10. Flavophosphine (Michrome); 11. Magdala Red (Schuchardt); 12. Methylene Blue (Grübler); 13. Neutral Red (Chinoin); 14. Phosphine 3R (Michrome); 15. Pyronine Y (Michrome); 16. Rhodamine B (Michrome); 17. Rivanol (Bayer); 18. Thioflavine T (Michrome).

After staining at room temperature the preparations were washed with water and buffer solution and mounted in distilled water.

c) *Fluorescence microscopic examination*

Fluorescence microscope: Zeiss HB 50. Filter combination: BG-OG and UG-GG. To increase the inducing light, a drop of glycerol was placed between the condensor front lens (aperture 1.4) and the slide. Photo: Practics, Orwocolor No. 16.

d) *Control procedures*

1. Digestion with ribonuclease (1 mg/ml distilled water at 37°C, for one hour (RNAse Reanal, Budapest)).

2. Digestion with hyaluronidase (75 I. U./ml solvent at 37°C, for three hours (Organon, OSS)).

3. Methylation (at 60°C, for two hours).

4. Demethylation (at room temperature, for 20 minutes).

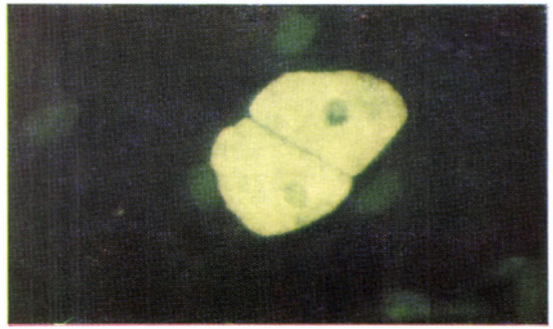

Fig. 13. Thioflavine T staining. Peritoneal mast cells. UG + GG filter combination. ×640

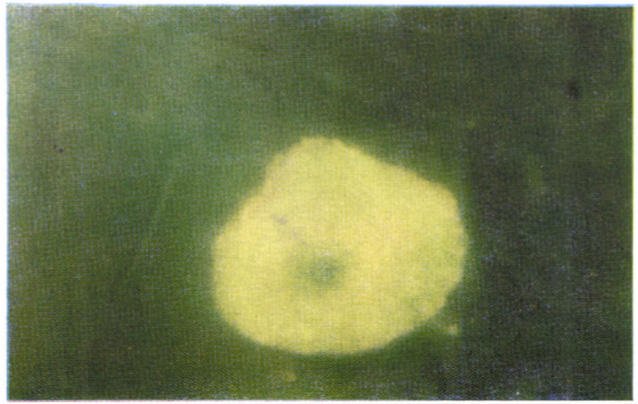

Fig. 14. Thioflavine T staining. Peritoneal mast cells. UG + GG filter combination. ×160

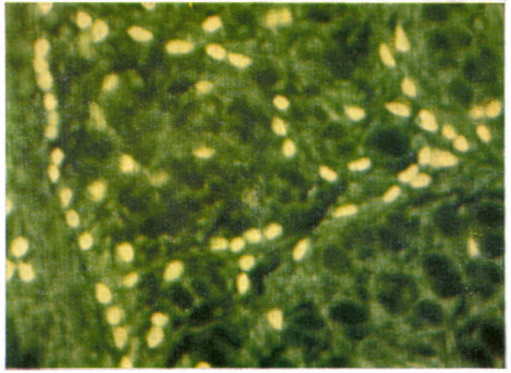

Fig. 15. Auramine O staining. Mast cells in stretch preparation. BG + OG filter combina tion. ×120

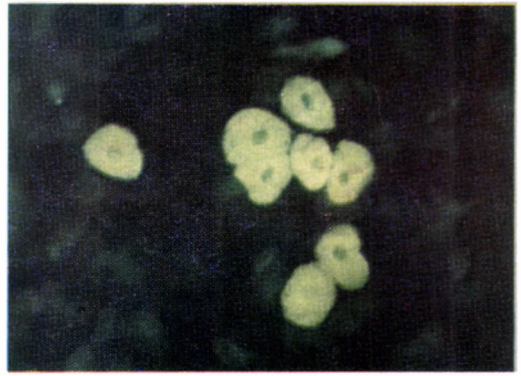

Fig. 16. Berberine staining. Peritoneal mast cells. UG + GG filter combination. ×400

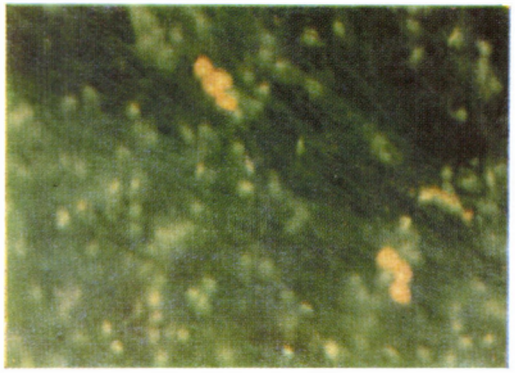

Fig. 9. Acridine yellow staining. Mast cells in stretch preparation. BG + OG filter combination. ×120

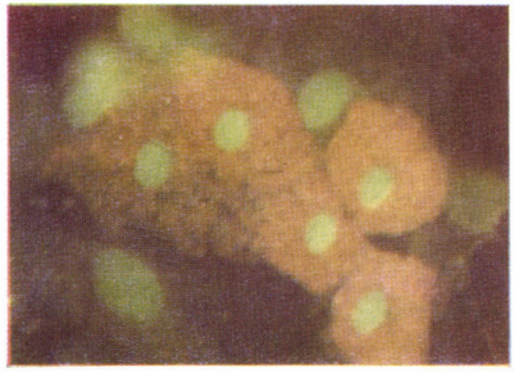

Fig. 10. Acridine yellow staining. Peritoneal mast cells. BG + OG filter combination. ×120

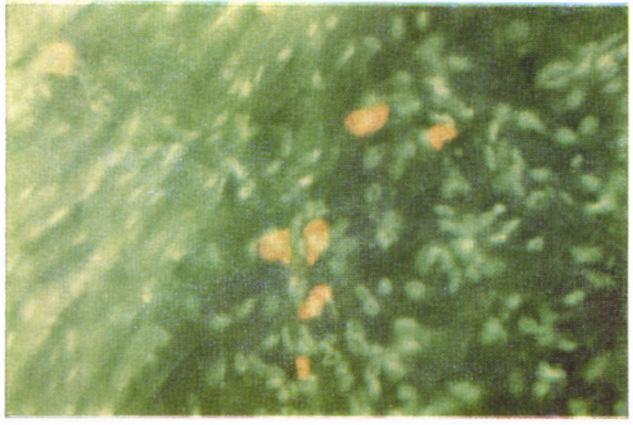

Fig. 11. Acriflavine staining. Mast cells in stretch preparation. BG + OG filter combination. ×120

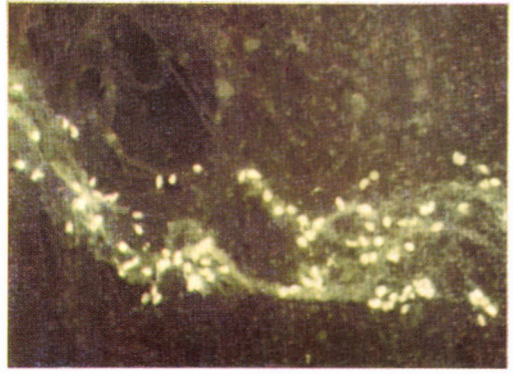

Fig. 12. Thioflavine T staining. Mast cells in stretch preparation. UG + GG filter combination. ×50

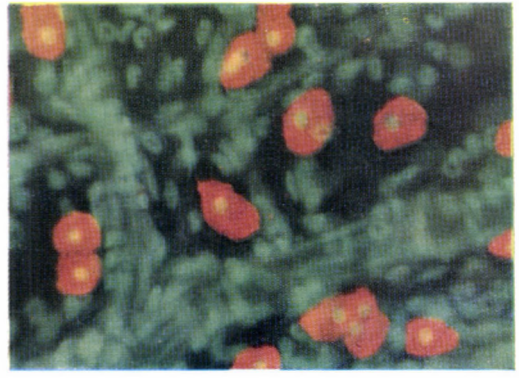

Fig. 5. Euchrysine 2GNX staining. Mast cells round an arteriolar bifurcation. Stretch preparation. BG + OG filter combination. ×400

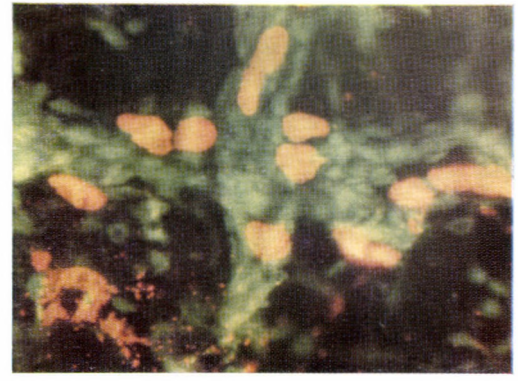

Fig. 6. Acridine orange staining. Mast cells around a crossing of arterioles. BG + OG filter combination. ×400

Fig. 7. Acridine orange staining. Peritoneal mast cells. BG + OG filter combination. ×150

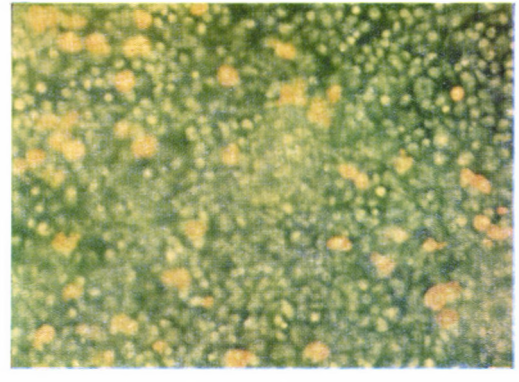

Fig. 8. Coriphosphine staining. Peritoneal mast cells. BG + OG filter. ×120

Fig. 1. Flavophosphine staining. Peritoneal mast cells. BG-OG filter combination. ×640

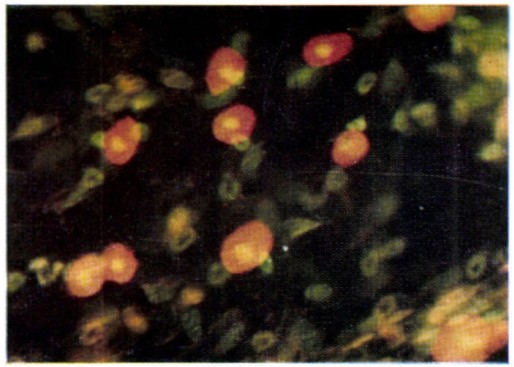

Fig. 2. Flavophosphine staining. Peritoneal mast cells. BG + OG filter combination. ×400

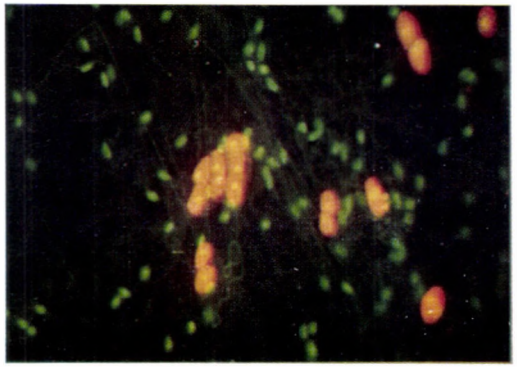

Fig. 3. Flavophosphine staining. Mast cells in stretch preparation. BG + OG filter combination. ×150

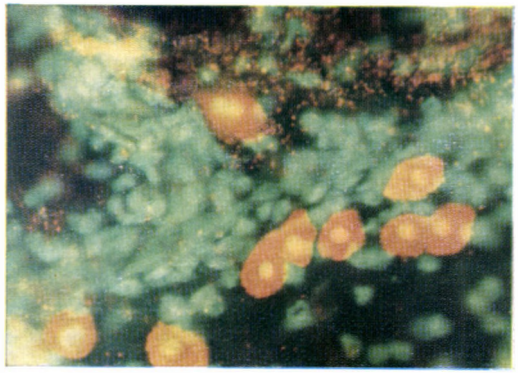

Fig. 4. Euchrysine 2GNX staining. Mast cells in stretch preparation. GB + OG filter combination. ×400

Results

Under the histotechnical conditions of our laboratory, optimal results were obtained with solutions containing 0.01% of dye dissolved in 10% acetic acid, after prefixation of the samples in 4% neutral formol or ethanol formol. Without previous fixation, at the low pH values applied the pattern of mast cells was not clearly visible. Of all the dyes tested, Magdala Red was the only non-selective one and the specificity of Phosphine RH and Pyronine Y was weak. Methylene Blue and Neutral Red yielded a specific reaction but their fluorochrome properties were poor. All the other dyes yielding secondary fluorescence gave a positive and selective result with the granular substance of mast cells. The fluorescence was not affected by ribonuclease and hyaluronidase but always disappeared after methylation. On demethylation the staining became similar to that of untreated preparations.

Heparin was selectively stained by the following dyes (optimal duration of staining is given in brackets). In blue light orange-red metachromasia fluorescence was obtained with Flavophosphine (10 sec) and with Euchrysine 2GNX (10 sec) (Figs 1, 2, 3, 4, 5), an orange-yellow fluorescence with Acridine Orange (10 sec) (Figs 6 and 7) and with Coriphosphine (3 min) (Fig. 8).

Acridine Yellow (3 min), Aurophosphine (3 min) and Acriflavine (6 min) produced in blue light a yellow-orange or yellow fluorescence of heparin (Figs 9, 10, 11). Orange-red fluorescence was produced with Astra Violet 3R Extra (2 min) in blue light. Rhodamine B (20 sec) showed a yellow fluorescence in violet light. Thioflavine T (2 min), Auramine O (2 min) and Berberine (2 min) produced in violet light a selective intensive lemon-yellow non-metachromasic fluorescence of the mast cell granules (Figs 12, 13, 14, 15, 16). The majority are acridines (Acridine Orange, Acridine Yellow, Acriflavine, Aurophosphine, Phosphine 3R, Rivanol), two of them are xanthenes (Pyronine Y, Rhodamine B) and two (Magdala Red and Neutral Red) azine dyes.

The rest of dyes used belong to various groups: Astra Violet 3R extra is a methyne, Berberine is a natural (alkaloid) dye, Auramine O is a phenylmethane, Thioflavine T a thiazole and Methylene Blue a thiazine dye. The cationic nature of all of these dyes is shown by the number of auxochrome (salt-forming) groups of basic character. These are amino groups quaternizing in solution, ensuring thus the dipolar character of the dye. In the list below we have given the amino group content of the dyes tested (giving in brackets the number of free and bound amino groups). The dyes have been listed also in the order of their histochemical value estimated on the basis of the experimental results.

Discussion

The heparin content of mast cells was verified by control procedures. Resistance to hyaluronidase and ribonuclease was considered the criterion of

Dye	Number of amino groups	Free	Bound
Flavophosphine	3	3	0
Euchrysine 2GNX	3	3	0
Acridine Orange	3	2	1
Acridine Yellow	3	3	0
Coriphosphine O	3	2	1
Acriflavine	3	3	0
Astra Violet 3R extra	2	1	1
Berberine	1	1	0
Auramine O	3	1	2
Thioflavine T	2	1	1
Rivanol	3	2	1
Pyronine Y	2	0	2
Rhodamine B	2	0	2
Phosphine 3R	3	3	0
Neutral Red	4	2	2
Methylene Blue	3	2	1
Magdala Red	4	2	2

the presence of heparin [13]. Methylation has a blocking effect on the reactive sulphates in the heparin molecule.

Satisfactory histotechnical conditions for heparin-selective reactions can be ensured by previous fixation, a low concentration of the dyes at low pH, and a short staining time.

Of the dyes tested the acridine showing metachromasic fluorescence (Auramine O, Astra Violet, Berberine and Thioflavine T) yielded the most specific and intensive reactions. Comparison of the histochemical value and cationic character of the stains revealed a parallelism between the number of free amino groups, proportion of free and bound amino groups on the one hand, and heparin selectivity on the other. Most of acridines have three free basic salt-forming groups and the most reliable fluorescent metachromatic reactions were obtained with these dyes. Acridine Orange having one free salt-forming group was the only exception to this rule. Still, Acridine Orange is widely used in fluorescence mucopolysaccharide histochemistry [2, 5, 9], probably because it is the best known and chemically standardized fluorochrome already popular in nucleic acid histochemistry. Our experience has, however, shown that the pronounced cationic Flavophosphine and Euchrysine 2GNX dyes ensure better results with acid mucopolysaccharides than Acridine Orange, and they give a strong metachromasic fluorescence with heparin. Euchrysine 2GNX had been known to have this effect [17] but Flavophosphine

has not been used for this purpose. The other acridine derivates are also selective for heparin but their fluorescence is inferior to that shown by Euchrysine and Flavophosphine. Since the metachromasia depends on certain conditions being fulfilled by the chromotrope and the dye, such as a dense anionic charge system on the chromotrope and a strong cationic activity on the flat dye molecule [10, 15], this type of reaction seems to be preferable for acid mucopolysaccharides. The fluorescent metachromasia of Acriflavine [3, 4, 11] and Coriphosphine [6] is inferior to that of Flavophosphine and Euchrysine 2GNX which may be due to a special electronic structure of the dye molecule.

Thioflavine T, Auramine O and Berberine, in spite of their being weak cations, yielded a selective, non-metachromasic staining of mast cells. So far, these dyes have not been employed for mast cell staining. As under certain circumstances these stains are heparin selective but show no metachromasia it seems that the strong cationic character is required first of all for the production of metachromasia and not for the binding of the dye to the chromotrope.

In spite of their selective heparin reaction the dyes which are not strong fluorochromes are not suitable for fluorescence histochemical assay.

The reactions obtained with all the above dyes are considered to be heparin-specific only under certain limited conditions as we do not know any stain that would be heparin or mucopolysaccharide specific *ab ovo*. Though it should always be borne in mind that all these staining processes are anionic-cationic group reactions a higher selectivity of acid mucopolysaccharides can be achieved by working at low pH values with a short duration of staining in diluted solutions, and the selection of fluorochromes rich in amino groups capable of producing fluorescence metachromasia.

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FLUORESZENZ-HISTOCHEMISCHE UNTERSUCHUNG DER STÜTZGEWEBE

III. Fluoreszenz-histochemische Untersuchung des Heparingehaltes von Mastzellen

L. MÓDIS und M. BATSCHWAROWA

Die Färbung des Heparins in den Mastzellen weißer Ratten wurde mit Hilfe von 18 verschiedenen Fluorochromverbindungen, bei unterschiedlichen histotechnischen Verhältnissen fluoreszenzmikroskopisch untersucht. Verfasser suchten die Zusammenhänge zwischen den vorteilhaften histochemischen Eigenschaften der Farbstoffe und ihrer chemischen Struktur aufzuklären. Sie nehmen an, daß zum histochemischen Nachweis des Heparins und darüber hinaus der sauren Mukopolysacharide die Fluorochromverbindungen mit starker Kationnatur und die Metachromasie herbeiführenden Farbstoffe am besten geeignet sind. Sie betonen, daß ihre Experimente Gruppenreaktionscharakter tragen, und die Ergebnisse der Färbungen bewerten sie nur unter den gegebenen histochemisch-histotechnischen Bedingungen als Heparin-spezifisch.

ФЛЮОРЕСЦЕНТНО-ГИСТОХИМИЧЕСКОЕ ИЗУЧЕНИЕ СОЕДИНИТЕЛЬНЫХ ТКАНЕЙ

III. Флюоресцентно-гистохимическое изучение гепарина в тучных клетках

Л. МОДИШ и М. БАЧВАРОВА

Окрашивание гепарина в тучных клетках белых крыс было изучено с помощью 18 различных соединений флюорохрома, при различных гистотехнических условиях в флюоресцентном микроскопе. Авторы искали связи между благоприятными гистохимическими свойствами красителей и их химической структурой. Предполагается, что для гистохимического выявления гепарина и, сверх этого, кислых мукополисахаридов, наиболее подходящими являются соединения флюорохрома с сильной катионовой природой и красители, обуславливающие метахромазию. Подчеркивается, что эксперименты по окрашиванию имели характер групповой реакции, и результаты расцениваются как гепарино-специфические лишь при данных гистохимических и гистотехнических условиях.

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FLUORESCENCE MICROSCOPIC METHOD FOR THE DIFFERENTIATION OF MAMMARY CONNECTIVE TISSUE COMPONENTS

L. MÓDIS and GY. MATOLAY

(Received January 27, 1969)

Two fluorescence microscopic methods suitable for the differentiation of special and general connective tissues of the female breast are described. The procedures are simple, selective and sensitive and give a good contrast effect. They allow the demonstration of certain stages of breast tumours.

Introduction

The normal ratio of the two kinds of connective tissue forming the mammary stroma, the special (periacinal) and general connective tissue was found to undergo a change in malignant diseases or in the stages preceding them. In certain precancerous stages an increase of the finely fibrillar connective tissue surrounding the acini was especially obvious. After the onset of cancerous proliferation the amount of special connective tissue was found to decrease again as observed by VAN DEN HOOFF [11], PROPST [7] and others in experimental skin tumours. In the case of breast cancer the connective tissue theory of neoplasms described recently by FROMME [2] should be taken in consideration; BERTELS [1] and KONJETZNY [6] also refer to the primary role of connective tissue in carcinogenesis.

Distinction between the two types of connective tissue components can be made in various ways.

a) Morphological differentiation. The special connective tissue is characterized by a fine fibrillar structure while the general stroma is rich in coarse, occasionally hyalinized, collagen fibres.

b) Histochemical differentiation. The special connective tissue stains metachromatically and gives a positive result with the reactions used for the demonstration of acid mucopolysaccharides [3, 10] and the general stroma contains PAS positive mucopolysaccharides.

c) Employment of staining methods used for differentiating precollagen from collagen fibres [4].

d) Polarization microscopic topo-optical reactions used for the differentiation of different types of collagen [8].

Methods

Two fluorescence microscopic methods have been worked out, allowing a simple and reliable differentiation between special and general connective tissue components in the precancerous female breast.

Method A

Fixation in Carnoy's fluid, embedding in paraffin. After deparaffination

1. staining in 0.005% aqueous solution of Thiazine Red (5 minutes);
2. rinsing in distilled water;
3. staining in 0.1% aqueous solution of Blankophor R (Bayer) (5 minutes);
4. washing in distilled water (3 minutes);
5. dehydration in alcohol, clearing with bergamot oil and mounting in Fluoromount (Michrome).

Evaluation of the results was made with a Zeiss fluorescence microscope type HBO 50 with filters UG + GG.

Results. The epithelium stained a rusty-red, the collagen fibres and basal membranes a bright whitish-blue and the finely fibrillar perinuclear connective tissue gave a faint fluorescence (Fig. 1).

Remarks. Blankophor R, a condensation product of 4.4' diamino-2.2' stilbene-disulphonic acid—phenylisocyanate, is a bleaching agent employed in the textile industry, *synonyms* are Lumisol RV, Blancol C, Leucofor R, Photin R, Phontamine White BR, Tintofen X, Rylux C. I. Fluorescent Brightening Agent 30.

Its identity with the Blankophorin R mentioned by ZIEGENSPECK [12] is questionable. According to our observations, Blankophor is selectively staining the old collagen elements of the cornea [9].

Method B

Fixation and embedding in paraffin as in Method A. After deparaffination,

1. staining in 0.1% aqueous Titan Yellow (Michrome) or Primulin (Chroma) solution (20 minutes);
2. washing in distilled water;
3. rapid alcoholic dehydration, clearing in bergamot oil;
4. mounting in Fluoromount.

Evaluation of the results was done as in the former procedure.

Results. Epithelium and special connective tissue stain a faint yellow or brownish-yellow. The coarse collagen bundles give a blue secondary fluorescence (Fig. 2 and Fig. 3).

Remarks. Staining with Titan Yellow gives a deeper colour than with Primulin. These colour effects are visible only if UG + GG filters are used.

Results

The described colour reactions, as most of the histological stainings, are due to an electrostatic binding. Binding of certain dye fractions might be responsible for the blue fluorescence observed on staining with Primulin and Titan Yellow. The exact chemical structure of Primulin is unknown and as a rule, it is the mixture of several fractions of different properties [5].

The following control results seem to be of interest. The fibres of special connective tissue react positively with alcian blue, show metachromasia with toluidine blue, and assume a greyish-black colour if impregnated with silver (Perdrau). The fibres of the general stroma are PAS positive and show a black colour on silver impregnation. The histochemical reaction used for the demonstration of protein (bromphenol blue) yielded a positive result with both kinds of connective tissue. Under a mercury vapour lamp no autofluorescence was observed in unstained sections.

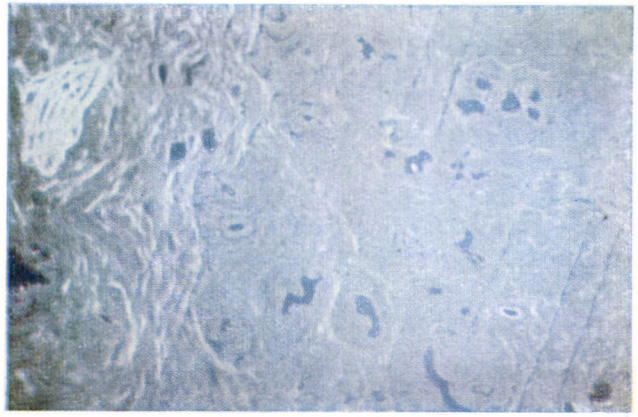

Fig. 1. Case No. 55. Fibrous mastopathy. Thiazine Red-Blankophor staining, UG + GG filters, ×30. The collagen fibres of the general stroma are light blue, the epithelium rusty red in colour. The fibres of the special connective tissue give a faint fluorescence

Fig. 2. Case No. 6. Cystic mastopathy and mammary mesoplasia. Primulin staining, UG + GG filters, ×30. The fibres of the general stroma are light blue and those of the special connective tissue are ochre yellow in colour

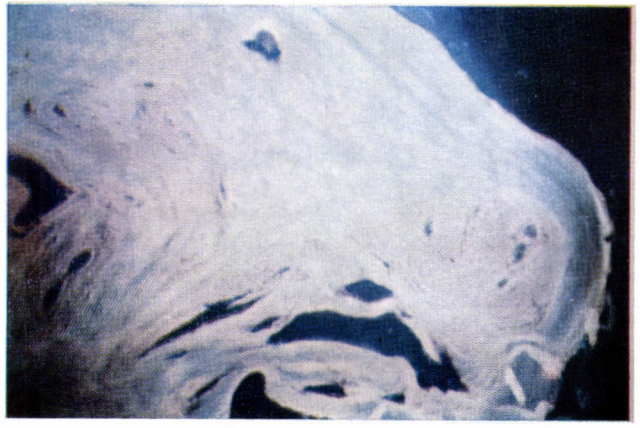

Fig. 3. Case No. 52. Mastopathic tissue adjacent to carcinomatous area. Titan Yellow staining, UG + GG filters, ×24. The general stroma gives a bluish and the special stroma a yellowish secondary fluorescence

From the above it may be concluded that the differences in fluorescence are first of all direct indicators of qualitative differences existing between the proteins of the special and general connective tissue. The role of mucopolysaccharides cannot be excluded but they seem to be — at least from the point of view of our reactions — of indirect importance since they seem to have no part in fluorochrome binding.

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FLUORESZENZ-MIKROSKOPISCHE VERFAHREN ZUR UNTERSCHEIDUNG DER BINDEGEWEBE DER MENSCHLICHEN MILCHDRÜSE

L. MÓDIS und GY. MATOLAY

Verfasser beschreiben zwei Verfahren zur Unterscheidung des spezifischen Bindegewebes vom allgemeinen Bindegewebe der Milchdrüse. Die von den Verfassern entwickelten fluoreszenz-mikroskopischen Färbungsverfahren sind selektiv, empfindlich, einfach durchführbar und kontrastreich. Sie eignen sich zur Untersuchung gewisser pathologischer Stadien.

ФЛЮОРОСЦЕНТНО-МИКРОСКОПИЧЕСКИЕ МЕТОДЫ ДЛЯ ОБОСОБЛЕНИЯ РАЗЛИЧНЫХ СОЕДИНИТЕЛЬНЫХ ТКАНЕЙ ГРУДНОЙ ЖЕЛЕЗЫ ЧЕЛОВЕКА

Л. МОДИШ и Д. МАТОЛАИ

Сообщаются два метода для обособления специфичной соединительной тканей грудной железы от общей соединительной ткани. Разработанные авторами флюоресцентно-микроскопические методы избирательны, чувствительны, просто осуществимы и богатые контрастами. Они пригодны для изучения определенных патологический стадий.

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ON THE ULTRASTRUCTURAL ORGANIZATION OF BIOLOGICAL MEMBRANES AS SHOWN BY TOPOOPTICAL STAINING REACTIONS

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Biological membranes show orientated dye binding reactions, characterized by strong anisotropy effects, with cationic dyes such as toluidine blue or rivanol if appropriate post-staining stabilization is applied. According to their different topooptical staining characteristics, three types of biological membranes could be distinguished. In each type the orientated dye binding of toluidine blue was dependent on the presence of structural lipids in the membranes. It could, however, be shown that the lipids were only playing a role in the orientation of the dye molecules which were bound by anionic dye binding sites on the non-lipid framework of the membranes.

Polarization optical data indicated that the dye molecules were intercalated with their planes in parallel between the hydrocarbon chains of the structural lipids. In this capacity the polar-apolar conformation and the flat molecular form of the dye molecules is decisive. By determining the ratio between anisotropy and optical density induced by the toluidine blue staining of different membranes, an indirect evidence of their ultrastructural density could be obtained.

The orientated dye binding reactions of the EP membranes in pancreas acinar cells, active fibroblasts, plasma cells and other protein secreting cells depended on the ionic strength of the dye solution, and that of the EP of the liver cells, in addition, on the reduced state and on the use of the electron acceptor ferricyanide as a post-staining precipitant. In this way indirect evidence of the ultrastructural organization of the RNA of the EP and of the oxidoreductive state of liver cell EP was obtained. Thus, the topooptical staining reactions provide a new approach to the study of the ultrastructural organization of biological membranes in physiology and pathology.

Evidence has been accumulating to show that biological membranes are built up of a bimolecular lipid leaflet sandwiched between two films of protein containing some other associated structural compounds such as mucoids (Lippman, 1968; Nakao and Angrist, 1968), glycoproteins (Eylar et al., 1962; Wallach et al., 1966) and RNA (Blobel and Potter, 1967a, b), and in addition a considerable amount of structured water (Hechter, 1965).

This structural pattern originally inferred from the physicochemical and functional characteristics of biological membranes, *e.g.* surface tension, semipermeability, conductivity (Danielli, 1935; Davson and Danielli, 1952), has been supported by the results of ultrastructural methods such as X-ray diffraction (Bear et al., 1941; Finean, 1953; Finean et al., 1966), polarization optics (Schmidt, 1936, 1940) and electron microscopy (Wolpers, 1941; Robertson, 1955, 1964; Stoeckenius, 1959, 1960, 1966). Recent electron microscopic observations have led to the suggestion that besides this lamellar membrane texture also globular lipoprotein subunits (Luzatti,

1962; SJÖSTRAND, 1963, 1964, 1968; LUCY, 1964; BRANTON, 1966; ROBERTSON, 1966; STAEHELIN, 1968) may contribute to the membrane structure, and a reversible dynamic transition between these two forms may be related to functional changes (KAVANAU, 1965). It has often been suggested that lipids are of basic importance in all biological membranes (WILLMER, 1961; VAN DEENEN, 1966; O'BRIEN, 1967), where they interact with structural proteins (HATCH and BRUCE, 1968) and membrane-bound enzymes and are crucial in many enzymatic activities (GARZÓ et al., 1952; REICH, 1961; OPARIN et al., 1966) in cation (JACOB et al., 1967) and electron transport function (GREEN and LESTER, 1959; REDFEARN, 1960; FLEISCHER et al., 1962; GREEN and FLEISCHER, 1963), and in phagocytic and pinocytic activities of the membranes (RUSTAD, 1959; KARNOVSKY, 1962; BRANDT and FREEMAN, 1967).

The ultrastructural organization of membrane lipids is not accessible to microscopic morphology. The lipid substances of the cytoplasm are, as often stated, "invisible". For this reason microscopic morphology could not be much interested in the ultrastructure of lipids, and attention has mainly been directed to the visible accumulation of lipid substances in the cytoplasm during fatty degeneration.

Even high resolution electron microscopy cannot afford a direct insight into the molecular organization of membrane lipids mainly because of the insufficient resolution power at the molecular level, and even the ultrastructural localization of lipid substances, based on their reactivity with OsO_4 seems to be problematic in view of the aspecific osmiophilic reaction of proteins (STOECKENIUS, 1962, 1966; ROBERTSON, 1964; NAPOLITANO and LEBARON, 1967).

The two methods capable of providing definitive indirect information about the molecular structural organization of membrane lipids are X-ray diffraction, and polarization optics.

Polarization optics has already been applied in the study of structural lipids in different membranes by several investigators (SCHMIDT, 1935, 1936; SCHMITT et al., 1936; ROMHÁNYI, 1949; MITCHISON, 1953; PONDER et al., 1956; ROLLHÄUSER, 1956; RÖHLICH, 1956; MISSMAHL, 1957, 1958; KAUTZ et al., 1957). All these investigations were performed on unstained substrates and based on the intrinsic birefringence of lipid molecules in the membranes and showed that the lipid molecules were arranged with their greater refractive index (their hydrocarbon chains) in perpendicular direction to the plane of the membranes. In a previous study of the ultrastructural basis of the metachromatic staining reaction [63] we briefly dealt with the staining reactions of structural lipids, which were found capable of binding some metachromatic cationic dyes in an ordered pattern, similarly to the metachromatic staining of other classic chromotropes such as acid mucopolysaccharides (MPS). This could, however, be achieved only when a post-staining stabilization of the

orientated dye-binding reactions with potassium ferricyanide was applied. When in the course of our studies it turned out that the orientated dye-stabilizing effect of potassium ferricyanide on the structural lipids was not satisfactory, we developed a more effective way to stabilize the anisotropic dye-binding reactions on structural lipids. This has made it possible to study, by means of topooptical staining reactions, the ultrastructural organization of the biological membranes, on which we shall report in this paper.

Materials and methods

Freshly fixed kidney, liver, pancreas and lung of rats and cats were investigated in frozen sections. Fixation was performed in 10% neutralized formol solution for 24 hrs. In the case of liver ergastoplasm membranes (EP), the anisotropic staining reaction of which was found to depend on the oxido-reductive state, fixation was carried out in formol solution [prepared with freshly-boiled (1/2 hr) distilled water], containing 0.05% sodium dithionite as a reductant, or in formol solution under anaerobic conditions (kept over a mixture of 20% pyrogallic acid and 10% sodium hydroxide and sealed in a reagent tube) for 24 hrs. Smears of human blood were fixed in 5% glutaraldehyde solution (buffered to pH 7.2 with 0.15 *M* phosphate buffer at 4°C for 1 to 24 hrs). To study the sensitivity of the topooptical staining reactions in demonstrating ultrastructural damage to biological membranes, rat kidneys were investigated after short-term ischaemia induced by clamping the vascular pole for 15, 20 and 30 minutes and killing the animals 2—24 hrs after releasing the clamping. To analyze the role of the different structural components of the membranes in the topooptical staining reactions the effect of lipid extraction and removal of RNA by ribonuclease and perchloric acid were tested.

Lipid extraction was made in two ways, *a)* by treatment of the frozen sections with absolute ethanol for 5 minutes, to extract the more labile lipids; and *b)* by treatment with choloroform—methanol (1 : 3) for 24 hrs, for complete extraction of the structural lipids. RNA was eliminated either by treatment with ribonuclease (RNAse, crystallized, Reanal Budapest, 2 mg/ml in neutral distilled water at 37°Cf or 4 hrs) or by extraction with 10% perchloric acid at 4°C, for 18 hrs [3]. The following topooptical-(staining)-reactions [63, 64] were applied.

Toluidine blue staining. Frozen sections were stained in 0.1% toluidine blue (buffered at pH 3.5—7.0 with Michaelis Veronal sodium acetate buffer), to which sodium chloride was added to adjust the ionic strength of the dye solution to 0.1—1.0.

The sections were stained on the slides for 10 minutes, blotted with filter paper and then treated with the following precipitants: *a)* 2% potassium ferricyanide, or *b)* a 7 : 1 mixture of potassium iodide (2%) and potassium ferricyanide (2%) (KJ/ferricyanide) to stabilize the dye molecules bound on the structure. The sections were then mounted in gum arabic, containing the precipitants at a concentration of 0.2%.

The gum arabic layer was allowed to dry. In this state the preparations could either be preserved for indefinite time or were sealed with Canada balsam and cover-slips.

However, when the sections were covered with cover slips, with the gum arabic still liquid, a slowly-progressing fading of the toluidine blue ensued in about a year, probably as a result of electron transport from the tissues to toluidine blue, mediated by ferricyanide. If, on the other hand, the gum arabic layer was allowed to dry, no electron transport could take place, and the staining reaction persisted constant for unlimited time.

Ethacridine (Rivanol)-staining (ALBERT, 1951) was carried out with a 1% aqueous solution. The staining procedure was similar to that with toluidine blue except that the sections were shortly rinsed after staining and before being treated with ferricyanide. Mounting was similar as in the case of toluidine blue. For comparison the following cationic dyes were used: methylene blue of the thiazine group, gentian violet, crystal violet and methyl violet of the triphenyl-methane group, and the copper phthalocyanin dye alcian blue.

Light microscope observations and polarization optical analyses were made with a Leitz Ortholux polarization microscope equipped with rotating compensators (18 mμ and 57 mμ) and a Berek compensator. Investigations in monochromatic light were made with a Zeiss interference filter, and a Leitz monochromator. Quantitative dye binding was estimated either with a Leitz cytophotometer of the "MPV" type, or calculated on the basis of the exposition times of an automatic exposure set (Leitz Orthomat) in the light microscope.

Observations

We found that in all biological membranes investigated so far the orientated binding of toluidine blue was dependent on the presence of structural lipids in the membranes. Besides this general condition, we found three types of membranes differing in their anisotropic staining reactivity with toluidine blue.

Type I membrane. The orientated dye binding of membranes of this type was not dependent (as in the Type II membrane) on the ionic strength of the dye solution and could best be stabilized by a post-staining treatment with KJ/ferricyanide. Many cytomembranes, cell membranes, nuclear membranes as well as the basement membrane belong to this type.

Type II membrane is represented by the RNA-rich EP membranes in pancreas acinar cells, plasma cells, active fibroblasts and protein-secreting glandular cells. Anisotropic staining of these membranes with toluidine blue was dependent, besides the presence of membrane-attached RNA, 1. on the presence of structural lipids, 2. on the ionic strength of the dye solution and 3. on a stabilizing effect best obtained by post-treatment with ferricyanide.

Type III membrane is represented only by the EP membranes of liver cells, the anisotropic staining of which was found to depend, besides the above conditions (1, 2 of Type II membrane) 4. on the oxido-reductive state of the structure and 5. could be stabilized exclusively by post-staining treatment with the electron acceptor ferricyanide.

Type I membrane

a) *Optimum way of dye stabilization*

The main characteristic of the anisotropic staining reaction of this type of membrane is the way in which optimum stabilization of the dye molecules on the structure can be achieved. Potassium ferricyanide, initially used by us as a general post-staining precipitant for anisotropic staining reactions with toluidine blue and Rivanol (ROMHÁNYI, 1963; ROMHÁNYI and DEÁK, 1967), had excellent effects in stabilizing the orientated dye binding of acid MPS, but did not produce standard effects on the lipid structures of different cytomembranes. When investigating the possibility of optimum dye stabilization on the structural lipids we tested different precipitants such as KJ, KSCN, potassium ferricyanide, potassium dichromate, ammonium molybdate, either alone or in their various combinations and we found that the best dye stabilization on the structural lipids of Type I membranes could be achieved by using a 7 : 1 mixture of potassium iodide (2%) and potassium ferricyanide (2%) (freshly prepared every 2—3 days). The effect of KJ alone was found not to be favourable, because of its tendency to form microcrystalline artefacts.

The superiority in dye stabilizing of KJ/potassium ferricyanide (7 : 1) over that of ferricyanide manifests itself in two effects: *a)* it exactly preserves the original shades of colour of metachromasia not only on the classic chromotropes (such as acid MPS) but also on structural lipids and can, therefore, be used for the study of visually weak metachromatic effects in light microscopy; and *b)* it results in optimum stabilization of the anisotropic effects of orientated dye binding on structural lipids. This effect is shown in Fig. 1a where the cytomembranes of the different segments of the nephron reveal uniformly strong birefringence due to the orientated binding of the toluidine blue molecules. After ferricyanide treatment (Fig. 1b) only the basement membranes and the brush-borders of the proximal tubuli stand out as a result of a strongly anisotropic staining, while the cytomembranes of the distal segments of the nephron are quite inconspicuous due to ineffective stabilization of the dye molecules on the structural lipids of these membranes, which appear in the light microscope as basal striations of the cytoplasm corresponding to the basal infoldings of the cytomembranes and to the membranes of interposed mitochondria as seen in the electron microscope. The effect of KJ/ferricyanide was found equally satisfactory on a large number of other cytomembranes. The intensive anisotropic staining reaction of the capillary endothelial cell membrane is noteworthy. Even the red blood cell membrane (MITCHISON, 1953), which has an extremely weak intrinsic birefringence, can readily be demonstrated by this method. Fig. 2 shows the strong anisotropic staining reaction of the human red blood cell membranes in a glutaraldehyde-fixed smear. This strong anisotropic effect of the staining is, in view of the extremely weak (unmeasurable) intrinsic birefringence of the red cell membrane, very remarkable and shows the possibilities which can be provided by the topooptical staining reactions in ultrastructural studies. This is further shown in Fig. 3 demonstrating the drastic decrease in anisotropic staining reaction of the brush-border and cytoplasmic membranes of the proximal renal tubuli as a result of early structural damage induced by 30 min ischaemia. At the same time the dye binding is not diminished, only the orientation of dye molecules is lacking. By contrast, the cytomembranes of the distal tubuli have retained their orientated dye binding capacity, as shown by their strong birefringence. These examples demonstrate that toluidine blue staining followed by post-staining treatment with KJ/ferricyanide can be regarded as a standard procedure for polarization optical analysis of the ultrastructural organization of membranes in physiology and pathology.

b) *Orientation of dye molecules with respect to the structural lipids in membranes*

Optical phenomena of anisotropy (dichroism and birefringence) induced by dye molecules bound on the membrane indicate that they are orientated

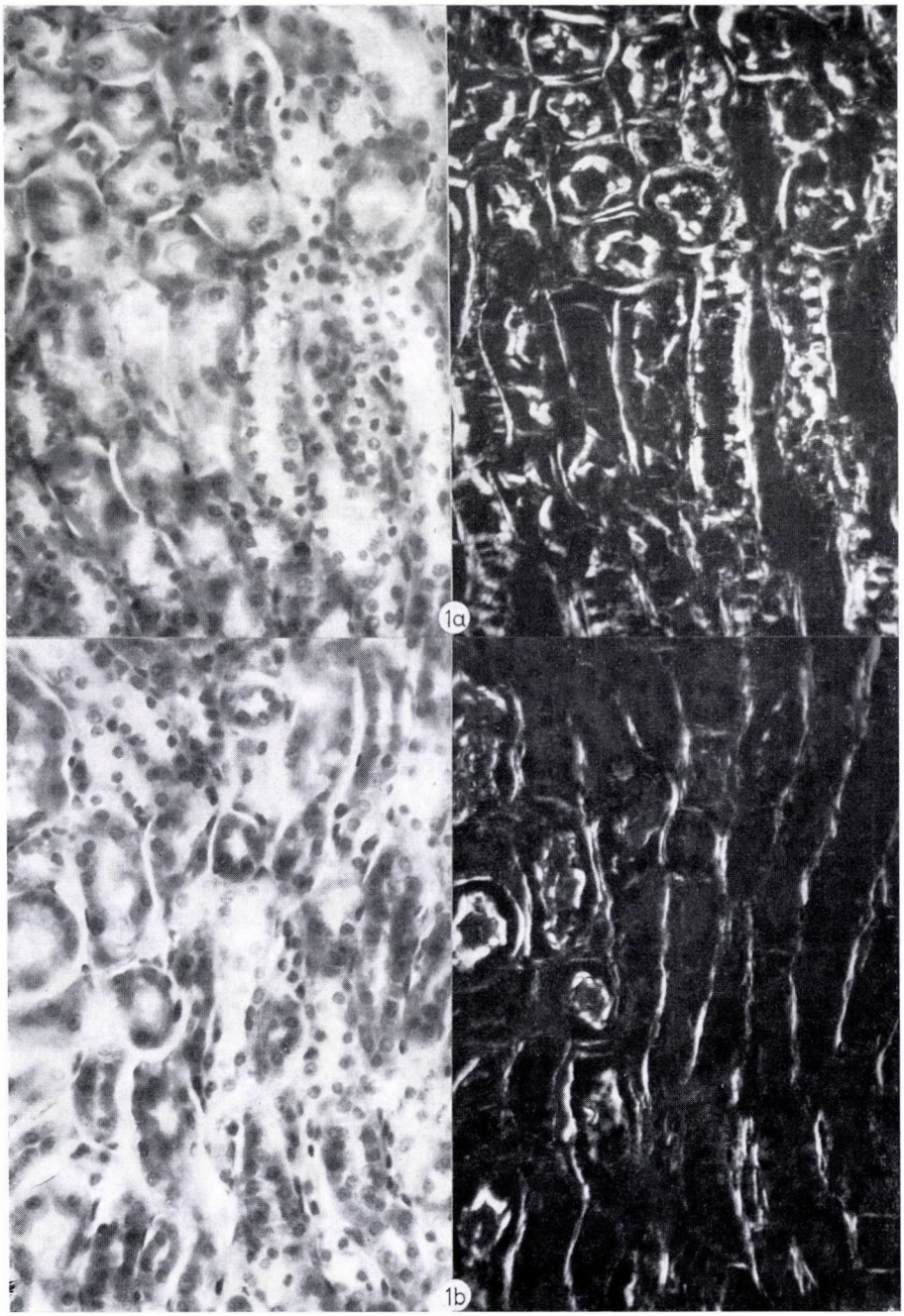

Fig. 1. Frozen sections of rat kidney stained with toluidine blue. *a:* post-staining fixation with KJ/ferricyanide (7 : 1), *b:* with ferricyanide. Pictures on the left were taken under the light microscope (λ 560 mμ) and those on the right are the same fields between crossed polaroids. There is a marked difference in anisotropic staining reaction between *a* and *b*. In *a* the brush borders, the cytomembranes and basement membranes are strongly birefringent as a result of the orientated dye binding. In *b* only the brush border and basement membranes are strongly birefringent, while the cytomembranes appear isotropic indicating a random binding of the dye molecules ($\times$300)

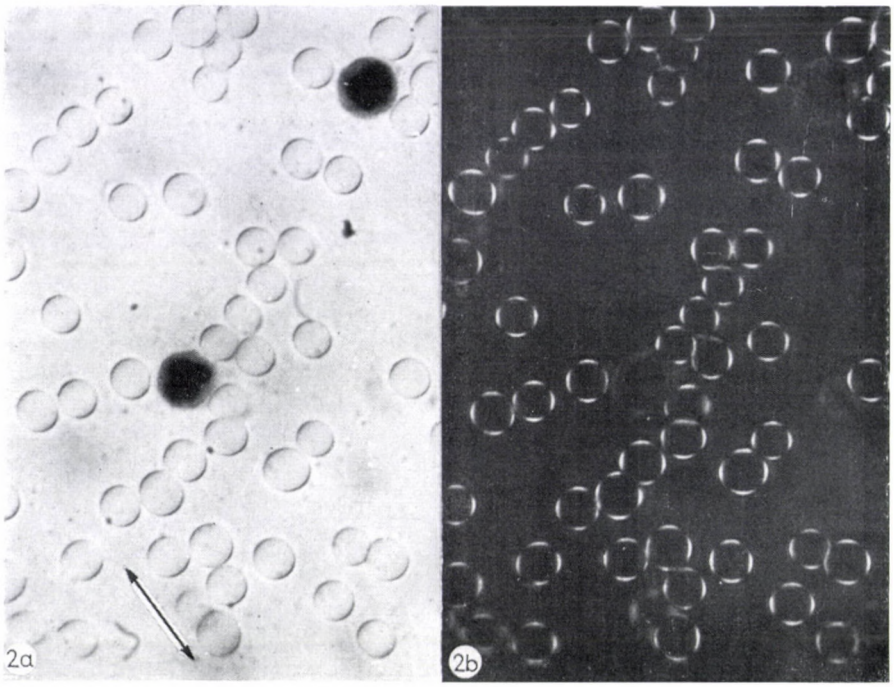

Fig. 2. Glutaraldehyde-fixed smear of human blood, stained with the toluidine blue KJ/ferricyanide method. *a)* under the light microscope in linearly polarized light (λ 560 mμ) the plane of which is marked by the arrow; *b)* the same between crossed polaroids. In *b)* the strongly birefringent red cell membranes are seen in the form of radially positive polarization crosses, indicating radially orientated dye molecules on the membranes. In *a)* radially positive dichroism of the red cell membranes can also be recognized. Both sectors of the red cell membranes, orientated perpendicularly to the plane (marked by an arrow) of polarization of the light, are dark, indicating that the dye molecules are aligned with their light absorbing bonds radially to the membranes. Two leukocytes seen in the picture show very weak anisotropy of their cytoplasmic membranes ($\times$1200)

with their light absorbing bonds (the planes of the dye molecules) parallel to the hydrocarbon chains of the lipid molecules in the membranes, inducing an additive type of anisotropic staining reaction. (For classification of the topooptical staining reactions, see Romhányi and Deák, 1967a.)

Dichroism. Toluidine blue-stained membranes revealed dichroism of different degrees, with maximum absorption for light polarized perpendicularly to the plane of the membranes, indicating that the light absorbing bonds of the dye molecules were arranged parallel to the hydrocarbon chains of the lipid molecules in the membranes (Fig. 4). Dichroism is however often weak or undetectable, in spite of the strong effect of birefringence in the same preparation. This is due to the fact that dichroism is very sensitive to the thickness of the specimen and is easily masked by the dye molecules of overlying layers with different orientation. By contrast, the birefringence induced

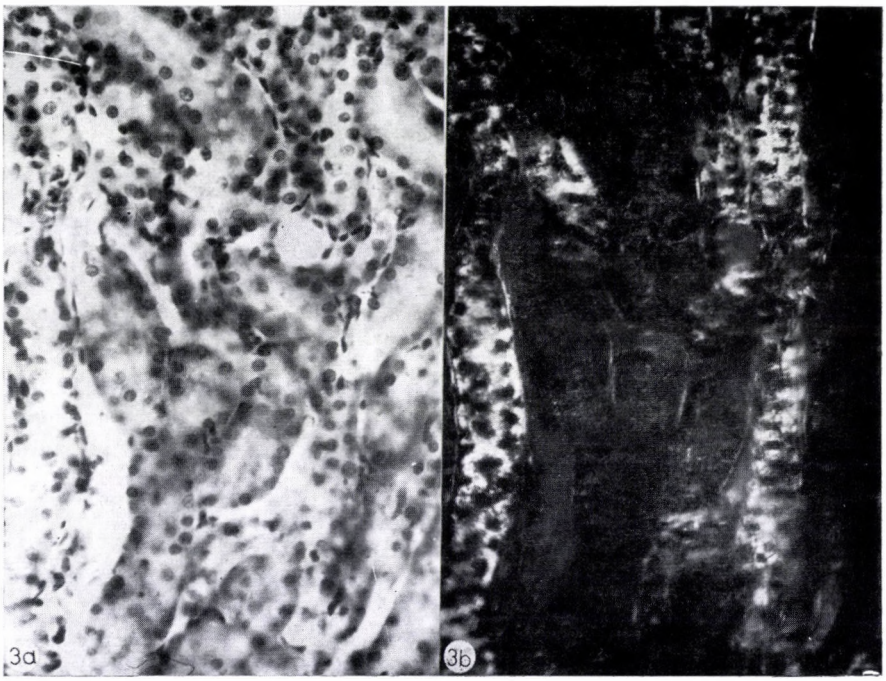

Fig. 3. Frozen section of rat kidney after 30 min ischaemia induced by clamping the vascular pole and investigated 2 hr after releasing the vascular clamping. Toluidine blue KJ/ferricyanide. *a)* Under the light microscope; *b)* the same field between crossed polaroids. In *b)* staining of the brush border and of the proximal tubuli is isotropic as an optical sign of early damage to the cytomembranes of these segments of the nephrons. Two distal tubuli show intensive birefringence in the cytoplasm indicating that the structural lipids have retained their ability to align toluidine blue in an orientated pattern. Staining of the proximal tubuli was orthochromatic, that of the distal tubuli metachromatic, under the light microscope. For comparison see Fig. 1*a* (×300)

by the orderly alignment of dye molecules on micellar textures is much less disturbed by such factors.

c) *Birefringence of toluidine blue-stained membranes*

The dispersion curve of birefringence of the toluidine blue-stained membranes shows a typical course (Fig. 5). There is increased negative birefringence with respect to the plane (tangent) of the membrane for light of the long wave side and positive birefringence for the short wave side of the spectrum [63]. From the curve it can be seen that in order to obtain standard comparable values for the anisotropy induced by the staining reaction, the measurements of retardation must be made at constant wave lengths. We used

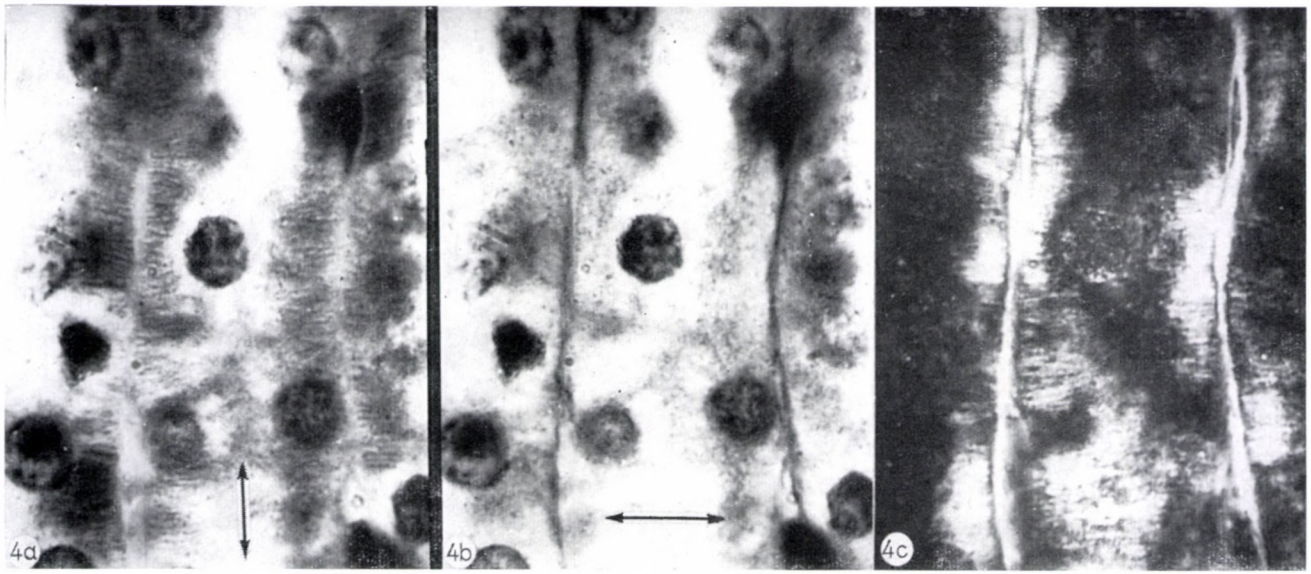

Fig. 4. Frozen section of rat kidney, thick Henle-segment. Toluidine blue KJ/ferricyanide. *a)* and *b)* the same field under the light microscope in linearly polarized light (λ 560 mμ), the plane of which is indicated by arrows; *c)* the same between crossed polaroids. The cytomembranes seen as "basal striations" appear in *a)* dark, in *b)* lighter; there is maximum absorption for light polarized perpendicularly to the length of the basal striations (negative dichroism). The basement membranes show negative dichroism with respect to their length, they appear in *a)* lighter, and in *b)* dark. The negative dichroism of both the cytomembranes and basement membranes indicates that the dye molecules are arranged with their light absorbing bonds (the planes of their molecules) perpendicularly to the cytomembranes as well as to the basement membranes *i.e.* parallel with the hydrocarbon chains of the structural lipids. In *c)* the strong anisotropic staining effect of the basal striations and basement membranes is seen, the optical character being negative with respect to their length as optical evidence that the dye molecules are orientated perpendicularly to the membranes ($\times$1200)

light of λ 580 mμ. The intensity of anisotropy of the staining reaction can be expressed by means of the index of anisotropy [63]:

$\frac{\text{induced anisotropy m}\mu}{\text{original anisotropy m}\mu}$, which varied from 5.0 to 0.2 with the different membranes.

The dispersion curve of birefringence (in Fig. 5) indicates that the dye molecules are aligned with their light absorbing bonds perpendicularly to the

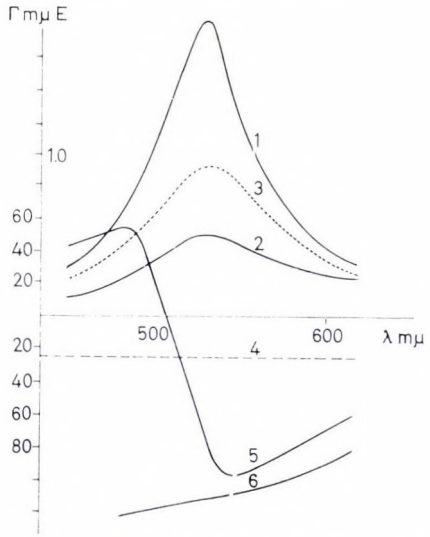

Fig. 5. The absorption and dispersion curves of birefringence of a toluidine blue-stained brush border of rat kidney. 1. Absorption curve for light polarized perpendicularly to the microvilli of the brush border. 2. Absorption curve for light polarized parallel with the microvilli of the brush border. 3. Absorption curve for unpolarized light. There is maximum absorption for light polarized perpendicularly to the length of the microvilli of the brush border. 4. Intrinsic birefringence of the unstained brush border (negative with respect to the length of the microvilli). 5. Dispersion curve of birefringence of the toluidine blue-stained brush border. There is an inversion of the negative birefringence to positive at λ 530 mμ (at the zone of the metachromatic absorption band of toluidine blue). 6. Spectral curve of birefringence of ethacridine-stained brush border. There is increased negative birefringence for the whole visible spectrum

membranes and in parallel with the hydrocarbon chains of the lipid molecules. Thus both dichroism and birefringence may be regarded as an indication that the toluidine blue molecules are arranged in parallel with the hydrocarbon chains of the structural lipids in the membranes.

d) *Optical effect of ethacridine of staining on membranes*

Ethacridine staining induces a strong increase in anisotropy of the membranes, which is, for the whole visible spectrum, negative in respect to the plane of the membranes (Fig. 5), indicating that the dye molecules are arrangad

with their greater refractive index (the plane of the dye molecules) perpendicularly to the membranes. However, the absorption band of ethacridine being in the near ultraviolet part of the spectrum — (between 430—360 mμ) the dispersion curve of birefringence of the ethacridine-stained membranes could not be followed in its whole course and owing to a similar fact dichroism, which would have been effective within the spectral region of the absorption band, could not be observed.

e) *The role of membrane lipids in the topooptical staining reactions*

Optical evidence indicates that the cationic dye molecules used (toluidine blue, ethacridine) are bound to the membrane structure in pattern orientated parallel with the hydrocarbon chains of the lipid molecules in the membranes. The question arises what role the lipids play in this phenomenon.

We found that lipid extraction, although it did not diminish quantitative dye binding, resulted in a complete lack of orientation of the toluidine blue molecules as shown by the isotropic staining reaction, in contrast to the anisotropic staining of membranes with lipids present in them (Fig. 6*a*, *b*). In lipid-extracted slices the staining of the cytomembranes was orthochromatic, in the control slices weakly metachromatic, indicating a random aggregation of the dye molecules in the lipid-extracted membranes and a micellar dye aggregation in the membranes of the control slices (Romhányi, 1963). Thus both polarization optics and the phenomena of orthochromasia-metachromasia provide evidence of the important role of structural lipids in the orientated dye binding of membranes in a remarkable way inasmuch as they are only essential for the orientation of dye molecules, but not for their binding, which is brought about by anionic groups of the nonlipid components of the framework of the membranes.

f) *Ethacridine staining after lipid extraction*

Lipid extraction did not abolish the anisotropic staining reaction of membranes with ethacridine, only diminished it to about 30%. This provides a possibility to study the topooptical staining reactions of biological membranes with ethacridine also in paraffin sections (Somogyi et al., 1967). Ethacridine molecules are bound to the membrane structure either by the negative side groups of the protein framework of the membrane or by membrane-attached RNA, which can be tested by ribonuclease treatment (Fig. 8). In contrast to toluidine blue, ethacridine molecules thus appear to be able to maintain an orientated arrangement on the protein structure of the membranes without the presence of lipids in them. This may be explained on a molecular structural basis, which will be discussed later.

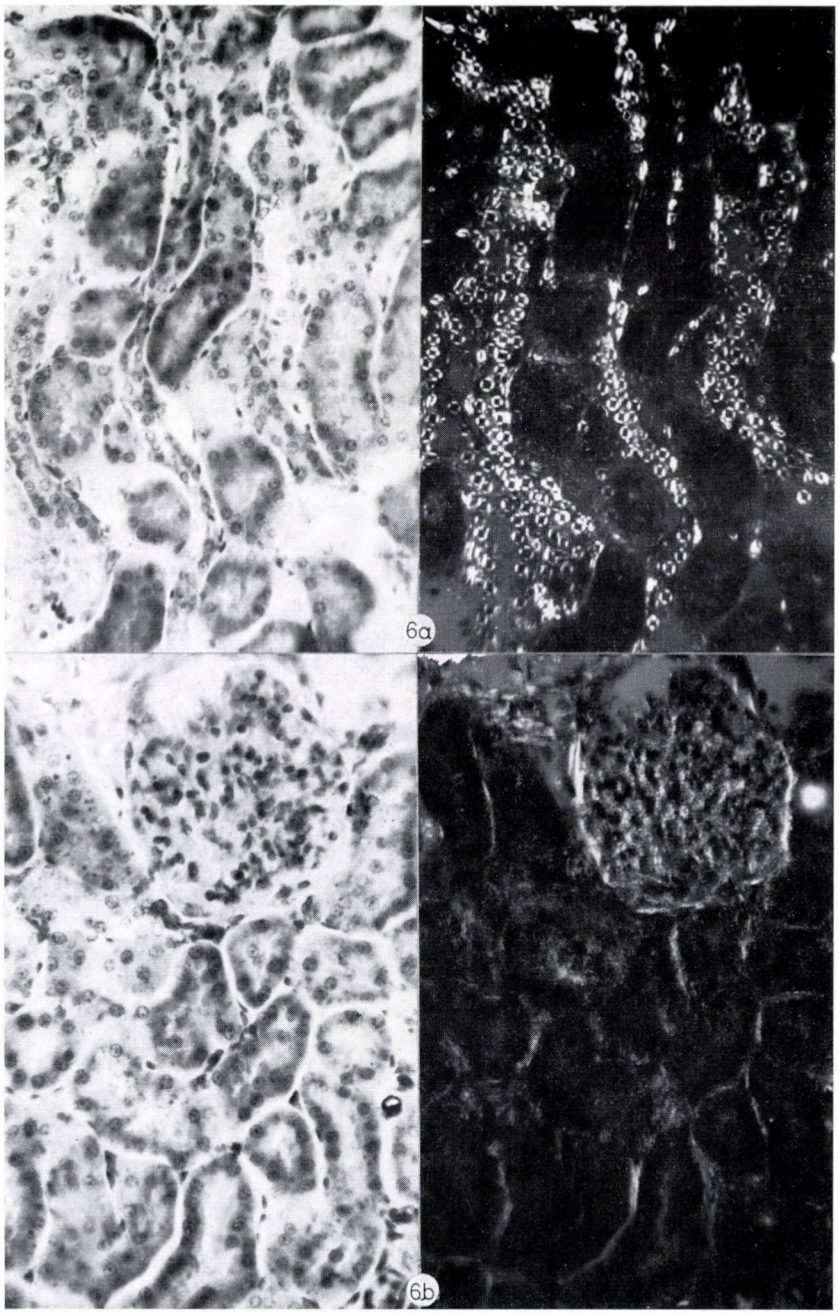

Fig. 6. Rat kidney. Toluidine blue KJ/ferricyanide. *a)* Incomplete lipid extraction (5 min absolute ethanol treatment). *b)* Complete lipid extraction with chloroform-methanol for 24 hr. In *a)* the proximal tubuli show isotropic staining as a result of the loss of their more labile structural lipids; the nuclear membranes of the distal tubuli, however, retained their anisotropic staining reaction indicating relative resistance to extraction of their structural lipids. In *b)* the staining is isotropic indicating random binding of the dye molecules. For comparison see Fig 1*a* (×300)

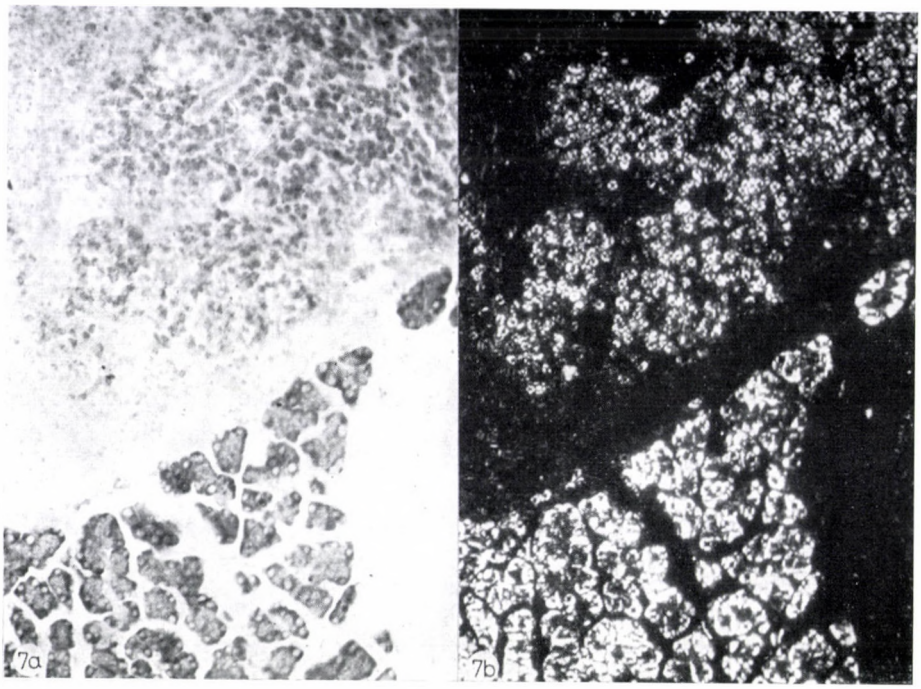

Fig. 7. Rat pancreas and surrounding lymph node tissue. Frozen section, toluidine blue/ferricyanide (at 0.6 *M* NaCl concentration of the dye solution). *a)* In the light microscope, *b)* the same between crossed polaroids. In *b)* the EP of pancreas acinar cells is strongly birefringent as a result of orientated dye binding on the membrane-attached RNA; in the lymphoid tissue strongly birefringent cells are seen which can be recognized in *a)* as plasma cells. The anisotropic staining of the plasma cells is due to their highly developed EP which can be abolished by treatment of the slices with ribonuclease. The orientated dye binding of the EP in pancreas acinar cells and plasma cells depended on the high ionic strength of the dye solution (0.6 *M* NaCl content) in which the cell nuclei of the pancreas acinar cells and those of the lymph node cells appear unstained (×150)

g) *Intercalation of dye molecules between the hydrocarbon chains of the structural lipids in the membranes*

The membrane orientation of the dye molecules by the structural lipids, as observed in our investigations, is indicative of a close molecular interaction between dye molecules and structural lipids. From this and the optical evidence indicating a parallel arrangement of the dye molecules with the hydrocarbon chains of the lipids one can conclude that the dye molecules are intercalated between the hydrocarbon chains of the membrane lipids. This capacity of the dye molecules is related to their polar-apolar molecular conformation, which enables them to be intercalated between the lipid molecules, which also have a polar-apolar molecular conformation (Fig. 12). Methylene blue for instance, a thiazine dye, structurally closely related to toluidine blue but

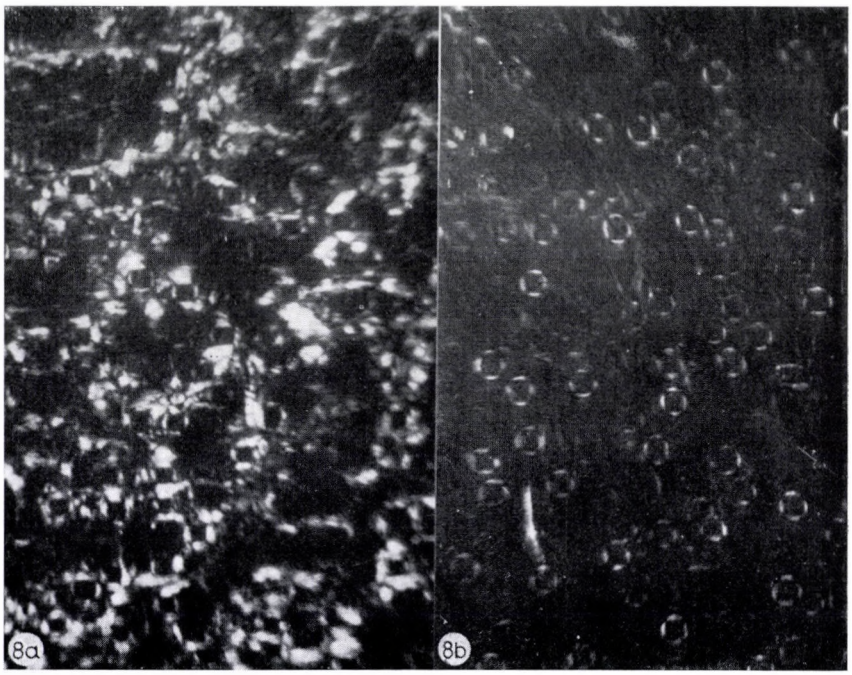

Fig. 8. Paraffin section of cat pancreas stained with ethacridine/ferricyanide and viewed between crossed polaroids, *a)* before, *b)* after ribonuclease treatment. *a)* shows the strong birefringence of the EP, optically negative with respect to the length of the membranes, induced by the orientated dye binding, which obscures the birefringence of the nuclear membranes; in *b)* the anisotropic staining effect of the cytoplasm disappeared as a result of the elimination of RNA, the anisotropic staining of the nuclear membranes persists although decreased in intensity. This may be taken as evidence of an orientated RNA layer on the nuclear membrane belonging to the EP, as known from electron microscopic observations. The remaining anisotropic staining reaction of the nuclear membrane is due to orientated dye binding on the negative side groups of the protein framework (×450)

with symmetrically distributed electrically charged groups on the molecule, was found incapable of being bound in an orientated pattern on biological membranes although its binding capacity was not less than that of toluidine blue.

The cationic dyes of the triphenyl-methane group and alcian blue were not able to induce an anisotropic staining reaction. It is probably for molecular steric reasons that they are unable to intercalate between the membrane lipids.

h) *Ultrastructural density of biological membranes*

The intercalation of dye molecules in the membrane structure is dependent not only on the conformation of the dye molecules but, similarly, on the

structural density of the membranes, which varies considerably with the different biological membranes. The degree of toluidine blue intercalation can, to some extent, be considered an optical sign of ultrastructural density of the membranes, which can be expressed quantitatively as a ratio between the anisotropy (A) and the optical density (D) of the staining reaction. As a standard reference value the $\frac{A}{D}$ ratio of toluidine blue KJ/ferricyanide films, often seen on a slide in the neighbourhood of the slices, was taken. In a number of measurements on such films a mean value of $\frac{25\ m\mu}{0.1}$ was found for $\frac{A}{D}$ which in the form of $\frac{A}{D \times 250} = \frac{25}{0.1 \times 250} = 1$. This can be taken as an optical unit value for a toluidine blue KJ/ferricyanide film texture with optimum orientation. It was now of interest to see what $\frac{A}{D}$ values could be found for different membranous structures when they were stained with toluidine blue KJ/ferricyanide. The following values were obtained: for brush border, $\frac{50\ m\mu}{0.4 \times 250} = 0.5$; for red cell membrane, $\frac{40\ m\mu}{0.5 \times 250} = 0.32$; for myelin, $\frac{40\ m\mu}{1.4 \times 250} = 0.12$; these ratios show that the degree of membrane orientation (intercalation) of dye molecules was different in the various membranes, the optimum value being found in the brush border, which, however, only reaches half the value of optimum dye orientation in the film. Myelin shows a very low degree of dye intercalation.

Type II membrane

The anisotropic staining reaction with toluidine blue of the EP of pancreas, plasma cells, fibroblasts and many other protein-secreting cells, was markedly influenced by the ionic strength of the dye solution. At low ionic strengths these membranes gave isotropic overstaining with toluidine blue (Fig. 9) but at higher ionic strengths the staining reaction was anisotropic. The maximum degree of dye molecule orientation on the EP appeared at high ionic strengths (0.6—1.0 *M* sodium chloride) of the dye solution, at which the cell nuclei remained completely unstained (Figs 7, 9). The orientated binding of cationic dyes by such membranes is due to their RNA content. Ribonuclease treatment or extraction of RNA with perchloric acid abolished this dye binding (Fig. 8). The RNA and lipid dependence of the anisotropic staining reaction of the EP of pancreas acinar cells reported in detail earlier (Romhányi and Deák, 1967b) gives some indirect indication of the ultrastructural organization of EP, which will be briefly discussed below.

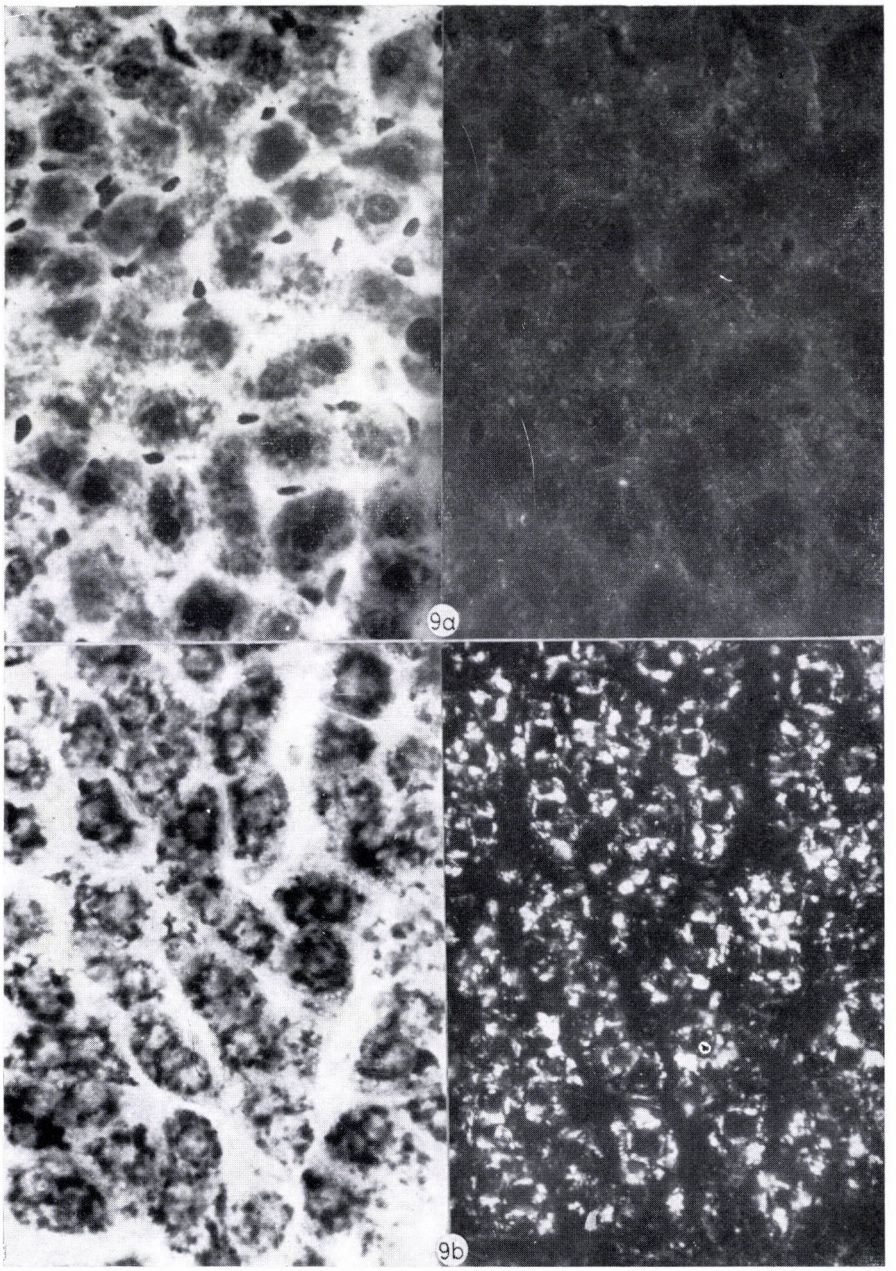

Fig. 9. Frozen section of rat liver stained with toluidine blue/ferricyanide in *a)* at low electrolyte concentration (0.02 *M* NaCl). *b)* the same staining at high electrolyte concentration (0.6 *M* NaCl). The pictures on the left were taken under the light microscope (λ 560 mμ) and those on the right between crossed polaroids. In the light microscopic picture of *a)* the cell nuclei and cytoplasmic granules show intensive staining which, between crossed polaroids, appears isotropic indicating a random binding of the dye molecules on these structures. The light microscopic picture of *b)* reveals selective strong basophilic

Type III membrane

The anisotropic staining reaction of the EP of the hepatic cell, the only representative of this membrane type, was found to depend, apart from the conditions described above for Type II membrane, on the oxidoreductive

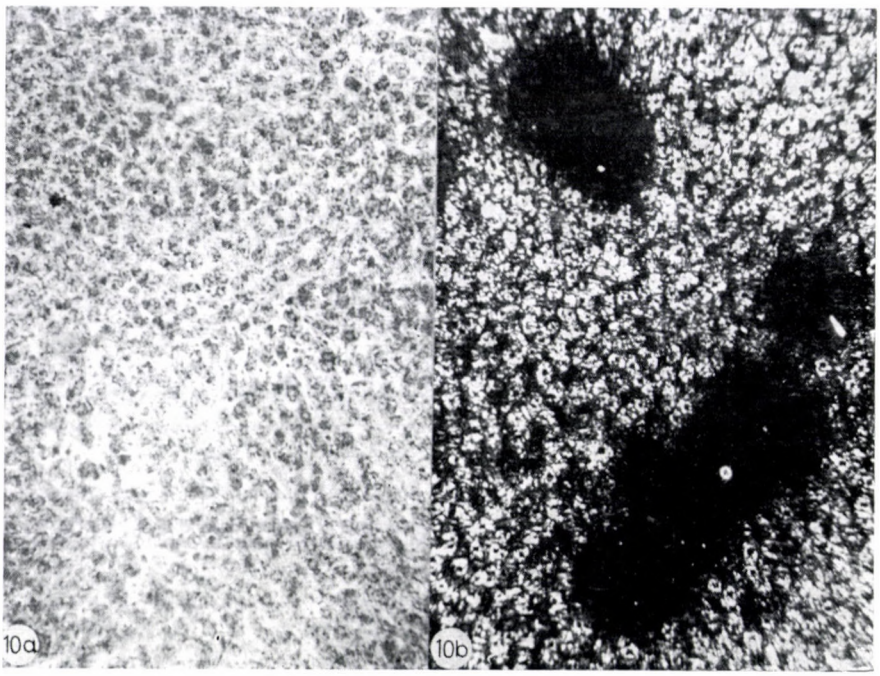

Fig. 10. Frozen section of formol-fixed rat liver, stained with toluidine blue/ferricyanide of 0.6 *M* NaCl content of the dye solution. The EP reveals strong birefringence as a result of an orientated dye binding of the membranes of the EP. In the two black areas the staining is isotropic as an optical sign of random dye binding on the EP resulting from spontaneous photooxidation of the slice in bright day light (3 hrs) (×120)

state of the EP. It should be pointed out that in this case the staining reaction could be stabilized exclusively by the electron acceptor ferricyanide. In the oxidized state the EP of liver cells showed isotropic staining, in the reduced state anisotropic staining (Figs 10, 11). In the former state the dye molecules

staining of the EP. At an electrolyte concentration of 0.6 *M* the cell nuclei remain unstained due to the lower critical electrolyte concentration of DNA; between crossed polaroids the same field shows intensive birefringence of the cytoplasm, resulting from an orderly alignment of the dye molecules on the EP membranes (×560)

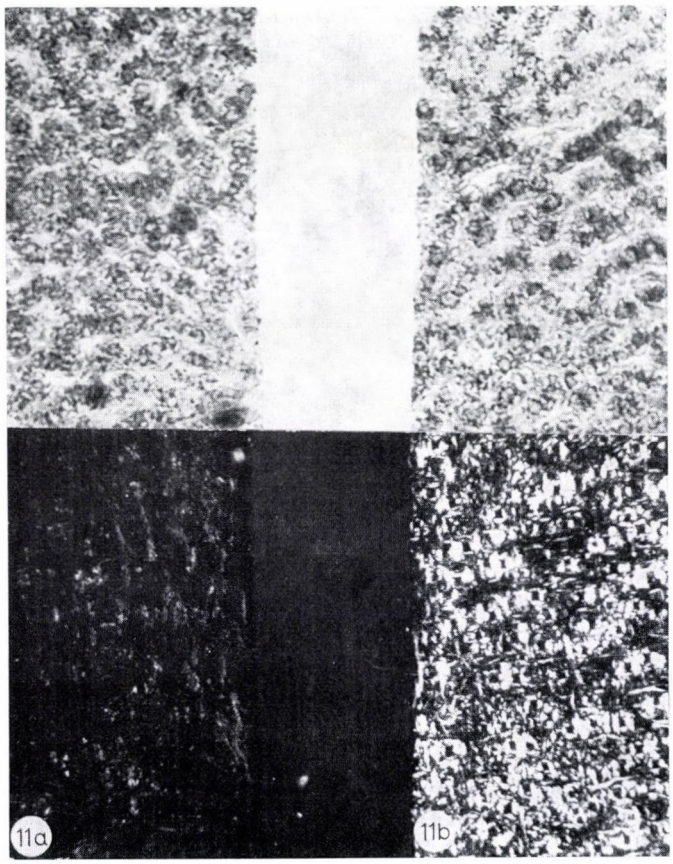

Fig. 11. Two halves of a frozen section of rat liver oxidized in 3% H_2O_2 for 5 minutes *(a)*, and then reduced with 0.1% sodium dithionite for 5 minutes *(b)*. Stained with toluidine blue/ferricyanide of 0.6 *M* NaCl content. Between crossed polaroids the staining in the oxidized half *(a)* is isotropic, indicating a random dye binding on the EP. In the oxidized-reduced half *(b)* the cytoplasm of the liver cells shows intensive birefringence as a sign of orientated binding of the toluidine blue molecules on the membrane ultrastructure of the EP in the reduced state (×200)

were bound at random on the RNA, but in the latter they were aligned in an orderly pattern, parallel to the hydrocarbon chains of the lipid molecules in the EP membranes. Thus an indispensable condition of orientation of the dye molecules in the EP membranes of the liver cell is the presence of electrons with which the electron acceptor ferricyanide can interact to bring about a membrane orientation of the dye molecules bound by the membrane-attached RNA (Fig. 13a). In the oxidized state (Fig. 13b), in which electrons are lacking, such an interaction cannot take place, and therefore the dye-ferricyanide complex bound to the RNA remains unorientated giving rise to isotropic staining.

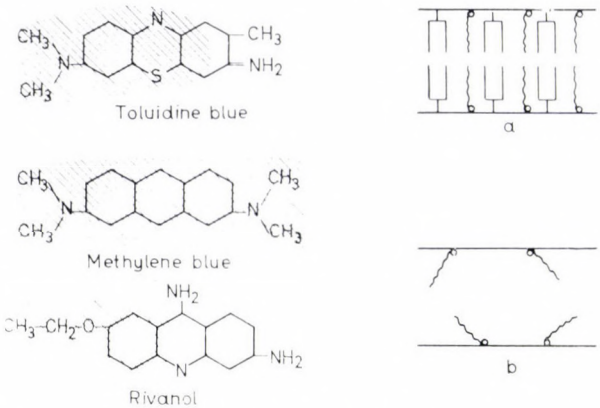

Fig. 12. Schematic drawing of the molecular conformation of the dyes used, showing the polar-apolar conformation of toluidine blue and ethacridine, which enables them to intercalate between the polar-apolar lipids of the membranes *(a)*. Methylene blue with its symmetrically distributed electric charge is not capable of membrane-intercalation. After lipid extraction *(b)* toluidine blue molecules bound by the anionic binding sites of the membrane-framework remain unorientated giving rise to an anisotropic staining reaction

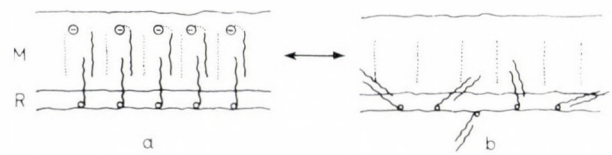

Fig. 13. Highly schematic structural model of the staining reaction of the EP in liver cell, depending on the oxidoreductive state. R: membrane-attached, and membrane-orientated ribosomal RNA, M: lipoprotein membrane. In *a)*, membrane orientation of the RNA-dye-ferricyanide complex resulted from the interaction between the electron acceptor ferricyanide and the electrons present in the reduced membrane. In *b)*, the RNA-dye-ferricyanide complex remains unorientated, resulting in isotropic staining because of lack of electrons to interact with ferricyanide

To preserve the physiological reduced state of the EP membranes of liver cells (RAPOPORT, 1966) it was necessary to apply either anaerobic fixation or a formol solution containing a reducing agent (sodium dithionite) and further to protect the slices from photooxidation by bright day light prior to the staining process. The oxidoreductive state, as reflected by the isotropic-anisotropic staining reaction of the EP, was reversible. More details are to be reported in a separate paper (ROMHÁNYI and DEÁK, 1969).

Discussion

The role of structural lipids in the topooptical staining of membranes

Polarization optical analysis of the orientated dye-binding reactions on biological membranes affords a new means of studying their ultrastructural

organization. A general characteristic of the anisotropic staining reaction of membranes with the cationic dye toluidine blue is the dependence of the reactions on the presence of structural lipids in the membranes, as shown by the fact that after lipid extraction (even after short ethanol treatment of formol-fixed slices) the ordered alignment of the toluidine blue molecules on the membrane structure is completely abolished, although dye-binding is not diminished (Fig. 6*b*). This suggests that the negative groups of choline containing phospholipids are not freely accessible to the cationic dye molecules in the membrane structure owing to steric factors, or because they are compensated with the electron-positive group of choline (BANGHAM, 1964; VAN DEENEN, 1966) and are present as non-charged lipids. This is noticeable as phospholipids in spot tests were found to give a considerable basophilic staining, although this was found also to depend on the state of aggregation (SINAPIUS, 1968). In our experiments, however, no basophilic staining of the structural lipids in the investigated membranes was demonstrable with toluidine blue. It appears, however, that in other membrane types, with more anionic phospholipids such as phosphatidyl glycerol and sulpholipids a basophilic staining may be observed in the lipid component itself. Thus one can conclude that, in general, the lipid molecules of membranes do not play a role in the binding of cationic toluidine blue molecules and are essential only for the orientation of dye molecules, bound by the negative side groups of the non-lipid framework (proteins, mucoproteins, RNA) of the membranes.

Intercalation of dye molecules in the membrane structure

Polarized light data indicate that in the orientated dye binding reactions of biological membranes the dye molecules are intercalated with their light absorbing (retarding) bonds (molecular planes) in parallel between the hydrocarbon chains of the lipids and, thus, their planes perpendicular to that of the membranes (Fig. 12a).

The ability of the dye molecules to intercalate between the structural lipids is largely dependent on their molecular conformation. Apparently only cationic dye molecules with polar-apolar conformation and a flat molecular shape are able to intercalate between the phospholipids of membranes whose molecules similarly possess a polar-apolar conformation.

As shown in the diagram in Fig. 12 the polar-apolar conformation of toluidine blue and ethacridine appears to render these molecules capable of intercalation between the lipid molecules of the membrane in such a way that their positively charged polar groups are confronted with the negatively charged polar groups of the lipids, and their apolar molecular parts intercalate between the apolar zones of the hydrocarbon chains.

Methylene blue, structurally closely related to toluidine blue, is not able to induce anisotropic staining of the membranes because it is unable to intercalate between lipids. This is probably due to the symmetrical distribution of the electrical charges on the methylene blue molecule, which renders it unable to intercalate in the lipid layer. Ethacridine, which has two electrically charged groups, located not symmetrically but diagonally on the molecule (Fig. 12), induces a strong anisotropic staining reaction of the membrane not only when the lipids are present, but even after lipid extraction, when its anisotropic effect has been decreased to about 30%, indicating that the molecule with its two diagonally located charged groups is able to maintain a certain degree of orientation even on the lipid-extracted framework of the membranes. Toluidine blue, on the other hand, with its single charged group located on one side of the molecule, is not able to maintain an ordered alignment on the membrane structure when the structural lipids are absent.

Ultrastructural density of biological membranes

The degree of intercalation of dye molecules between structural lipids, as reflected by the ratio $\frac{A}{D}$, may be regarded as an indication of the structural density of different membranes. The factors assumed to play a role in determining the structural density of membranes (O'BRIEN, 1965, 1967; VAN DEENEN, 1966) are 1. the length of the hydrocarbon chains of lipid molecules; 2. the number of unsaturated double bonds of the hydrocarbon chains that cause their bending, which inhibits the intercalation of dye molecules; 3. the cholesterol content, which has a condensing effect on the structure of the lipid leaflet; and 4. the number of side chains of proteins bending back into the interior of the membrane (STOECKENIUS, 1960, 1962; HATCH and BRUCE, 1968).

On the basis of the available data O'BRIEN suggested that myelin, as a metabolically inert membraneous structure which contains a large amount of cholesterol responsible for about 50—60% of the birefringence of myelin (BUBIS and WOLMAN, 1962), could be regarded as one of the most tightly-structured biological membranes. While cytomembranes such as mitochondrial and ergastoplasmic membranes with relatively low cholesterol and high phospholipid contents (TAKEUCHI and TERAYAMA, 1965; WHITTAKER, 1966) represent, in the view of O'BRIEN (1965), a loosely-structured membrane type. We found the lowest $\frac{A}{D}$ value for myelin (0.12), which seems to indicate a low degree of dye intercalation in it. This agrees with the dense ultrastructural pattern as suggested by O'BRIEN for myelin. On the other hand, the membrane of red blood cells, which was regarded by O'BRIEN as a compact type of membrane, showed a strong anisotropic staining reaction and a relatively

high value for $\frac{A}{D}$, in contrast to its extremely weak intrinsic birefringence (SCHMITT el al., 1936; BEAR, 1941; MITCHISON, 1953; PONDER and BARRETO, 1956). This suggests a rather more loosely-structured membrane type in the red blood cells. This indirect evidence is at variance with the view of O'BRIEN but seems to agree with the electron microscopic observations revealing a more porous structural pattern in lipid-extracted ghosts (HOFFMAN, 1956).

The role and nature of non-lipid dye-binding sites in membranes

Further studies are needed precisely to clarify the nature of the negatively-charged dye-binding sites of the non-lipid framework of different membranes such as protein, RNA (ROMHÁNYI and DEÁK, 1967b) and mucoproteins (GIELEN, 1968; NAKAO and ANGRIST, 1968). It remains especially to be clarified what role the acid groups of mucoproteins (sialic acid or neuraminic acid), as components of different cellular membranes (EYLAR et al., 1962; PATTERSON and TOUSTER, 1962; WALLACH et al., 1966; GIELEN, 1968; KEMP, 1968), play in the topooptical staining reactions of membranes.

The significance and nature of the acid groups of the EP in the orientated binding of cationic dyes (toluidine blue, or ethacridine) is evident since their anisotropic staining was found to be dependent on the presence of membrane-attached RNA, and to be abolished by ribonuclease treatment or extraction of RNA with perchloric acid. A special characteristic of the anisotropic staining reaction of the EP with toluidine blue was its dependence on the ionic strength of the dye solution. In agreement with electron microscopic and functional data, analysis of the topooptical staining reactions of the EP of the pancreas acinar cell, has led to the conclusion [66] that the RNA of the EP is membrane-bound and membrane-orientated, which is related to its functional activity in protein synthesis (SZÉKELY, 1965; HENSHAW et al., 1963; HENDLER et al., 1964; HENDLER, 1965; BLOBEL and POTTER, 1967a, b). The topooptical staining reactions of the EP of the pancreas acinar cell, dealt with in a previous paper (ROMHÁNYI and DEÁK, 1967b), will be discussed here briefly.

The membrane attachment of ribosomal RNA has been inferred from our finding that toluidine blue molecules bound by RNA are orientated by the structural lipids of the EP membrane. This could be taken as evidence of a close molecular structural relation between RNA and membrane structure. Furthermore, it is obvious that membrane-attached ribosomal RNA can serve as a basis for orientated dye-binding only when it itself possesses a membrane-orientated molecular ultrastructure. Free, not membrane-bound, ribosomes (25% of the total ribosomal population, BLOBEL and POTTER, 1967a, c), distributed at random contribute to the overall basophilic staining of the cytoplasm but not to the anisotropic staining effect, which is brought

about only by the membrane-attached ribosomal RNA (75% of the total ribosomal population in the liver cell) with its membrane-orientated molecular conformation. Such an assumption of the ultrastructural organization of membrane-attached ribosomal RNA, with its membrane-orientated molecular structure, seems to be in agreement with what is suggested by functional and electron microscopical observations (SABATINI et al., 1966; BLOBEL and POTTER, 1967b; FLORENDO, 1969). Based on their studies on the binding of ribosome to the membrane, BLOBEL and POTTER (1967b) suggested that the ribosome had specific binding sites to interact with specific receptor sites on the membrane, and that these binding sites are topographically related to other binding sites on the ribosome for mRNA and transfer RNA in such a way, that they become orientated in a definite way when the ribosome is bound to the membrane.

This orientation then may sterically facilitate the complex molecular interactions during protein synthesis. In this connection the ribonuclease resistance of membrane-attached ribosomal RNA is of special interest. This may be the result of molecular conformational changes of RNA induced by the binding to the membrane, which renders the RNA resistant to the enzyme reaction (BLOBEL and POTTER, 1967b).

Another point of interest is the dependence of the orientated dye binding of EP on high electrolyte concentrations of the dye solution. The isotropic staining of EP observed at lower electrolyte concentrations (Fig. 9*a*) appears to indicate an overcrowding of the RNA surface with dye molecules, which cannot be orientated by the membrane lipids. At high electrolyte concentrations, however, a great number of the negative side groups of the RNA are neutralized by adsorbed electrolyte cations so that the dye molecules bound to the remaining negative binding sites can be orientated by the structural lipids of the membranes (Fig. 9*b*). SCOTT and WIKETT (1966) have shown that RNA has a higher critical electrolyte concentration than DNA and is therefore able to bind cationic dye molecules at electrolyte concentrations at which DNA remains unstained, and the staining of RNA is only moderately decreased. Our observations show, however, that at electrolyte concentrations over 0.4 it is not simply a quantitative change in dye binding that takes place, but also a qualitative one, i.e. a transformation of the random binding of dye molecules into a paracrystalline ordered dye association on the ergastoplasmic structure (Fig. 9*b*).

Evidence is accumulating to show that cations (Mg^{++}) play an important role in the structural organization and functional activity of ribosomes in protein synthesis (BREILLATT and DICKMAN, 1966; DALLNER and NILSSON, 1966; MOORE and ASANO, 1966). YOUNG SUNG CHOI and CARR (1967) suggested that the structural stabilization of ribosomes by Mg^{++} was not simply a random aggregation of subunits, but that the subunits associated in an organ-

ized pattern to form the biologically active particles in the E. coli cell. Mg^{++} can be displaced by other cations (monovalent, divalent) or by spermine or protamine. The said authors assumed that such a competitive binding of cations on ribosomes might be involved in the cellular control mechanism of protein synthesis. It would be tempting to speculate about whether the structural organizational effect exerted by cations on an orderly pattern of dye binding on the EP could be compared to the organizational effect of cations on the ribosomal structure *in vivo*.

Role of electrons in orientated dye binding of the EP membranes of liver cells

The conditions of orientated dye binding by the EP membranes in liver cells are of special interest. The EP in liver cells, unlike those in other cells such as pancreas acinar cells, plasma cells or fibroblasts was found to bind toluidine blue molecules in an orientated pattern only in the reduced state, and only if the electron acceptor ferricyanide was applied as a post-staining precipitant. The explanation of the role of ferricyanide might be that, as a very active electron acceptor, it interacts with the electrons present in the reduced membrane structure and, in this way, a membrane orientation of the dye-ferricyanide complex is brought about (Fig. 13). It is of interest to note that in this orientated dye binding reaction of the EP membranes of liver cells a complex is formed in which the following structural components are involved: 1) membrane-attached RNA which binds the dye molecules, 2) structural lipids which play a role in the orientation of the dye-ferricyanide complex, 3) the electron acceptor ferricyanide, which interacts with the dye molecules and with 4) the electrons present in the reduced membrane structure. The result is a multifactorial complex of a highly ordered structure dependent on the electrons present in the reduced state of the membrane. When all factors, except electrons, involved in this orientated dye binding reaction are given, no membrane orientation of the dye-ferricyanide complex is possible because in the oxidized state no electrons are available for the ferricyanide molecules to interact with, and therefore the RNA-dye-ferricyanide-lipid complex remains unorientated (Fig. 13*b*). This type of topooptical staining reaction is a special characteristic of the EP of liver cells since EP in all other cell types failed to show such a dependence of the anisotropic staining reaction on the oxidoreductive state. The physiological reduced state of the EP membranes of the liver cell can be assumed to result from the constant electron flow (RAPOPORT, 1966) during the metabolic dehydrogenating processes governed by the enzymes of the electron transport chain. The EP of liver cells is known to be rich in electron transporting enzyme systems, especially in flavoproteins, involved in different metabolic dehydrogenating processes (ERNSTER et al., 1962; NISHIBAYASHI et

al., 1963; ERNSTER and ORRENIUS, 1965; KAMIN et al., 1965). The orientated dye binding of the EP of liver cells depending, among other ultrastructural factors, on the reduced state, provides an essentially new approach for the study of the functional activity of a biological membrane at the ultrastructural electronic level (ROMHÁNYI and DEÁK, 1969).

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DIE ULTRASTRUKTURELLE ORGANISATION BIOLOGISCHER MEMBRANEN AUF GRUND VON TOPOOPTISCHEN FARBREAKTIONEN

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Biologische Membrane zeigen mit kationischen Farbstoffen (Toluidinblau oder Ethacridin) orientierte, durch starke anisotrope Effekte gekennzeichnete Farbstoffbindungsreaktionen, falls nach der Färbung eine entsprechende Fixierung vorgenommen wird. Auf Grund ihrer verschiedenen topooptischen Farbreaktionen ließen sich die biologischen Membranen in drei Typen unterteilen. Bei allen drei Typen war die orientierte Bindung des Farbstoffs (Toluidinblau) an die Anwesenheit von Strukturlipiden gebunden. Es ließ sich indessen nachweisen, daß die Lipide nur in der Orientierung jener Farbstoffmoleküle beteiligt waren, welche durch die anionischen Farbstoffbindungsstellen am nichtlipiden Gerüst der Membranen gebunden wurden.

Die polarisationsoptischen Angaben deuteten darauf hin, daß die Farbstoffmoleküle zwischen den Wasserstoffketten der Strukturlipide mit ihren Flächen parallel eingeschaltet waren. Die polar-apolare Konformation und die flache molekulare Form der Farbstoffmoleküle spielt hierbei eine entscheidende Rolle. Durch die Bestimmung des Verhältnisses der Aniso-

tropie zu der Toluidinblau-Färbung-bedingten optischen Dichte der verschiedenen Membranen, konnte ein mittelbarer Beweis ihrer ultrastrukturellen Dichte erbracht werden.

Die orientierten Farbbindungsreaktionen der EP-Membranen von Pankreas-Acinuszellen, aktiven Fibroblasten, Plasmazellen und anderen proteinsezernierenden Zellen hing von der Ionenstärke der Farbstofflösung ab, und diejenigen der EP-Membranen von Leberzellen, darüber hinaus, auch von ihrem reduzierten Zustand und von der Verwendung des Elektronenakzeptors Ferrocyanid als Fällungsmittel nach der Färbung. Auf diese Weise wurden indirekte Beweise der ultrastrukturellen Organisation der Ribonukleinsäure des EP und des oxydoreduktiven Zustands der Leberzellen-EP erhalten. Die topooptischen Farbreaktionen liefern mithin eine neue Annäherungsmöglichkeit bei der Untersuchung der ultrastrukturellen Organisation von biologischen Membranen unter physiologischen und pathologischen Bedingungen.

УЛЬТРАСТРУКТУРНАЯ ОРГАНИЗАЦИЯ БИОЛОГИЧЕСКИХ МЕМБРАН НА ОСНОВЕ ИХ ТОПООПТИЧЕСКИХ ЦВЕТНЫХ РЕАКЦИЙ

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Биологические мембраны дают с катионными красителями (толуидиновая синька и этакридин) ориентированные, характеризуемые сильными анизотропными эффектами, реакции связывания красителя, если после окрашивания применяется соответствующая фиксация. На основе различных топооптических цветных реакций удалось обособлять три типа биологических мембран. Ориентированное связывание красителя (толуидиновой синьки) зависит у всех трех типов от присутствия структурных липидов. Однако, удалось доказать, что липиды участвуют лишь в ориентировании тех молекул, которые связываются местами связывания анионных красителей на не-липидном остове мембраны.

Поляризационно-оптические данные указывают на то, что молекулы красителя располагались между углеводными цепями структурных липидов параллельно их плоскости. Решающую роль играют при этом полярно-аполярная конформация и плоская молекулярная форма молекул красителя. Определением соотношения между анизотропией и оптической плотностью, обусловленного окрашиванием различных мембран толуидиновой синькой, было получено косвенное доказательство их ультраструктурной плотности.

Ориентированные цветные реакции ЕР-мембран ацинозных клеток поджелудочной железы, активных фибробластов, плазматических клеток и прочих протеиносецернирующих клеток зависит от ионной силы раствора красителя, а реакции ЕР печеночных клеток, сверх того, также от редукционного состояния и применения электронно-акцептора феррицианида в качестве преципитатора после окрашивания. Таким образом было получено косвенное доказательство ультраструктурной организации РНК ЕР и окислительно-восстановительного состояния ЕР печеночных клеток. Следовательно, топооптическое окрашивание предоставляет новую возможность изучения ультраструктурной организации биологических мембран при физиологических и патологических условиях.

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TERMINAL ARBORIZATIONS IN SPECIFIC AFFERENTS IN THE SPECIFIC THALAMIC NUCLEI

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Branching pattern and shape as well as size of arborizations of specific sensory as well as specific non-sensory afferents have been studied in the thalamic and geniculate nuclei of the adult cat. The brush-like arborizations known from the classical description correspond to preterminal arborizations. Their terminal parts, the so-called terminal branching units, are described and some speculations about the significance of these branching patterns for synaptic linkage are presented.

The specific afferents are easy to define in the specific sensory nuclei where the optic fibres, for example, are the specific afferents of the lateral geniculate body (LGB), or the lemniscal fibres are the specific afferents of the ventrobasal nucleus (VPL and VPM). If used in such a restricted sense, the term "specific" would have little if any meaning as one might then use the term sensory (secondary or of *n*th order) afferent as well. This would even have the advantage of being more straightforward and unequivocal. The term "specific" aims at giving a somewhat more general meaning to the classcifiation of pathways or fibres. It signifies a judgement or opinion that the connexion which is labelled specific is characteristic solely of the nucleus in question. It also includes the statement that the connexion in question is instrumental in leading to (or from) the nucleus the decisive information processed there. Conversely "non-specific" would mean connexions which are not unique and characteristic and which do not convey the decisive information processed in the nucleus under consideration. — In this sense the main afferent systems of the non-sensory relay nuclei, for example the cerebellar afferents of the ventralis lateralis nucleus (VL) and the mamillo-thalamic afferents of the anterior nuclear complex can be regarded as specific. They have obviously to be separated from descending cortical or subcortical as well as from intrathalamic connexions.

This paper aims at clarifying, whether the specific afferents in the above sense of specific thalamic nuclei have some common features and whether they can be distinguished on the basis of their caliber, branching pattern, size or shape of the terminal arbor and whether on this basis they could be distinguished from other types of connexions in the same nuclei. The study has been based chiefly on Golgi preparations and may be considered an essen-

tial preliminary step for any attempt at a better understanding of the numerical and spatial divergence and convergence or what this amounts to at understanding the topology as well as the geometry of the connectivity of the relay from specific afferents to cortical afferents.

Due to difficulties in the Golgi impregnation of specific thalamic and geniculate afferents, data concerning their arborizations are scarce and apply mainly to the undeveloped thalamus of newborn animals (CAJAL, 1911). Some more information has been offered by the studies of O'LEARY (1940), SZENTÁGOTHAI (1963), GUILLERY (1966, 1967) and PETERS and PALAY (1966) on the arborization of optic fibres. Specific afferents of the medial geniculate body (MGB) have recently been analyzed by MOREST (1963, 1964, 1965) and by MAJOROSSY and RÉTHELYI (1968), those of the VPL by SCHEIBEL and SCHEIBEL (1966) and TÖMBÖL (1966/67, 1968). — The present study, although comprising some material of newborn or few days old animals, has been based mainly on findings on adult or near adult (2—3 months) material, in which the arborizations had undoubtedly reached full maturity.

Methods

The present study has been based on 150 brain series from adult cats and 40 series from kittens. For kittens either the original rapid Golgi procedure (double impregnation) or its modification by VALVERDE (1962) was applied. As seen in Fig. 1, these methods ensure an excellent all-over picture of the preterminal arborization, without staining the real terminal expansions. This led to misinterpretations of the relation between specific afferents and thalamic geniculate neurons, considered by CAJAL (1911) pericellular nets or nests, a description still influencing the authors (SCHEIBEL and SCHEIBEL, 1966). Specimens from two to three months old cats were treated with a new perfusion Golgi—Kopsch procedure (SZENTÁGOTHAI, 1963; TÖMBÖL, 1966/67) which — as seen in Figs 2 and 3 — is inferior to the original rapid procedure in staining preterminal arborizations, but much superior in demonstrating the real terminal parts of the arborization. Thus, both methods are essential for an understanding of the arborization as a whole.

Results

From all the terminal axonal or collateral arborizations in the specific thalamic nuclei the specific afferents are showing the most characteristic branching patterns with almost individual specificities in the nuclei. In spite of some similarities the arborizations are different in the case of the specific sensory afferents and the specific non-sensory afferents.

1. *Specific sensory afferents.* The all-over shape of the arborization is that of a cone. In the newborn or young animal the cones are pointed (Fig. 1) and are showing the brush-shaped telodendria so clearly described by CAJAL (1911) and more recently by SCHEIBEL and SCHEIBEL (1966). In the adult cat, the arbors assume their final shape of a broad cone 300—400 μ in diameter, and around 200—250 μ in height. It is, of course, rare that a sufficiently well stained complete cone should be traced in the same preparation (Fig. 2). Therefore, little can be said about the variations in shape and size

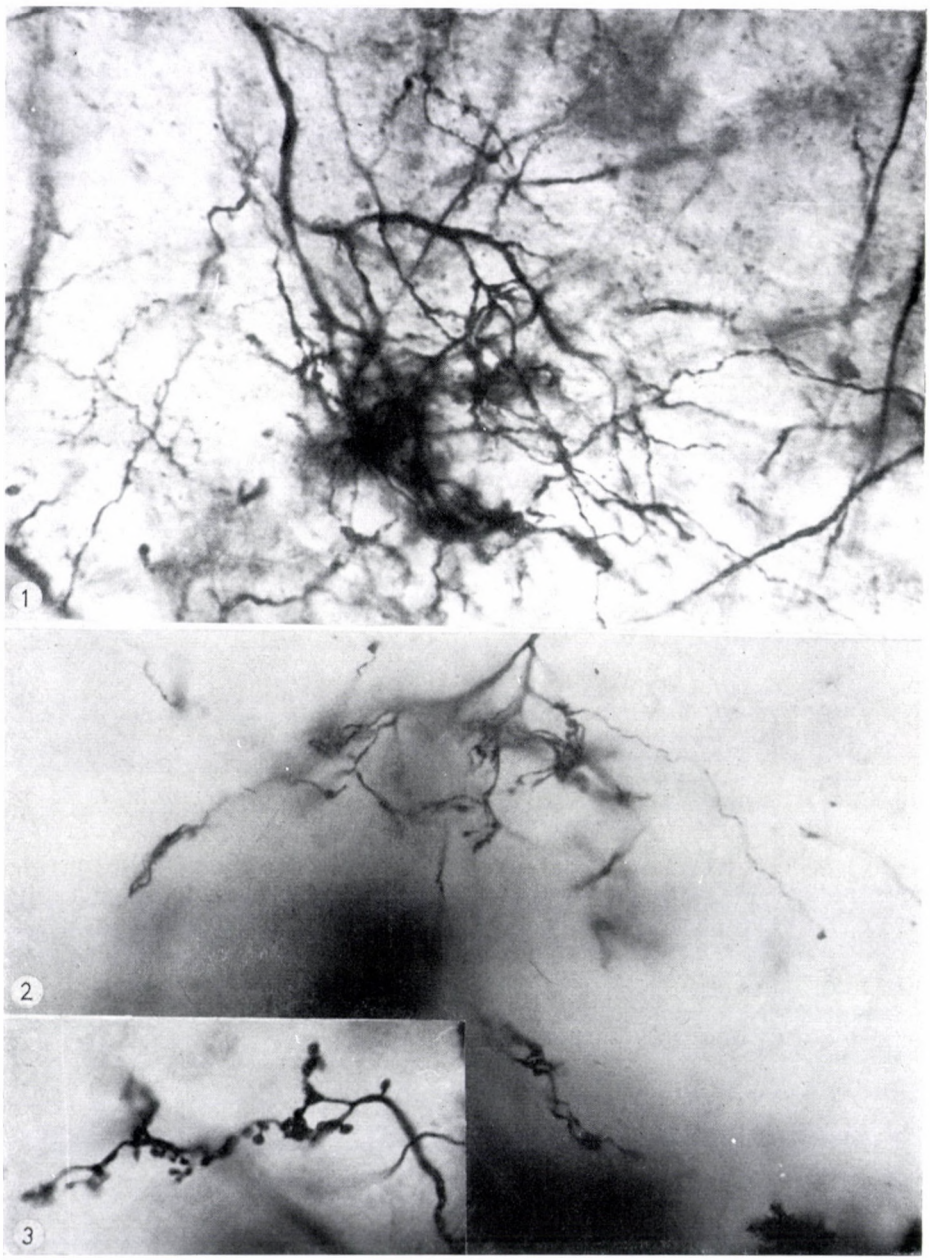
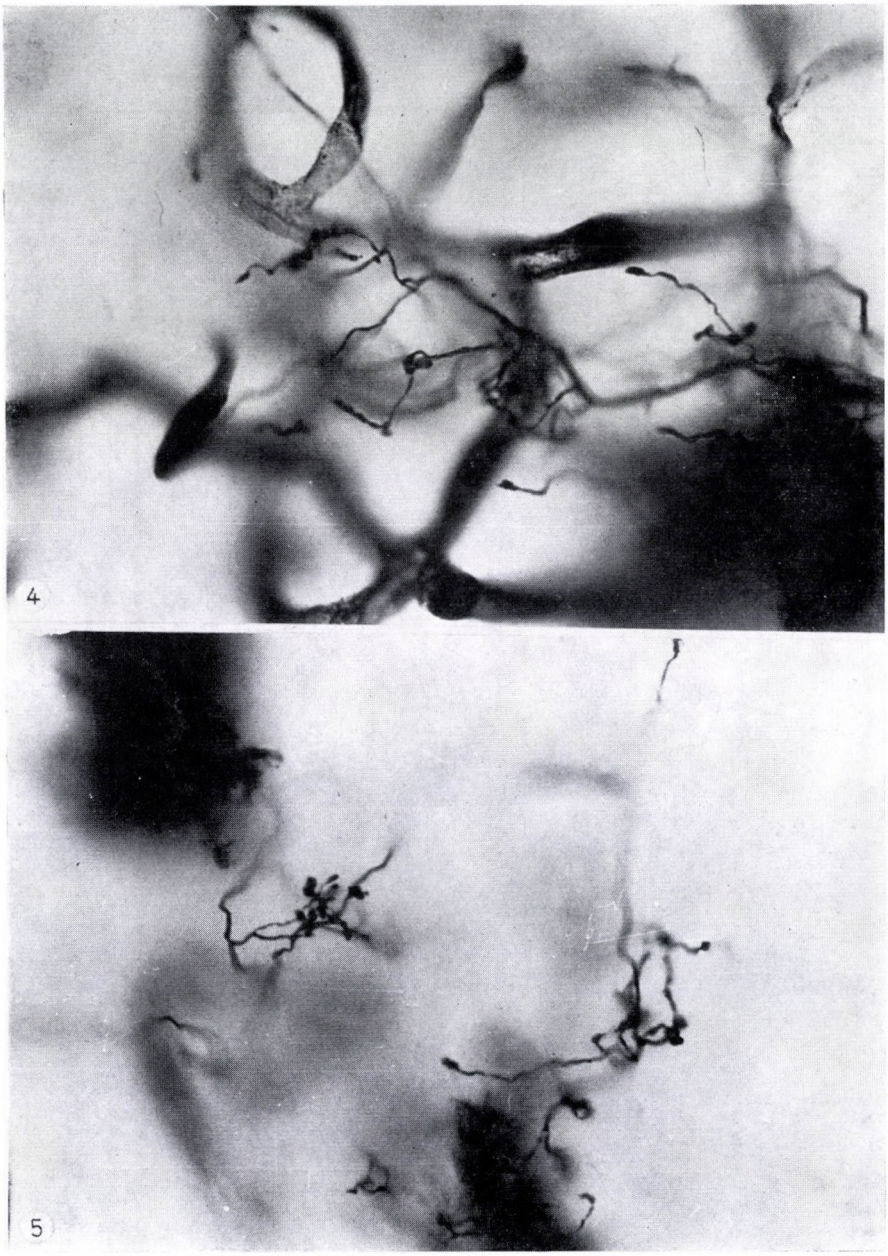
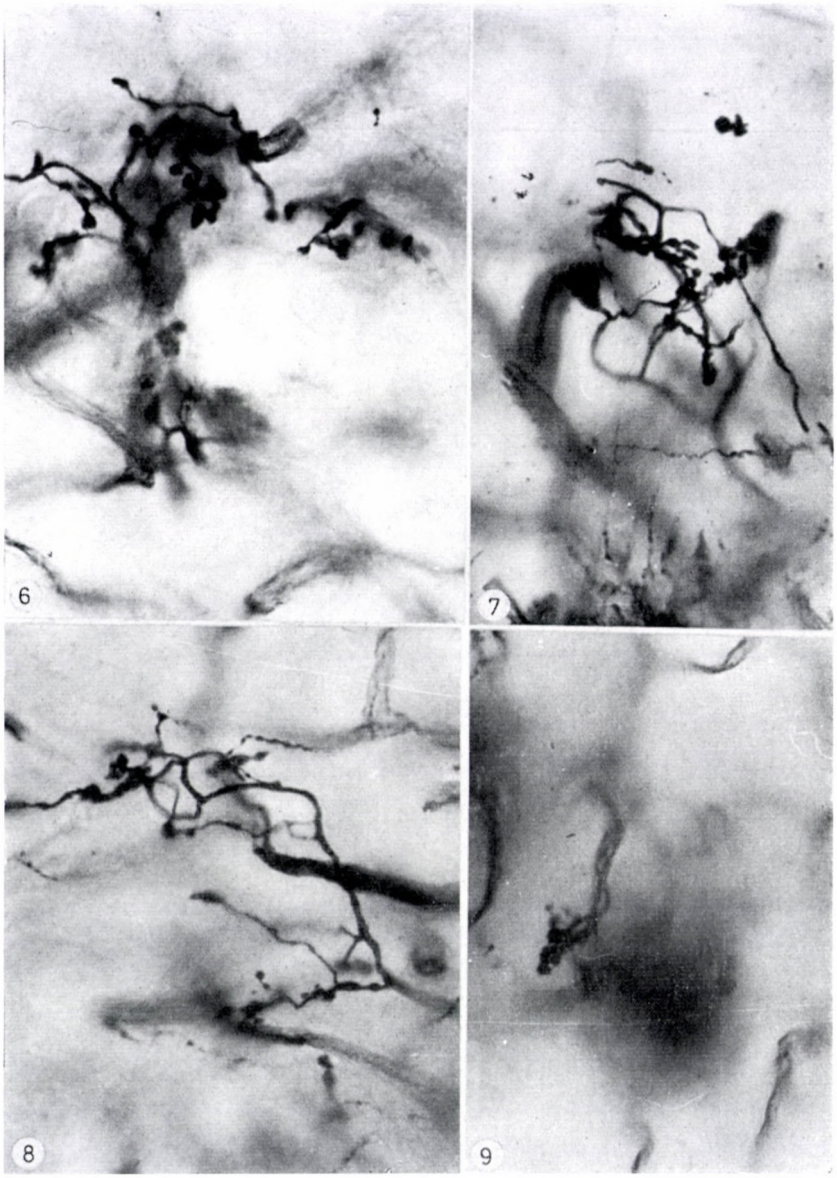


Fig. 1. Arborization pattern of lemniscal fibre in VPL. Rapid Golgi picture, ×800
Fig. 2. Arborization pattern of lemniscal fibre in VPL. Golgi—Kopsch picture, ×500
Fig. 3. Detail of terminal arborizations of optic fibre in CGL. Golgi—Kopsch preparations, ×800



Figs 4—5. Details of terminal arborizations of lemniscal fibre in VPL. Golgi—Kopsch preparations, ×500



Figs 6, 7, 8, 9. "Terminal units" of optic fibres. Golgi—Kopsch preparations, ×500

of the cones apart from that their base is not circular but elliptic. The difference, nevertheless, between the brushes in the newborn and the relatively broad cones shows clearly the considerable transformation occurring during postnatal growth and the later development of the neuropil.

The remarkable new information supplied by the perfusion Kopsch procedure in the adult material is the separation from the preterminal arboriza-

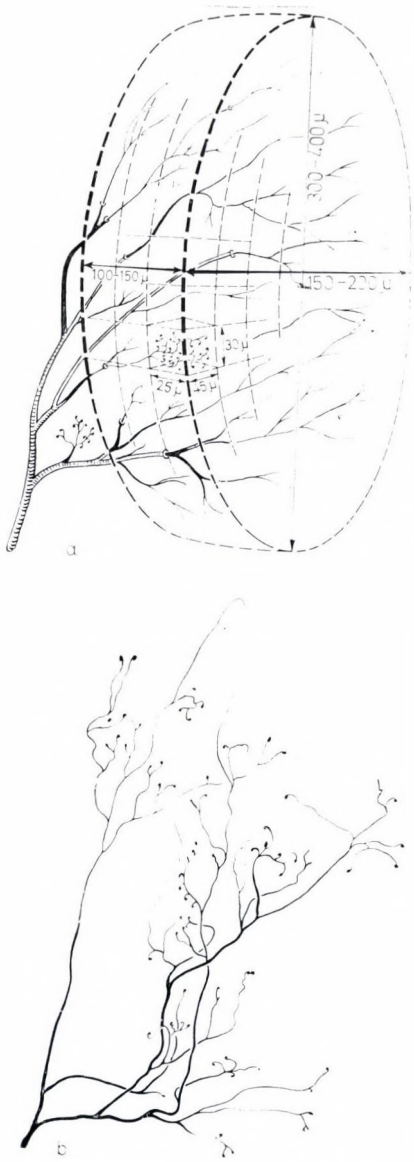


Fig. 10a. Arborization tree of specific fibre shown diagrammatically
Fig. 10b. Arborization tree of lemniscal fibre shown diagrammatically

tion cone of the real terminal units (Fig. 3). The terminal part of the ramifications termed terminal units are grape-like arborizations of varying density and size. An average terminal unit measures 20—45 microns in diameters but some may be as large as 60 microns and others considerably smaller (10—30 microns). Each of the final branches terminates in a club-shaped thickening.

Various terminal units of lemniscal fibres of the VPL are shown in Figs 6, 7, 8 and 9. Terminal units do not occur in the whole space occupied by the arborisation cone. The apical part of the cone is usually devoid of terminal units and cannot, therefore, be considered a synaptically active part of the arborization. As indicated diagrammatically in Fig. 10, the terminal units are confined to a disk-shaped basal part of the cone 100 to 150 microns high.

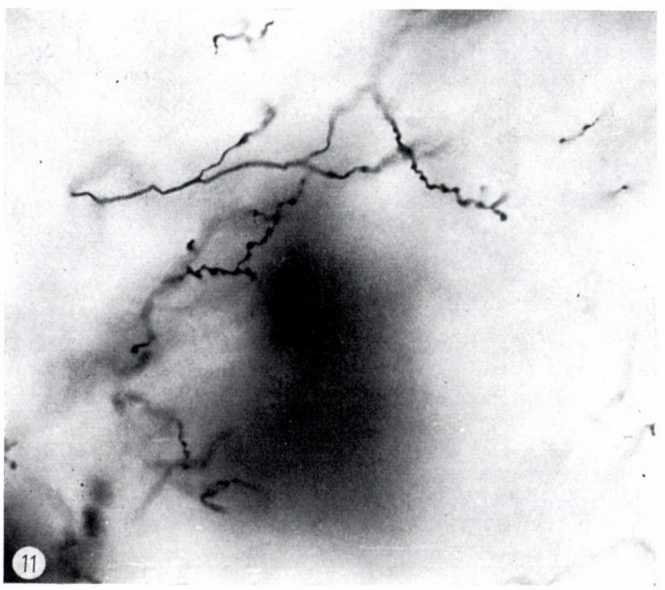
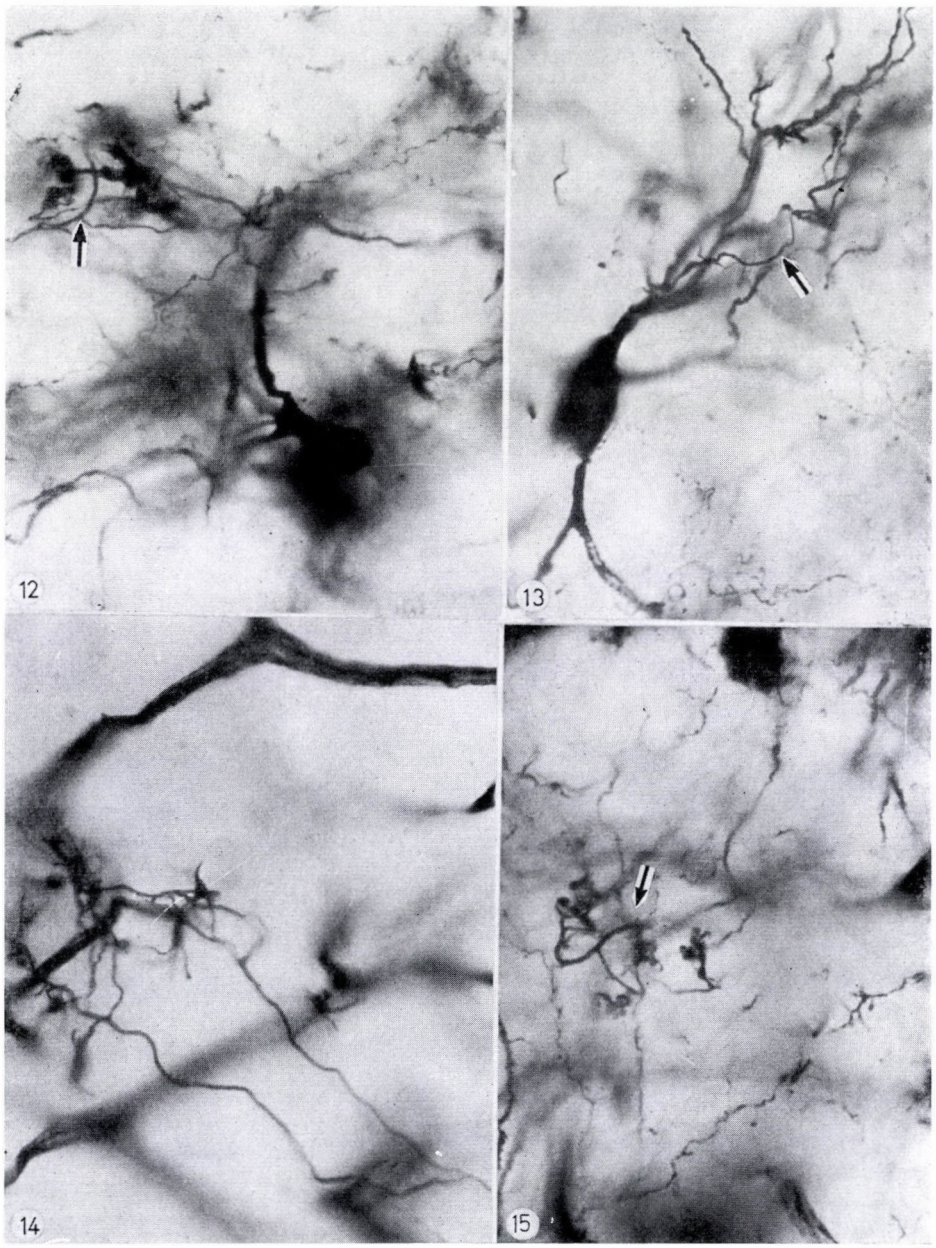


Fig. 11. Ramification of spino-thalamic fibre. Golgi—Kopsch preparation, ×500

This disk-shaped region is the space in which the specific afferent is synaptically active. The number of terminal units within a specific afferent arborization varies considerably between 50 and 150.

The arborization of spino-thalamic fibres in the VPL differs considerably from that of lemniscal fibres, by expanding diffusely over a much larger space. A diffuse lattice is established by such fibres which is not confined to the boundaries of the nucleus. Terminal knobs are also arranged diffusely and no accumulation of a larger number of boutons from the same afferent occurs within any restricted space (Fig. 11).

2. *Specific non-sensory afferents.* The specific non-sensory relay nuclei are in many respects similar to the specific sensory afferents, being either large or medium size fibres with a definite arborization shape. Only in the medial dorsal (MD) and the ventralis lateralis (VL) nucleus are the arborizations somewhat more diffuse and uncertain in shape and pattern. Arborizations are less rich in the non-sensory relay nuclei and most of them occupy a smaller space than in the sensory relay nuclei. A particularly characteristic pre-



Figs 12, 13. Terminations (arrows) of non-sensory specific fibres in AV. Golgi—Kopsch pictures, ×500

Fig. 14. Terminal arborization of non-sensory specific fibre in AD. Golgi—Kopsch picture, ×500

Fig. 15. "Terminal unit" of non-sensory specific fibre in VL. Golgi—kopsch preparation, × 500

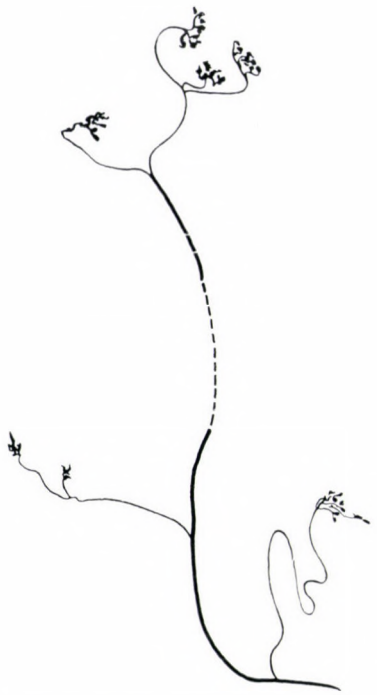


Fig. 16. Arborization pattern of non-sensory specific fibre, in VL, shown diagrammatically

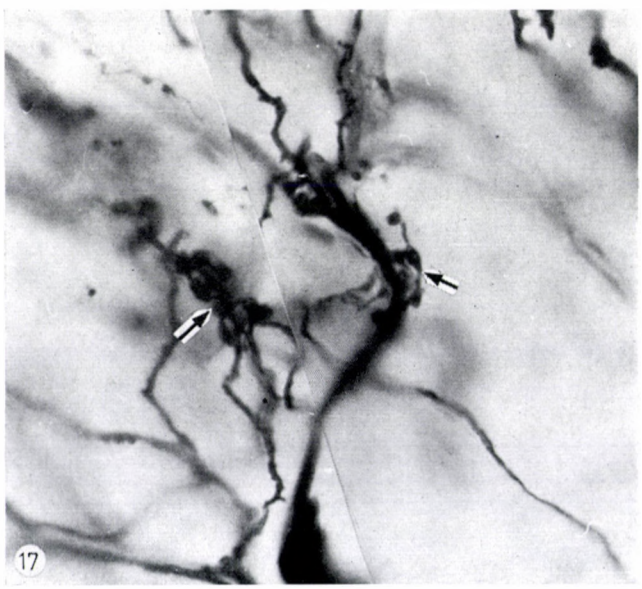


Fig. 17. Terminal unit "arrows" of optic fibre articulating with dendritic tuft of relay neuron in CGL. Golgi—Kopsch preparation, ×800

terminal course can be observed in the anterior nuclei where the preterminal part of the mammillo-thalamic fibres is often tortuous or curved. No real arborization cones are formed. The terminal units or expansions are also characteristic and resemble in shape the mossy fibres of the cerebellar cortex (Figs 12, 13, 14), as correctly observed already by CAJAL (1911). The arborizations of the cerebellar afferents in the VL are more closely resembling the lemniscus afferents, particularly as concerns the terminal units (Fig. 15). The preterminal arborization is more elongated, having two to three final branches with as many and similar terminal units as the ends of two to three collaterals given off at a distance from the final branching. Thus, the arborization of the cerebellar afferents is not confined to disk-shaped spaces as seen in the case of the sensory afferents but to an indistinct elongated region cutting through part of the nucleus (Fig. 16).

Discussion

The introduction of the perfusion Kopsch procedure in the study of Golgi architectonics in the thalamus and geniculate bodies of adult animals has clearly shown that the classical description of the specific afferent arborization needs some reconsideration. The brush-like arborizations revealed in young animals by the rapid Golgi procedures correspond to preterminal arborizations, neither does the description by CAJAL (1911) of pericellular nests as the mode of synaptic connexions do justice to reality. The scarcity in all specific thalamic and geniculate nuclei of axo-somatic synapses as revealed by the EM (SZENTÁGOTHAI et al., 1966; MAJOROSSY and RÉTHELYI, 1968) shows also that pericellular baskets, nests or calyces cannot be the mode of articulation between specific afferents and relay cells. In the MGB, MAJOROSSY and RÉTHELYI (1968) have shown that the main dendrites of the relay cells are almost completely devoid of synaptic contacts, which suddenly start in large abundance in the regions of the dendritic tufts, *i.e.* where the main dendrite breaks up into several secondary dendrites. In fact it is most common to observe the dense parts of what has been described as terminal units (Figs 4 to 9) to interdigitate intimately with the dendritic tufts (Fig. 17).

The size of the terminal units agrees well with the size of the dendritic tufts and the presence of numerous dendritic protrusions in the regions of the dendritic tufts. This situation often observed in the perfusion Kopsch picture has been interpreted by SZENTÁGOTHAI (1963), SZENTÁGOTHAI et al. (1966) and MAJOROSSY and RÉTHELYI (1968), in agreement with the results of EM analysis, by assuming that the predominating site of synaptic articulation between specific afferents and relay cells are the dendritic tufts of the latter. From the functional point of view the intimate interdigitation between a

dendritic tuft and a terminal unit would, by numerous contacts established between the branches of the same axon on one and the same dendrite on the other side, ensure an effective contact. SZENTÁGOTHAI (1967) has based some interesting speculations on the mode of synaptic action of a single terminal unit on a number of relay cells and interneurons, if the distribution of these elements were at random. Taking, however, into account the extreme degree of specificity that seems to emerge from everything what recent physiological evidence is constantly bringing up in the field of connexions between specific afferents and relay cells, it is probably better to abandon in further speculations the idea of randomness and to assume a high degree of specificity.

Starting from the data obtained for the specific sensory afferents in the VPL or the LGB, according to which the number of terminal units — and thus the number of potential major (multiple) synaptic sites — would be between 50 and 150 per afferent, and assuming that all these would be in contact with dendritic tufts of relay cells, the number of relay cells contacted by a specific afferent would be around 5—20. This number follows from the fact that each relay cell has about 5—10 main dendrites and as many dendritic tufts. This calculation, however, is based on two unlikely assumptions, *viz.* *(i)* that all dendritic tufts of any given relay cell are occupied by the terminal units of the same afferent and, *(ii)* that the same dendritic tuft is involved with a single terminal unit. If these assumptions would not hold true — as is probably the case — the number of relay cells contacted by a single sensory afferent must be still higher. It is impossible to proceed further without having data on cell numbers and dendrites. However, so much is clear from these considerations, that the anatomical findings presented are irreconcilable with the assumption of a one-to-one relation (or one close to this figure) between afferents and relay cells.

The differences between specific sensory and non-sensory afferents are only quantitative and concern the shapes of the tissue area encompassed by the afferent axon arborization. Particularly the cell groups contacted by the individual mammillo-thalamic afferents may be smaller or at least narrower. The tortuous course of the afferents does not suggest that any simple geometric grouping could be present in the anterior nuclei. In the VL, the arrangement of relay cells contacted by the same afferent are relatively narrow groups longitudinally arranged in ateroposterior direction.

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CHARAKTERISTISCHE ENDVERZWEIGUNGSMUSTER DER SPEZIFISCHEN AFFERENTEN FASERN IN DEN SPEZIFISCHEN THALAMUSKERNEN

THERESE TÖMBÖL

Das Verzweigungsmuster sowie auch die Form und das Ausmaß der Arborisation der spezifischen sensorischen wie auch der spezifischen nichtsensorischen afferenten Fasern wurde in den Thalamuskernen, ferner in den Kernen des Corpus geniculatum erwachsener Katzen untersucht. Die aus der klassischen Beschreibung bekannte bürstenartige Arborisation entspricht nur der präterminalen Verzweigung. Verfasserin beschreibt die Endteile der Arborisation, die sog. terminalen Verzweigungseinheiten, und stellt Theorien in bezug auf die Bedeutung dieser Verzweigungsmuster in der Herstellung synaptischer Verbindungen auf.

ХАРАКТЕРНЫЕ СВОЙСТВА КОНЦЕВЫХ РАЗВЕТВЛЕНИЙ СПЕЦИФИЧЕСКИХ АФФЕРЕНТНЫХ ВОЛОКОН В СПЕЦИФИЧЕСКИХ ЯДРАХ ТАЛАМУСА

Т. ТЁМБЁЛ

Разветвление, форма и размер арборизации специфических чувствительных и специфических нечувствительных волокон изучались в ядрах таламуса и коленчатого тела взрослых кошек. Известные из классического описания арборизации в виде щетки соответствуют лишь предконцевым разветвлениям. Дается описание концевых частей арборизации, так наз. концевых единиц разветвления и выдвигаются теории о значении этих арборизаций в создании синаптических связей.

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TWO TYPES OF SHORT AXON (GOLGI 2ND) INTERNEURONS IN THE SPECIFIC THALAMIC NUCLEI

THERESE TÖMBÖL

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Two types of short axon (Golgi 2nd) interneurons can be distinguished in perfusion Golgi—Kopsch preparations in the specific nuclei of the cat's thalamus. Distinction of the two types is based primarily on the length of the axon. The axon-arborization of the Golgi 2nd type *a* neuron does not extend beyond the span of the dendritic tree, whereas the axon of the Golgi 2nd type *b* neuron ramifies in a considerably larger area. The axon of this type connects different intranuclear regions and layers. — Connections of the Golgi 2nd type *a* neurons both with relay and with other Golgi 2nd type interneurons have already been established but those of the type *b* interneurons have not yet been clarified.

Introduction

Beside the relay or projective neurons in the specific thalamic nuclei, the second main cell type are relatively short interneurons corresponding in many respects to Golgi 2nd type neurons. They were described in the last century but their functional significance has been brought into focus only recently by physiological observations. As these neurons by their locally branching axons can only affect their immediate neighbourhood, they might be considered an important factor of intranuclear synaptic organization. MONAKOW (1895) termed them "Schaltzellen", a name supported by the morphological observation of specific afferents terminating on these cells in some of the nuclei. This was, however, not corroborated by the observations of ANDERSEN et al. (1964, 1966).

The Golgi 2nd type interneurons are small or medium size cells with long and sparsely branching dendrites usually covered by spines. Their axons arborize profusely but the arborization as a whole is confined to the immediate neighbourhood of the cell. RAMÓN Y CAJAL (1911) did not distinguish the two main neuron types in all thalamic nuclei. Both in the description and in the figures reference is made to small irregular cells having axons that ramify immediately after their origin from the cells. In recent Golgi studies (MOREST, 1964, 1965; GUILLERY, 1966, 1967; SCHEIBEL and SCHEIBEL, 1966) these small cells are mentioned without giving details.

The ratio of the two main cell types — relay and Golgi 2nd type neurons — was established by counting the large and small cell bodies in cresyl-

violet stained preparations (Mc LARDY, 1963; TÖMBÖL et al., 1968). The assumption that the axon of Golgi 2nd type cells terminates mainly on relay neurons has been verified in Golgi studies (TÖMBÖL, 1965, 1966/67, 1968; SZENTÁGOTHAI et al., 1966).

It has been recognized during these studies that two types of short axon interneurons can be distinguished in most of the specific thalamic and geniculate body nuclei. A detailed account of this is presented below.

Methods

For studying the thalamic interneurons various modifications of the Golgi method were applied. The observations are based on 150 complete series of the thalamus of cats around or slightly over 2 months of age stained with the perfusion Kopsch procedure and on 40 rapid Golgi stained (classical double procedure or Valverde's modification, 1962) series of newborn to one week kittens. The interneurons stain well with both the classical Golgi or its Valverde modification and the perfusion Kopsch procedure but the latter technique usually warrants a better impregnation of some important structural details such as the dendrites, dendritic spines, as well as of the axonal arborizations of the Golgi 2nd type neurons.

Results

A survey of the main characteristics of a great number of interneurons with short or relatively short axons gave the impression that two different types of such neurons might be distinguished on the basis of their axon arborization pattern. Specific observations have shown that such a distinction is justified and the two types have been termed Golgi 2nd type *a* and Golgi 2nd type *b* respectively (Figs 1a, b).

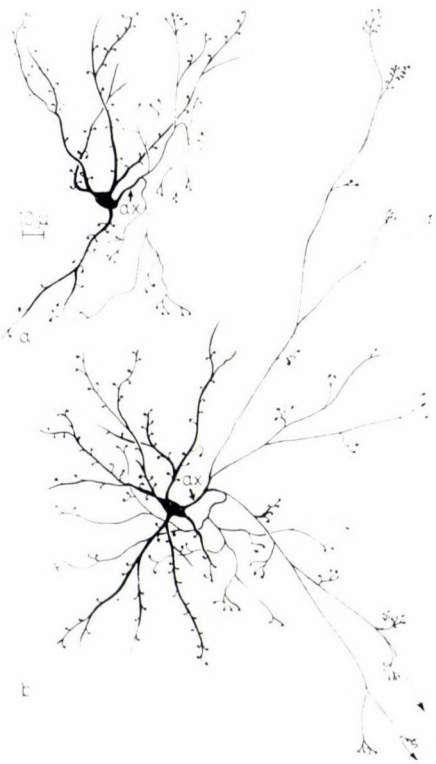
The size of the cell-body of interneurons shows considerable variations of the two (the short and the long) diameters (8—12 μ and 12—18 μ, respectively). The only difference between the two subtypes with respect to size of the cell body is that the cells of subtype *a* may have any size within the above range, whereas the size of subtype *b* is always on the upper side of the scale.

No essential difference between the two subtypes can be noticed in dendrites, dendritic spines or general pattern of dendritic arborization. Corresponding to the larger size of the cell body, the dendrites of subtype *b* are often somewhat thicker than those of subtype *a*. There are some minor differences in dendritic arborization and dendritic spines both being somewhat more profuse or aberrant in subtype *b*. This, however, would not suffice to separate the two subtypes (Figs 2a, b).

In the lateral geniculate body (LGB), where a specific kind of dendritic spines can be observed in subtype *a*, the two kinds of interneuron might also be differentiated on the basis of the dendritic tree (Fig. 3).

The main difference between the two kinds of interneuron is in the character of the branching of the axon, particularly in the distance bridged

by the whole ramification. The axon of subtype *a*, originating either from a principal dendrite or directly from the cell body, ramifies usually by repeated dichotomy into branches of equal thickness immediately after its origin. The space engulfed by the total ramification is small and does not, in general, exceed the size of the dendritic tree. The arborization is dense (TÖMBÖL,



Figs 1a, b. Golgi 2nd *a* and *b* subtype neurons shown diagrammatically

1966/67) having the shape of a more or less regular disk (Fig. 4). Owing to the small number of dendrites, the dendritic tree does not show a spheric arborization. The axon ramification of subtype *a* may partly share the space with the dendritic area, or may be shifted partially or totally in parallel to the latter. Less often the main diameter of the two arborizating disks, that of the dendritic and the axonal, may deviate considerably in direction. The two (the long and the short) diameters of the axonal discoid ramification spaces if elliptic measure 300—400 μ and 150—200 μ, respectively, and are about 50—100 μ in height (Fig. 5).

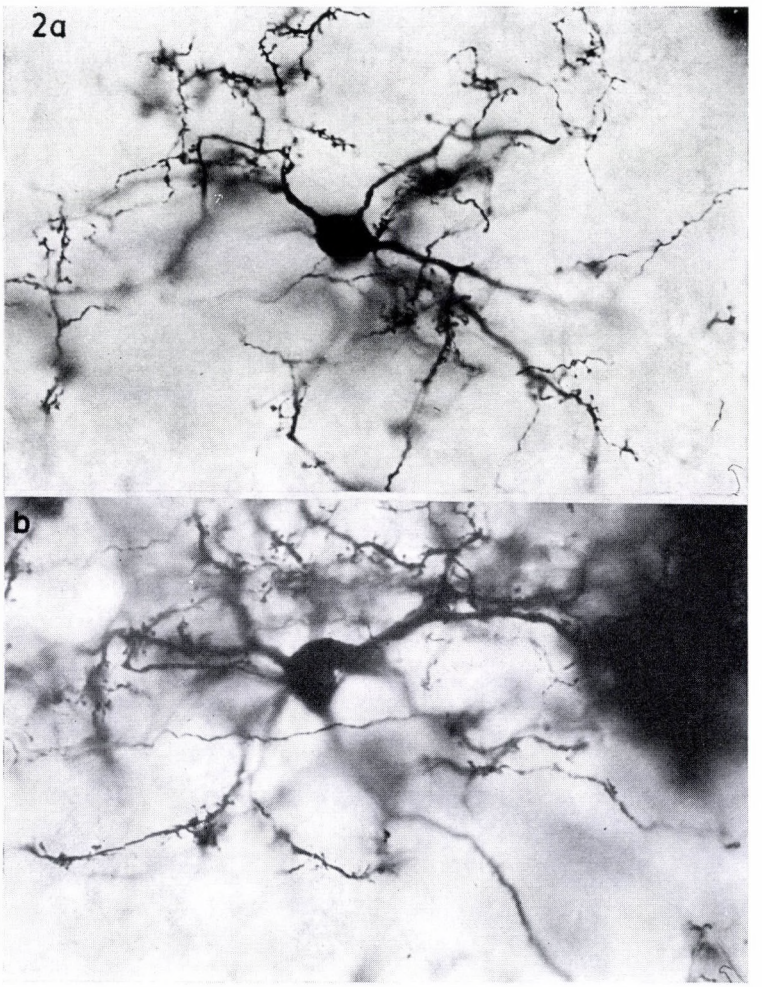


Fig. 2a. Golgi 2nd subtype *a* neuron, Golgi—Kopsch preparation, ×500
Fig. 2b. Golgi 2nd subtype *b* neuron, Golgi—Kopsch preparation, ×500

The axon of subtype *b* can be traced as far as 700—800 μ (Figs 6 to 9). Its axon may originate directly from the soma or from one of the main dendrites. The axon is somewhat thicker than that in subtype *a*. The axon's arborization begins either immediately after its origin, or at a distance from the cell. In the first case two main branches of equal thickness arise from the axon; they can both be traced as far as 700—800 μ. One of the secondary branches, however, gives rise to a secondary branch which terminates in a dense arborization in the close neighbourhood of the cell. The main axon

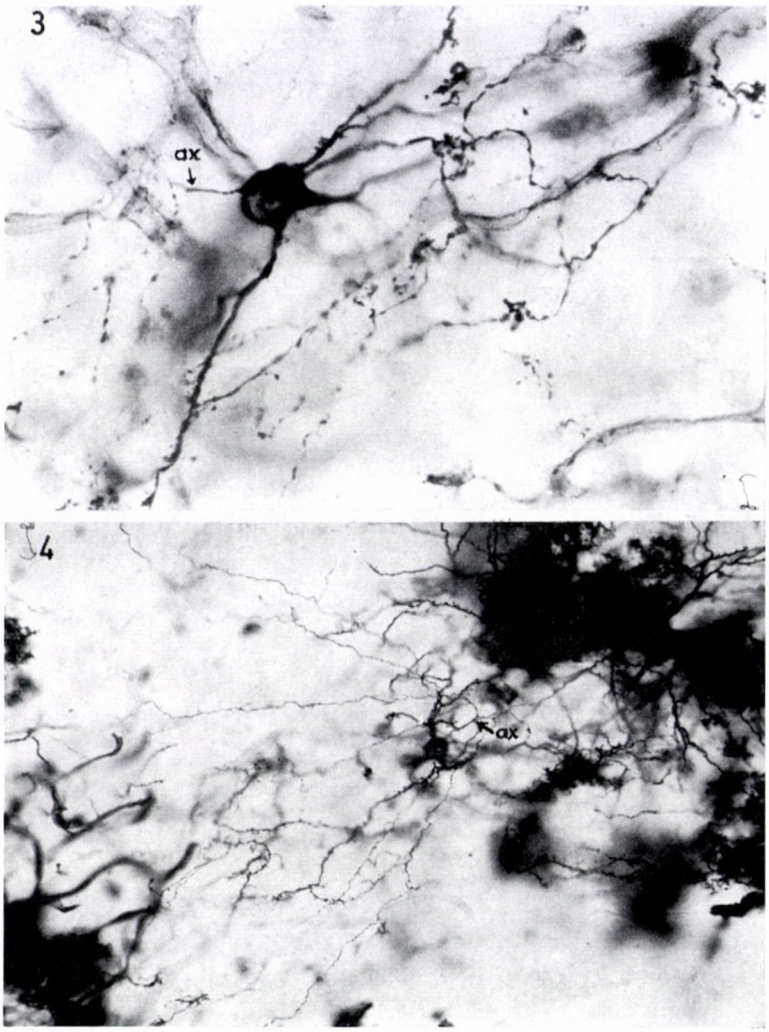


Fig. 3. Golgi 2nd subtype *a* neuron in CGL. Arrow shows the axon and its ramification. Golgi—Kopsch preparation, ×500
Fig. 4. Golgi 2nd subtype *a* neuron in VPL. Arrow shows the axon and its ramification. Golgi—Kopsch preparation, ×200

branches are gradually tapering, giving rise to numerous short and poorly ramifying side branches. The terminal ramifications of the main branches form similar terminal arborizations (Figs 1b, 10, 11, 12). In the second case only few collaterals arise from the main axon to ramify near the cells or at a small distance from their origin. The stem of the axon leaving the territory of the dendritic tree has a sparse terminal ramification.

Connexions between the axon ramifications of subtype *a* neurons and the relay neuron have been shown both in Golgi and EM studies (TÖMBÖL, 1965, 1966/67, 1968; SZENTÁGOTHAI et al., 1966) (Figs 13, 14). The synaptic relations of the axons of subtype *b* interneurons are more difficult to evaluate, as due to the larger expansion of the terminal ramification of the axon, one is less likely to find convincing synaptic contacts in the Golgi pictures. The number of terminal knobs counted in the axon arborizations of interneurons is considerable: 20—50 in subtype *a* and 20—70 in subtype *b*.

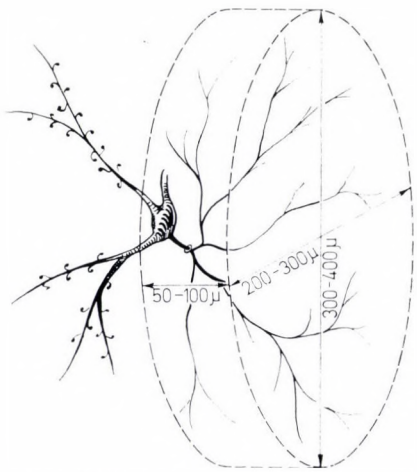
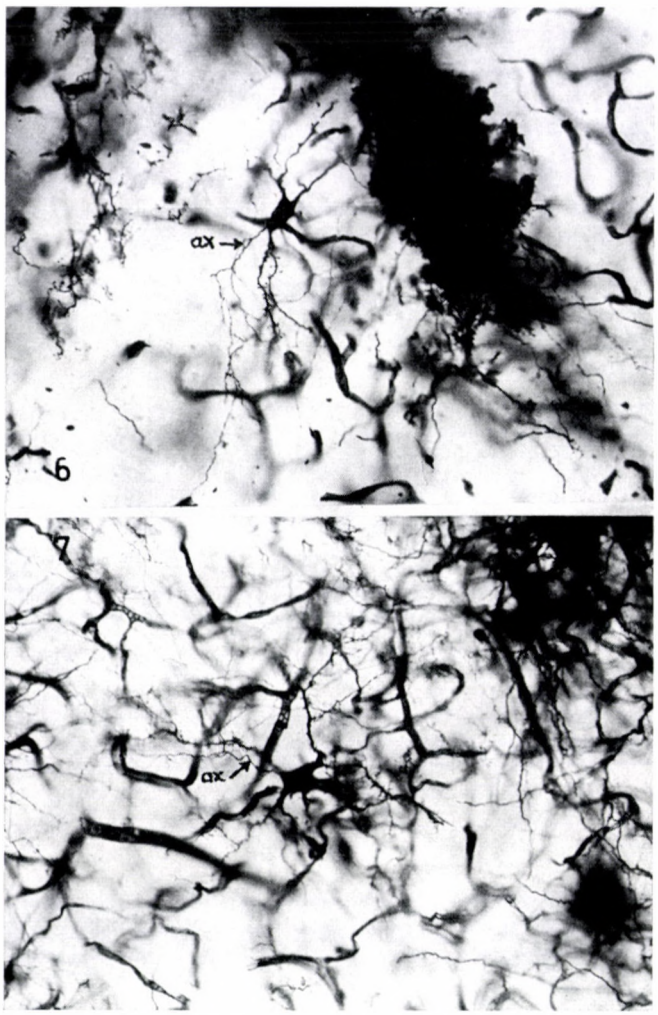


Fig. 5. Ramification space of Golgi 2nd subtype *a* cell axon shown diagrammatically

Whether or not a neuron with short axon is considered a Golgi 2nd type neuron is often somewhat arbitrary. Subtype *a* corresponds in every respect to the classical definition of Golgi 2nd type neurons with respect to both the immediate arborization of its axon after its origin and the formation of a relatively dense terminal arbor in the close vicinity of the cells of origin. This is not so simple in the area of subtype *b*, which satisfies neither of these criteria. On the other hand, the similarity in various characteristics of the perikaryon and the dendrites would certainly favour the view that the two subtypes belong to the same category of neurons. The material studied offered convincing evidence that the axons of subtype *b* although by no means short in the sense of Golgi 2nd type neurons, does not leave the nucleus on which the cell is located. They do, however, establish connections between different divisions of the same nucleus. In the ventrobasal nucleus (VPL) dorsally situated subtype *b* interneurons send their axons to ventral parts of the nucleus and the reverse. In the LGB the axons of subtype *b* interneurons cross the borders between neighbouring layers (from A to A_1 in the cat) and

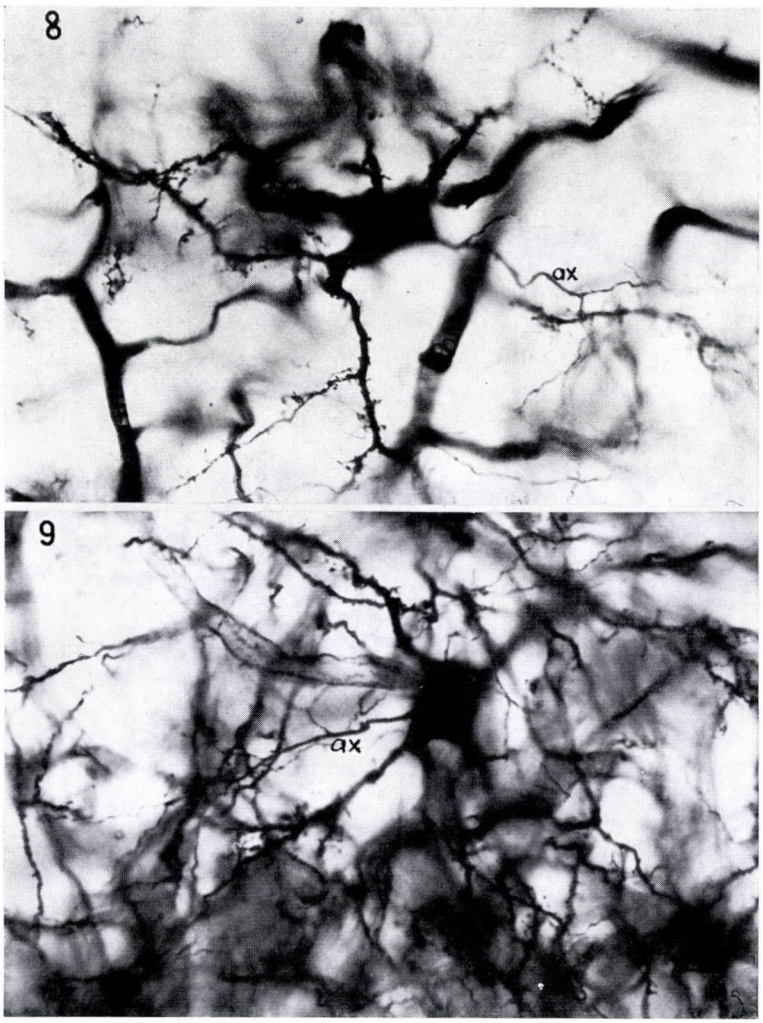


Figs 6, 7. Golgi 2nd subtype *b* neurons in VPL and MD. Arrows indicate the axons. Golgi—Kopsch, ×200

vice versa. In the MGB even axons from cells in the medial division have been traced into the dorsal division of the same nucleus. These observations may offer some clues for understanding the functional significance of the two kinds of interneuron.

Discussion

There is, in most cases, no direct approach to the functional interpretation of structural details of any definite network, apart from the obvious conclusion that the presence of local interneurons means local interactions



Figs 8, 9. Golgi 2nd subtype *b* neurons in MD and VPL. Axons and their ramification are indicated (ax). Golgi—Kopsch, ×500

between the neurons of this network. Further conclusions have to be based on indirect evidence, analogies and an attempt to compare physiological facts with structural data.

The characteristically Golgi 2nd subtype *a* interneuron has a narrow field of action that might affect 5—10 other neurons, from which 1—3 (TÖMBÖL, 1968) might be other interneurons and the rest relay cells. There is some evidence to the effect that interneurons with short axons are often inhibitory in function. The existence of inhibitory interneurons in the VPL has been

postulated by ANDERSEN et al. (1964), to explain the observation of intranuclear recurrent inhibition, and a probable anatomical pathway *via* recurrent axon collaterals of relay cells, Golgi 2nd type interneurons, and back to the relay cells, has been shown to exist (TÖMBÖL, 1966/67). The observations by ANDERSEN and ECCLES (1966) that part of this recurrent inhibition is presynaptic, might be explained by axo-axonic synapses in the VPL, although there is no evidence that in these axo-axonic synapses the terminals of local interneurons are presynaptic to the specific afferents of this nucleus.

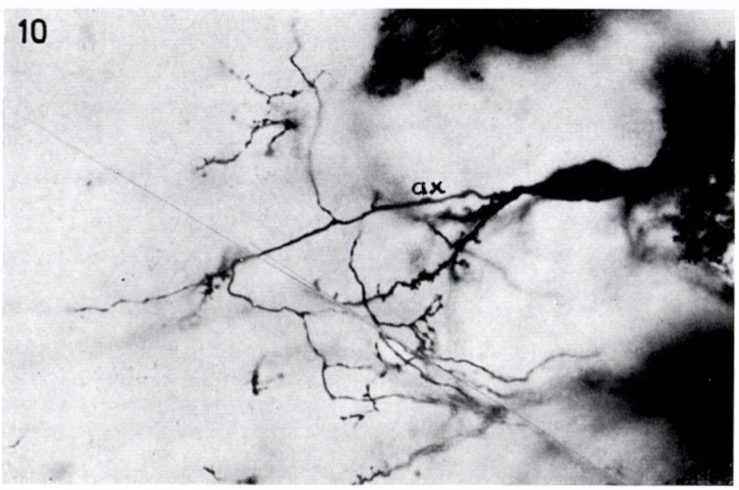


Fig. 10. Axon ramification of Golgi 2nd subtype *b* neuron in VPL. Golgi—Kopsch, ×500

The abundance of local interneuron terminals in the glomerular synaptic complexes in the LGB (SZENTÁGOTHAI et al., 1966) and in the synaptic clusters of the MGB (MAJOROSSY and RÉTHELYI, 1968) is in agreement with the large number in these nuclei of subtype *a* interneurons (TÖMBÖL, 1965, 1968). The small and often flat vesicles of these terminals (PECCI-SAAVEDRA and VACCAREZZA, 1968; MAJOROSSY and RÉTHELYI, 1968) would strongly suggest their inhibitory character — provided that UCHIZONO's concept (1965) is correct. It would be difficult to believe that this great wealth of inhibitory terminals should be serving exclusively recurrent inhibitory phasing of the discharge of relay cells. Various other possibilites have been discussed by SZENTÁGOTHAI (1967, 1968). Collateral inhibition, assumed in the VPL by POGGIO and MOUNTCASTLE (1963) and sharpening of contrast in the LGB (JUNG, 1960) might be one of these, but many other transform functions could be envisaged.

No functional explanation can be offered for subtype *b* of interneurons. Not having identified their synaptic contacts under the light microscope and

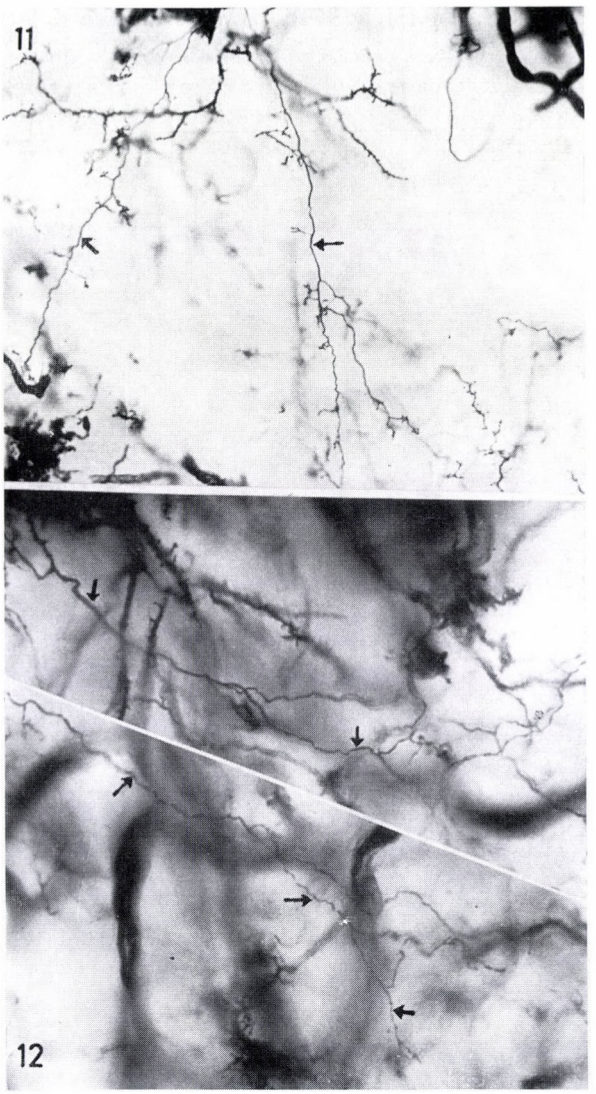
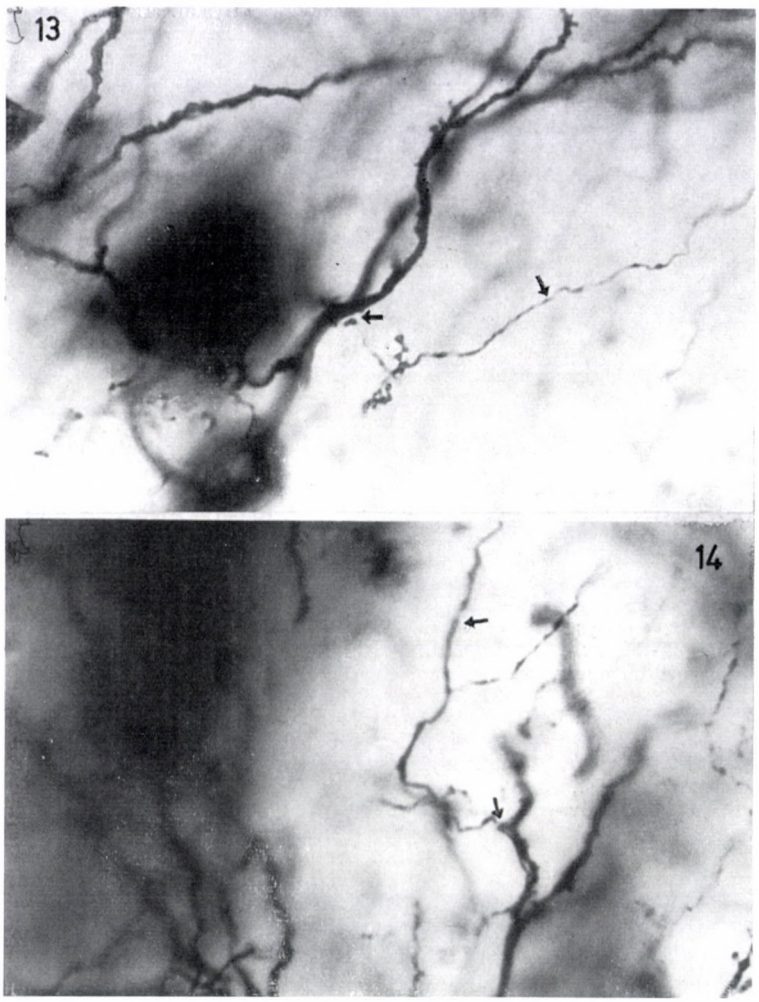


Fig. 11. Arrows indicate axon ramification of Golgi 2nd subtype *b* neuron. Golgi—Kopsch, ×200

Fig. 12. Arrows indicate axon and its ramifications in Golgi 2nd subtype *b* neuron. Golgi—Kopsch, ×800

particularly under the EM it is not even known on what type of neuron they might act. There is, however, one piece of information available which might prove as a hinge by which the whole question can be removed from its present deadlock. The fact that subtype *b* interneuron axons cross from one layer of the LGB into another, points clearly to their role in binocular interaction



Figs 13, 14. Contacts (arrow) with dendrites of relay cells of Golgi 2nd subtype *a* neurons in CGL and MD. Golgi—Kopsch, ×800

on the geniculate level. Of this, so far little is known apart from some evidence of it being largely inhibitory (FREUND et al., 1969). This would be in accord with the similarity of the cells and dendrites of subtype *b* and *a*, and their Golgi 2nd character.

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ZWEI TYPEN VON KURZEN AXON (GOLGI 2)-INTERNEURONEN IN DEN SPEZIFISCHEN THALAMUSKERNEN

T. TÖMBÖL

An Perfusionspräparaten nach Golgi—Kopsch lassen sich in den spezifischen Thalamuskernen der Katze zwei Typen von kurzen Axon(Golgi 2)-Interneuronen unterscheiden. Die Unterscheidung der beiden Typen beruht hauptsächlich auf der Länge der Axonen. Die Axonarborisation der Golgi-2-Neuronen vom Typ »a« erstreckt sich nicht über die Ausdehnung des Dendritenbaumes hinaus, während sich die Axone der Golgi-2-Neuronen vom Typ »b« erheblich weiter verzweigen. Die Axone dieses Typs stellen die Verbindung zwischen verschiedenen nuclearen Bereichen und Schichten her. Verbindungen zwischen den Neuronen Golgi 2 Typ »a« sowohl mit den Relay-Interneuronen als auch mit den Interneuronen von anderen Golgi-2-Typen wurden bereits früher nachgewiesen, während die Verbindungen der Interneuronen vom Typ »b« bisher noch nicht geklärt werden konnten.

ДВА ТИПА АКСОНО-(ГОЛЬДЖИ 2)-ИНТЕРНЕЙРОНОВ В СПЕЦИФИЧЕСКИХ ЯДРАХ ТАЛАМУСА

Т. ТЁМБЁЛ

На перфузионных препаратах по Гольджи—Кропшу в специфических ядрах таламуса кошек можно обособлять два типа коротких аксоно-(Гольджи 2)-интернейронов. Обособление двух типов основывается главным образом на длине аксонов. Аксоноарборизация нейронов Гольджи 2 типа «а» не распространяется за простиранием дендритического дерева, тогда как аксоны нейронов Голджи 2 типа «б» разветвляются на гораздо большем расстоянии. Аксоны этого типа соединяют различные внутриядерные зоны и слои. Связи между нейронами Гольджи 2 типа «а» с реле-интернейронами и с интернейронами другого типа Гольджи 2 были выяснены уже раньше, в то время как связи между интернейронами типа «б» до сих пор еще не удалось выяснить.

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QUANTITATIVE ASPECTS OF NEURON ARRANGEMENT IN THE SPECIFIC THALAMIC NUCLEI

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Quantitative studies on the specific thalamic nuclei gave the following results.

1. The ratio of thalamo (geniculo)-cortical relay cells to Golgi 2nd type neurons varies between roughly 2 : 1 and 3 : 1 in the relay and on the basis of more indirect reasoning approximately 4 : 1 in the elaborative (associative) nuclei.

2. Cell nuclear volume measurements reveal the presence of two distinct cell groups in the relay nuclei. No such separation of two cell populations appears in the nuclear spectra of the elaborative (associative) nuclei.

3. The spheric dendritic tree of the relay neurons can be divided on the basis of its synaptic relations into three zones, *(a)* a central space; *(b)* an interior zone; and *(c)* an exterior zone.

4. No such division into zones is possible with the dendritic arborization of Golgi 2nd type neurons.

Introduction

Correct interpretation of the functions of various neuron networks depends on the understanding of the interneuronal connexions. Comprehension of the geometry and topology of a neuron network as a connectivity system is greatly facilitated by the regularity of arrangement. The regular arrangement of the neuronal elements facilitated, for example, in the cerebellum the early development of synaptology (Ramón y Cajal, 1911) and later its structural analysis on the ultrastructural level and its functional elucidation (literature summarized by Eccles et al. in 1967).

A simple regularity in the geometric sense is not found at all or is rarely recognizable in the arrangement of the elements of the specific thalamic nuclei. Yet, even if there is no true geometrical regularity one might look for some general principles of structure or arrangement that could help determining — at least in some approximation — the general synaptic and connection arrangements. Several authors have realized (Jung, 1960; Andersen et al., 1962, 1964; Mountcastle et al., 1963) that the arborization pattern of the specific afferents must be the most important factor of neuron connectivity. Szentágothai (1967) has tentatively proposed a model of connectivity in the specific thalamic nuclei, emphasizing that the features of crucial importance are 1. the arborization pattern of specific afferents; 2. the types of neurons, their relative numbers and densities; 3. the pattern of their dendritic ramifica-

tion; and 4. the local connexion between the different types of neurons. The model of SZENTÁGOTHAI (1967) was based on the assumption of a quasi random spatial distribution of the terminal ramifications of specific afferents relative to the dendritic arborization of the nerve cells. As shown, however, in a preceding paper (TÖMBÖL, 1969), even the final parts of the axonal arborizations, the so-called terminal units, are not of random distribution. An investigation into the quantitative aspects of the neuron network seemed therefore to be of even greater significance.

Methods

Part of the measurements was made on Golgi preparations, for which the modified perfusion Kopsch procedure proved to be the most appropriate (SZENTÁGOTHAI, 1963; TÖMBÖL, 1966/67). On these preparations the diameters of the cells, the length of the dendrites and their different portions were measured with a simple ocular micrometer, and the dendritic ramifications were counted. The ratio of different cell types was established by cell counts in the cytoarchitectonic picture (Nissl and HE preparations) on the basis of cell size and shape. Another approach to the differentiation of the cell types recognized in the Golgi preparations, were discriminative measurements of the nuclear volume. The data obtained were analyzed by different statistical procedures. PALKOVITS—CSAPÓ's microprojection table (1968) was used for nuclear volume measurement. In each of the thalamic nuclei the diameter and volume of 500—1000 nuclei were examined. Statistical analysis was aimed at showing that — in some of the thalamic nuclei at least — the two main cell types recognized in the Golgi as well as in the cytoarchitectonic picture can be differentiated also on the basis of nuclear size and excentricity.

Results

As in every central nervous tissue, in the specific thalamic nuclei Golgi preparations proved to be the most appropriate for the recognition of various types of neurons. In these nuclei two main types of cells can be distinguished: thalamo-cortical relay neurons and Golgi 2nd type interneurons (CAJAL, 1911; O'LEARY, 1941; SZENTÁGOTHAI, 1963; MOREST, 1963/64, 1965; GUILLERY, 1966/67; SCHEIBEL and SCHEIBEL, 1966; TÖMBÖL, 1966/67, 1968) (Figs 1a, b). The size of the cell body of both neuron types was measured in Golgi and Nissl preparations and there was no significant difference between the data obtained with the two techniques. The two — the longest and a perpendicular short — diameters of the relay cells were 18—30 μ, and 12—22 μ, those of the Golgi 2nd type neurons 8—15 μ and 10—18 μ respectively.

The ratio of the two main cell types was established in Nissl preparations. In each nucleus, in 100—150 visual fields chosen at random, the proportion of relay and Golgi 2nd neurons was consequently identical. In the relay nuclei, both in the sensory relay nuclei: lateral geniculate body (LGB), medial geniculate body (MGB), ventrobasal lateral (VPL) and medial (VPM), and in the non-sensory relay-nuclei: nucleus anterior ventralis (AV); nucleus anterior medialis (AM), nucleus ventralis lateralis (VL) and ventro-medialis (VM), nucleus medialis dorsalis (MD), the proportion of the relay and Golgi

2nd neurons varies between 2 : 1, and 3 : 1. In the elaborative (associative) nuclei: nucleus lateralis posterior (LP), nucleus lateralis dorsalis (LD), the pulvinar (Pu) and the nucleus anterior dorsalis (AD), the ratio of relay cells to Golgi cell type cells is 4 : 1.

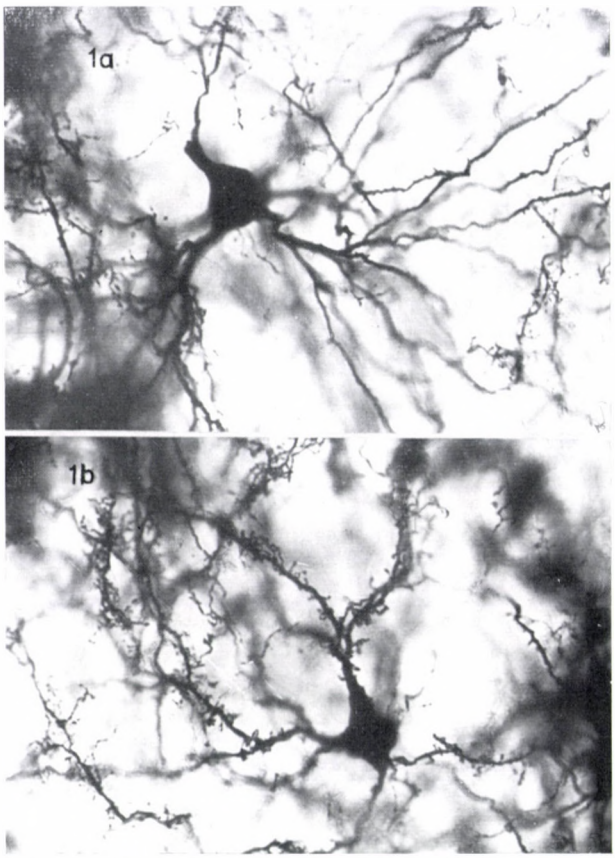


Fig. 1. a) Relay neuron (tufted type) in VPL; *b)* Golgi 2nd type neuron in VPL. Golgi—Kopsch method, ×500

An attempt was made to verify the somewhat subjective judgement of the cytoarchitectonic pictures by systematic measurement of nuclear size. The rough data were analyzed and compared by various statistical and plotting procedures. In the nuclear groups (sensory, relay, non-sensory relay and elaborative nuclei), data of the longitudinal diameter of the nuclei were plotted against the logarithm of nuclear volume (Fig. 2). The dot diagrams thus obtained show two neuron populations in the sensory (Fig. 2a) and in the non-sensory relay nuclei (Fig. 2b) while no such subdivision is apparent in the elaborative nuclei (Fig. 2c). In order to establish the boundary between the

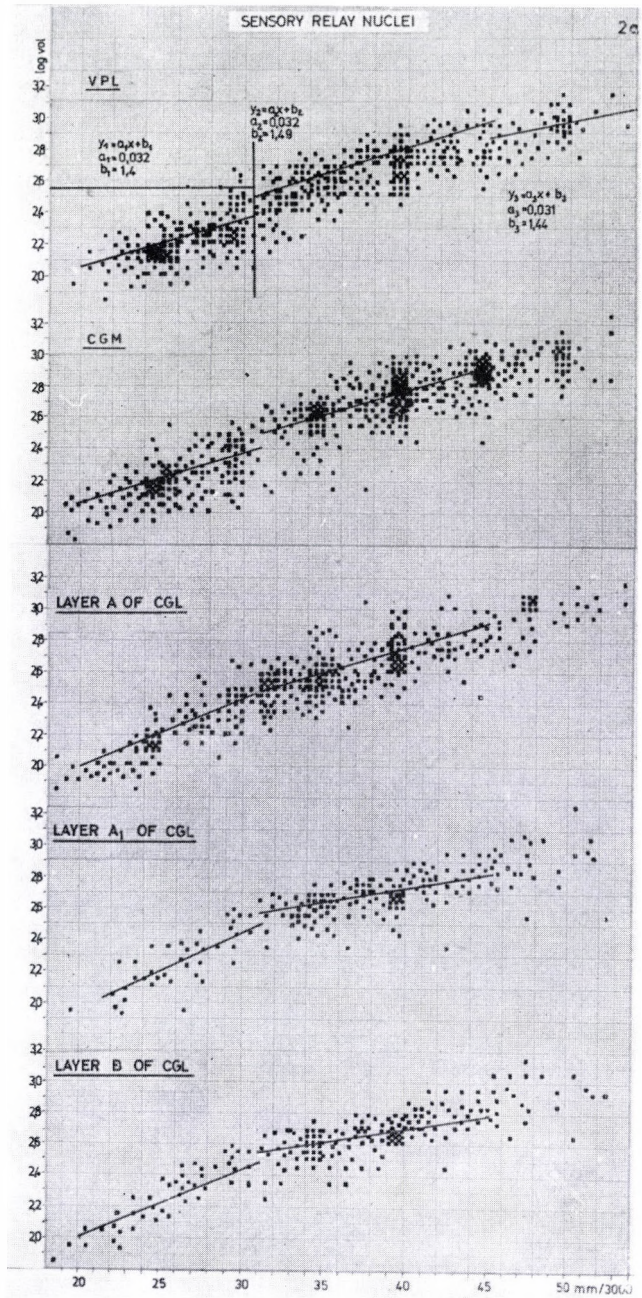


Fig. 2. a) Dot diagram of cell nucleus groups in the sensory relay nuclei

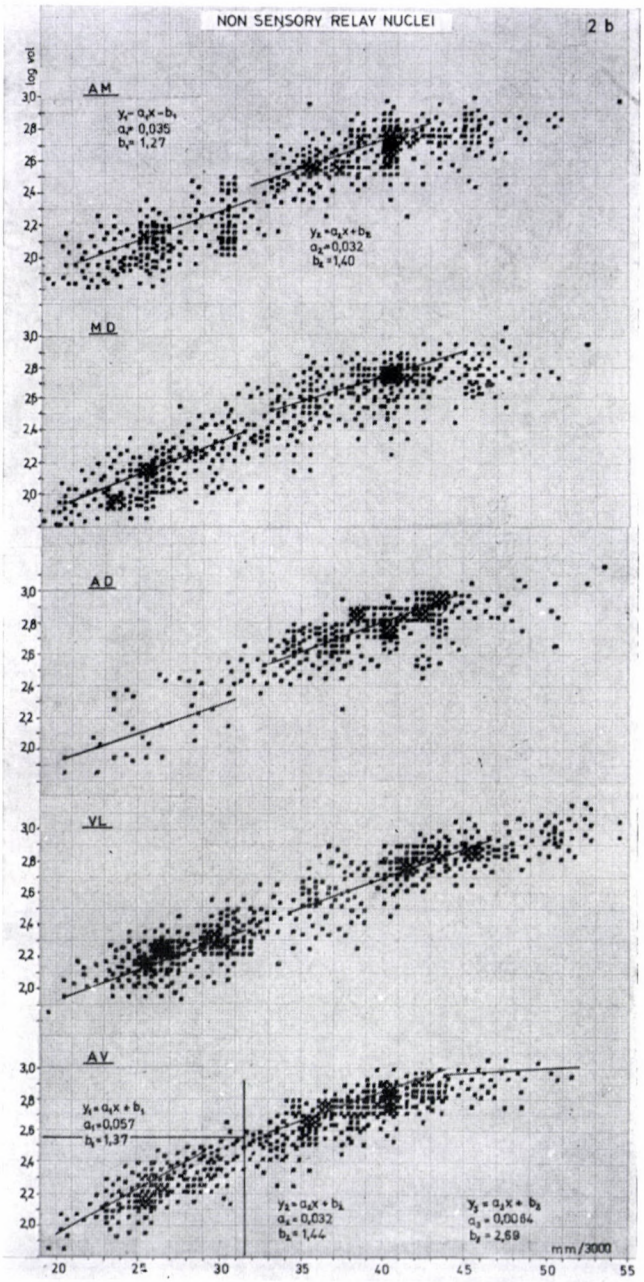


Fig 2. b) Dot diagram of cell nucleus groups in the non-sensory relay nuclei

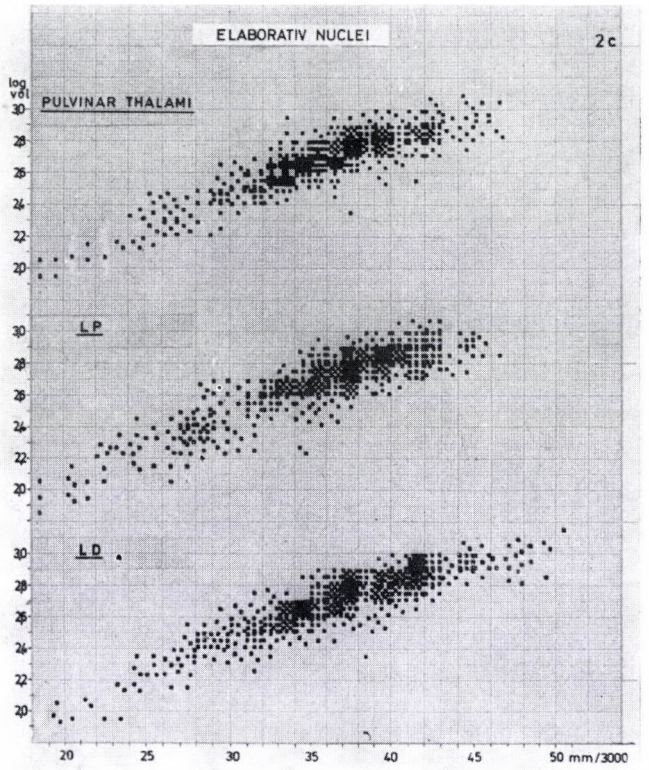
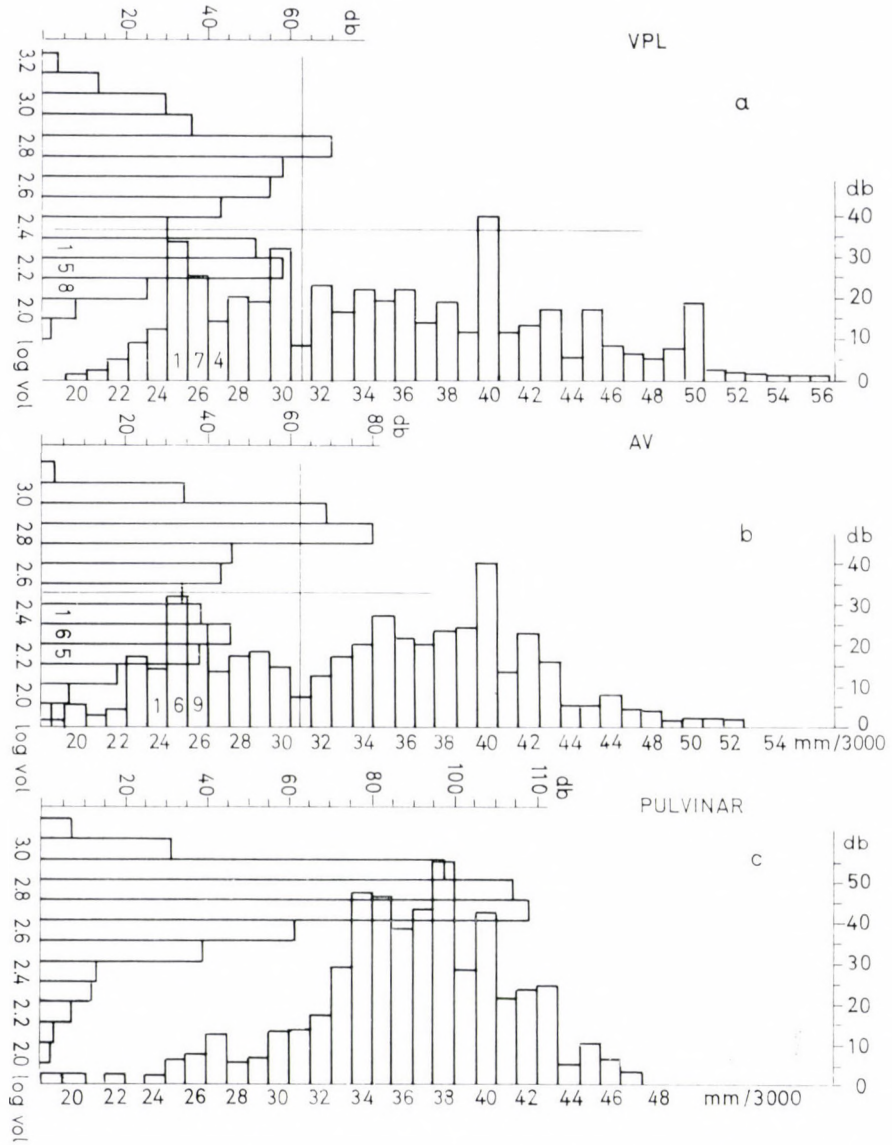


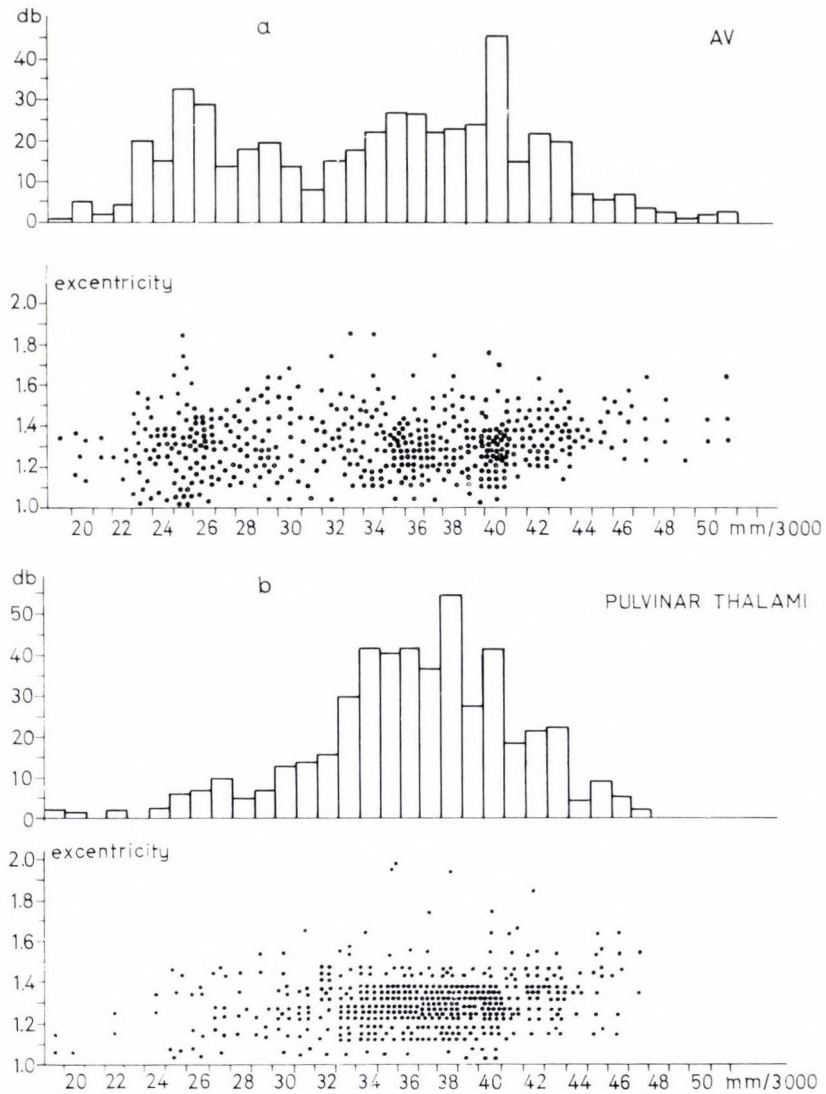
Fig 2. c) Dot diagram of cell nucleus groups in the elaborative nuclei

two cell types, another plotting technique was used. On Figs 3a, b and c the data of the cell nuclei of representative nuclei of each group (VPL, AV and Pu) were confronted as histograms of the long diameter of the cell nuclei represented on the abscissa while the histograms of the logarithm of nuclear volume was represented on the ordinate. Both histograms of the nuclear volumes show two elevations in the sensory and the non-sensory relay nuclei but none in the elaborative nuclei. The empirically found lines of separation between the two elevations of the histograms (thin lines in Fig. 3a and b) were then transferred to the point diagrams of Fig. 2a and b and the regressions were calculated for the two nuclear size groups. The regression straights show significant breaks at the border between the two groups. These breaks are due either to the directional tangent ("*a*") of the line, or to the shift of the intersection of the axis *y* ("*b*"), or possibly to the change of both factors. On the basis of these calculations, the cell nuclei belonging to the highest classes of the long diameter ought to be considered as a separate group of cells.



Figs 3a, b, c. Histograms of the long diameter of cell nuclei and of the logarithm of nuclear volume, in VPL, AV and pulvinar

Fig. 4 shows the distribution of the cell nuclei according to shape in two representative nuclei. The long diameter of the cell nuclei was marked on the abscissa, and on the ordinate the excentricity was plotted corresponding to the ratio of the long versus the short diameter; the latter being the diameter perpendicular to the middle point of the long diameter. This graph



Figs 4a, b. Distribution of the excentricity of cell nuclei by means of dot diagrams and histograms in the AV and pulvinar thalami

reveals two groups in the sensory and non-sensory relay nuclei (shown here only for the AV) and a continuous distribution of cell nuclei in the elaborative nuclei. The graphs are compared with the respective long diameter histograms.

It may be surmised that one population of the cell nuclei observed in the sensory relay nuclei is identical with the interneurons, the nuclear volume is 170—350 μ^3 and the diameter varies between $6\times8\,\mu$ and $8\times10\,\mu$. (The

given numbers are extreme values.) The other group of nuclei, corresponding to the nuclei of relay-cells, has a volume of 350—1400 μ^3, the diameters vary between 8×11 μ, and 12×17 μ. With respect to excentricity, the cell nuclei are slightly ovoid in shape, but among those of small size there are also round nuclei.

In the non-sensory relay nuclei, in the group corresponding to the interneurons, the nuclear volume is 150—300 μ^3, and the diameter measure 7×8 μ and 7×10 μ. Nuclear volume in the other cell group is 450—1100 μ^3, with diameters of 8×12 μ and 12×16 μ, respectively. Occasional nuclei 11—17 μ in diameter are found in the latter group which, as judged from the difference between the diameters are probably nuclei of large integrative cells. According to excentricity, slightly ovoid and ovoid shapes are found in every order of magnitude, in the AM, MD and VL; in the AD and AV, however, only slightly ovoid shapes occur.

In the elaborative nuclei, nuclear volume of the cells varies in a wide range but a clear separation into groups cannot be ascertained by this approach. With respect to excentricity, the nuclei are slightly ovoid.

Although the nuclear volume of the specific thalamic nuclei in itself does not allow a clear separation of the two main cell types, it completes the conclusions drawn from Golgi preparations and supports the separation of the two types in the architectonic picture. Differentiation of the two cell groups merely on the basis of numerical data is not possible because they are overlapping.

Analysis of the dendritic arborization of the relay neurons

Beside data on size and type of neurons as well as ratios between groups, some numerical observations on the dendritic arborizations were found to be informative. Length of the dendrites was measured in perfusion Kopsch preparations.

The length of the dendrites of the relay cells differs slightly in the three arborization types (tufted, transitory, radiated) (Figs 5a, b, c), either are essential differences in the various nuclei. The neurons of the tufted and transitory dendritic type have less principal dendrites (5—8) of 12—15 μ length. From the tufts of each principal dendrite 6—8 secondary dendrites take their origin. Characteristic dendritic protrusions can be observed on the initial 10—25 microns of the secondary dendrites. The all-over length of the dendrites is about 140—160 μ. With the exception of relay neurons having spatially oriented dendrites the dendritic tree is of spheric shape. In the cells with radiate dendritic arborizations, 10 to 15 principal dendrites emerge in every direction. The length of the undivided part of the primary dendrites

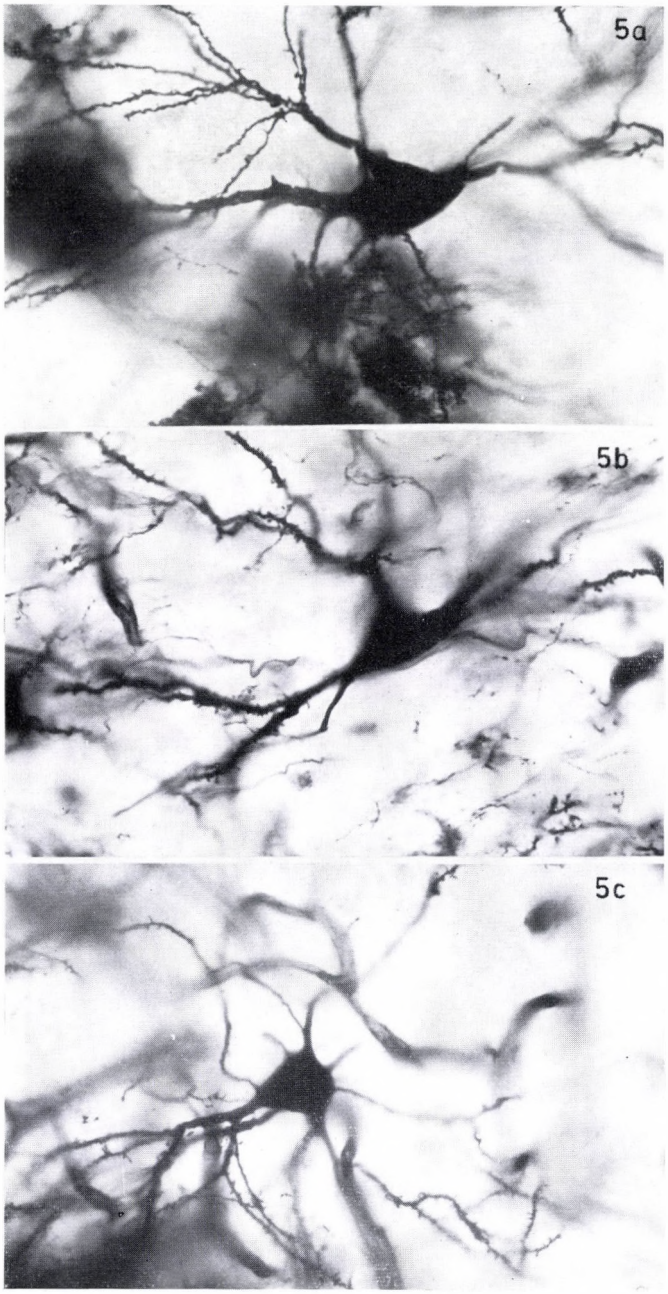


Fig. 5. a) Tufted type of arborization of dendrites of relay neuron in VPL. Golgi—Kopsch method, ×500; *b)* Transitory type of arborization of dendrites of relay neuron in pulvinar. Golgi—Kopsch method, ×500; *c)* Radiated type of arborization of dendrites of relay neuron in AV. Golgi—Kopsch method, ×500

varies considerably and they divide dichotomically. Protrusions are found only occasionally in the initial portions of their secondary dendrites. The all-over length of dendrites in this type does not differ significantly from that of the other types.

The spheric dendritic space measures about 300—400 μ in diameter in all the three relay-cell types. With respect to the predominant site of the contacts with the afferent fibres, three zones of relay neurons may be distinguished: 1. the central space containing the cell body and the principal dendrites; 2. an interior zone or interior shell; and 3. an exterior zone or spheric shell

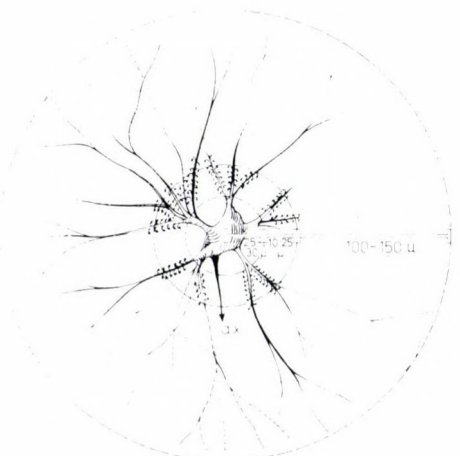


Fig. 6a. The three zones of the shperic dendritic space of the tufted type relay neuron shown diagrammatically

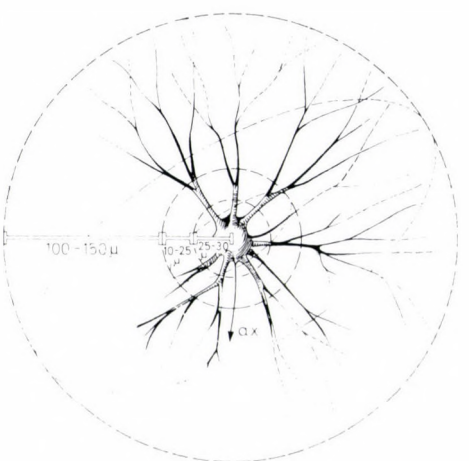


Fig. 6b. The three zones of the spheric dendritic space of the radiated type relay neuron shown diagrammatically

(Figs 6a, b). The central space measuring about 50—60 μ in diameter is poor in synaptic contacts. The interior zone is the predominant site of the termination of specific afferents. It embraces the ramifications of the principal dendrites and the proximal portions of the secondary dendrites. The thickness (10—25 μ) of the interior zone corresponds to the proximal part of the secondary dendrites which are covered with protrusions. The third, exterior, zone of the dendritic tree encompasses the distal part of the dendritic tree. Nonspecific afferents appear to terminate mainly here. In width, this zone is more variable then the two internal zones.

The central space and the exterior zone show little if any difference in the several types of relay neuron. The interior zone, where the primary dendrites ramify, differs however, fundamentally in the three types. In the tufted and the transitory cell types it contains the dendritic tufts or dendritic baskets

with the numerous protrusions of the initial parts of the secondary dendrites. The synaptic entanglement between the specific afferents and the dendritic tufts occurs here (Fig. 7) with repeated contact between the branches of the "terminal units" of the afferent and the branches of the dendritic tufts. On the interior zone of the radiate type of relay-cells, where the arborization of the dendrites occurs progressively and at random sites, there is no concentration of synaptic contacts at any particular zone of the dendritic tree. Neither are there protrusions which are considered to represent specific receiving surface differentiations.

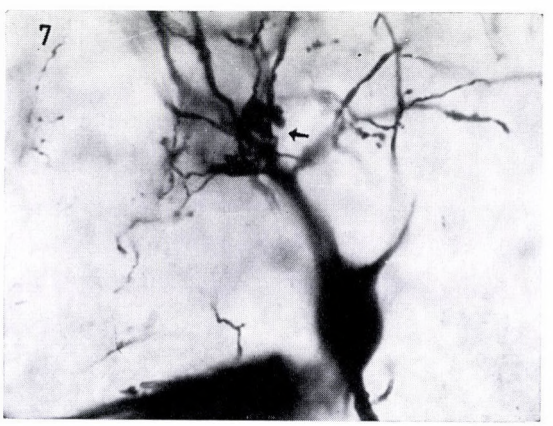


Fig. 7. Terminal unit of optic fibre entangled with dendritic tufts of relay neuron in CGL. Golgi—Kopsch method, ×800

Analysis of the dendritic arborization of Golgi 2nd type neurons

Two parts can be distinguished in the dendritic tree, a central space lacking or poor in spines and the space of the spiny dendrites. The dendritic tree of Golgi 2nd type neurons is rarely if ever spheroid, as a consequence of the small number of dendrites and of their poor ramification. The central space having a diameter of 25—30 μ contains the cell body and the initial parts of the dendrites. The number of the axosomatic synapses is low also in the Golgi 2nd type. The distal spiny dendritic portions vary widely in length, they may reach in extreme cases 300—400 μ, although the distances bridged, due to the wavy course, may measure not more than 80—100 μ (Fig. 8). The numerous spines of the dendrites as well as their surface proper may get into contact with cortical fibres, initial collaterals of relay neurons, and also with specific afferents.

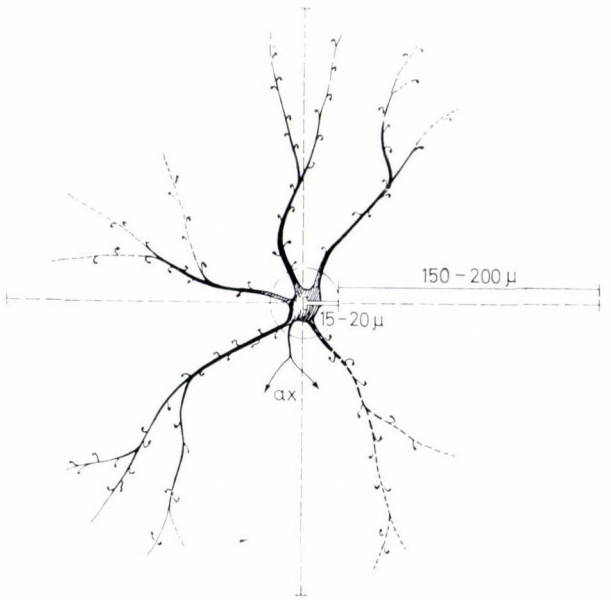


Fig. 8. Dendritic arborization space of Golgi 2nd type neuron shown diagrammatically

Discussion

UTTLEY (1955) has developed mathematical models for estimating the probability of synaptic contacts established between dendritic and axonal arborizations of various shapes and densities. The speculations of SZENTÁGOTHAI (1967) were based on the assumption that a larger number of synaptic contacts between any given axon and the dendrites of a given neuron would secure a better transmission between these two elements, than could be secured if only few chance contacts were present. Otherwise the distribution of dendritic arborizations and axon ramifications was considered to be random in the network model of SZENTÁGOTHAI (1967). Our recent observation reported in a previous paper (TÖMBÖL, 1969) has, however, led us to assume that the relative positions of these arborizations are not random but fairly specific not only in a more general topographic sense but also in the sense that each specific afferent must specifically be connected with a set of relay and possibly also of Golgi 2nd type interneurons. The Golgi findings of MOREST (1964, 1965) and of SCHEIBEL and SCHEIBEL (1966) are also better understood on the basis of such an interpretation.

Unfortunately, the density and general spatial distribution of neurons in most of the thalamic nuclei is subject to various non essential — quasi chance — circumstances such as number, fasciculation and direction of fibre systems running through, etc. Corresponding to this there are many distor-

tions in shape and distribution of the ramifications of the specific afferents. From the density of the so-called terminal units of specific afferents, — *i.e.* from the average distances between the terminal units belonging to the same afferent — it can be deduced that various dendritic tufts of the same relay cell are unlikely to be contacted (in a major degree) by two or more terminal units of the same afferent. This would mean that there is a convergence of as many specific afferents upon the average relay cell as it has dendritic tufts.

On the other hand, the same terminal unit of an afferent may be in intimate and multiple contact with two or more dendritic tufts of different cells. In this way, the divergence from a specific afferent could be considerably larger numerically than the number of its terminal units. But, obviously, there are clusters of relay cells, and attached to them groups of interneurons, which share a finite number of common specific afferents. Size and shape of such cell clusters would be difficult to judge as long as one does not know how complete — or conversely how much shifted — the overlap is between the terminal arborizations of the specific afferent arbors that share common spaces.

An approximate measure of this overlap is given by the 1. all-over size of the specific afferent arborizations as compared with the 2. space of the whole nucleus and 3. the number of specific afferents. Data concerning (1) have been given in a previous paper (TÖMBÖL, 1969) and (2) could be calculated, but as regards (3), we have only a vague idea about its order of magnitude. Further efforts along these lines might be rewarding.

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QUALITATIVE VERHÄLTNISSE DER NEURONENVERTEILUNG IN DEN SPEZIFISCHEN THALAMUSKERNEN

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Die quantitative Untersuchung der spezifischen Thalamuskerne erbrachte folgende Ergebnisse:

1. Das Verhältnis der thalamo-(geniculo)-kortikalen Relay-Zellen zu den Neuronen vom Typ Golgi 2 variiert in den Relay-Kernen zwischen 2 : 1 und 3 : 1. In den Assoziations-Zellkernen beträgt es auf Grund einer indirekten Überlegung approximativ 4 : 1.

2. Aus Messungen des Zellkernvolumens geht hervor, daß in den *Relay-Kernen* zwei verschiedene Zellgruppen vorliegen, während bei der Analyse des nuclearen Spektrums der *Assoziationskerne* keine derartige Absonderung von zwei Zellpopulationen nachgewiesen werden kann.

3. Der sphärische Dendritenbaum der Relay-Neuronen läßt sich auf Grund seiner Synapsen-Verbindungen auf drei Zonen aufteilen: *a)* einen zentralen Teil, *b)* eine Innenzone und *c)* eine Außenzone.

4. Bei der Dendritenarborisation der Neuronen vom Typ Golgi 2 ist eine derartige Zonenunterscheidung nicht möglich.

КОЛИЧЕСТВЕННЫЕ УСЛОВИЯ РАСПРЕДЕЛЕНИЯ НЕЙРОНОВ В СПЕЦИФИЧЕСКИХ ЯДРАХ ТАЛАМУСА

Т. ТЁМБЁЛ, Л. УНГВАРИ, Ф. ХАЙДУ, М. МАДАРАС и Д. ШОМОДЬИ

1. Соотношение между таламо(геникуло)-кортикальными реле-клетками и нейронами типа Гольджи 2 варьирует в реле-ядрах между 2 : 1 и 3 : 1, а в ассоциативных ядрах оно составляет на основе более косвенного размышления — 4 : 1.

2. Измерения объема клеточного ядра выявили присутствие двух различных клеточных групп в *реле-ядрах;* в ядерном спектре ассоциативных ядер такого обособления двух клеточных популяций не наблюдалось.

3. Сферическое дендритическое дерево реле-нейронов можно делить на основе синаптических связей на три зоны: а) на центральную часть, б) на внутреннюю зону и в) на внешнюю зону.

4. Такое деление на зоны невозможно в случае дендритической арборизации нейронов типа Гольджи 2.

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A SIMPLE METHOD FOR CUTTING SEMITHIN SERIAL SECTIONS

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(Received June 30, 1969)

A simple method is described for cutting semithin serial sections. The metal knife is replaced by a glass knife in the Reichert rotary microtome, and a large trough is attached to the angle of the glass knife. This permits to collect long ribbons of serial sections onto a film of liquid, and provides an easy way to mount the ribbons on the slide.

The investigation of tissue sections of a thickness between the range of traditional light microscopic and that of electron microscopic sections, commonly designated as semithin sections, has met with but moderate interest. This may partly be due to the difficulties in preparing such sections with traditional cutting techniques, and to the situation that new techniques for ultrathin and semithin tissue sections have been introduced with the advent of electron microscopy which directed the investigation of cytological problems into a new line of histological methodology. There are, however, a number of special problems for which light microscopical investigations performed with a less laborious technique would provide more informative data, if sections thin enough could be made in an easily reproducible way. To prepare semithin sections with the ultramicrotome would mean an uneconomical use of this costly instrument, and also little saving of labour. Žlabek [1] has recently described a simple device which is attached to the knife of a Spencer rotary microtome, and permits to collect semithin sections onto a film of liquid even in ribbons of serial sections. The level of the fluid on which the sections are floated has to be altered with every downward and upward stroke of the specimen block to avoid picking up of the last section by the block face at the upward stroke. This causes complications which reduce the effectivity of the technique in terms of successful sections in unit of time, and permits to make only very short ribbons when cutting in series. By combining some of the advantages of ultramicrotomy, i.e. cutting with glass knife and collecting the sections onto the surface of liquid, with traditional cutting techniques we succeeded in elaborating a method which overcomes these difficulties. With no more practice than required for ordinary microtomy, semithin sections of specimens embedded either in paraffin, celloidin-paraffin or in epoxy resin

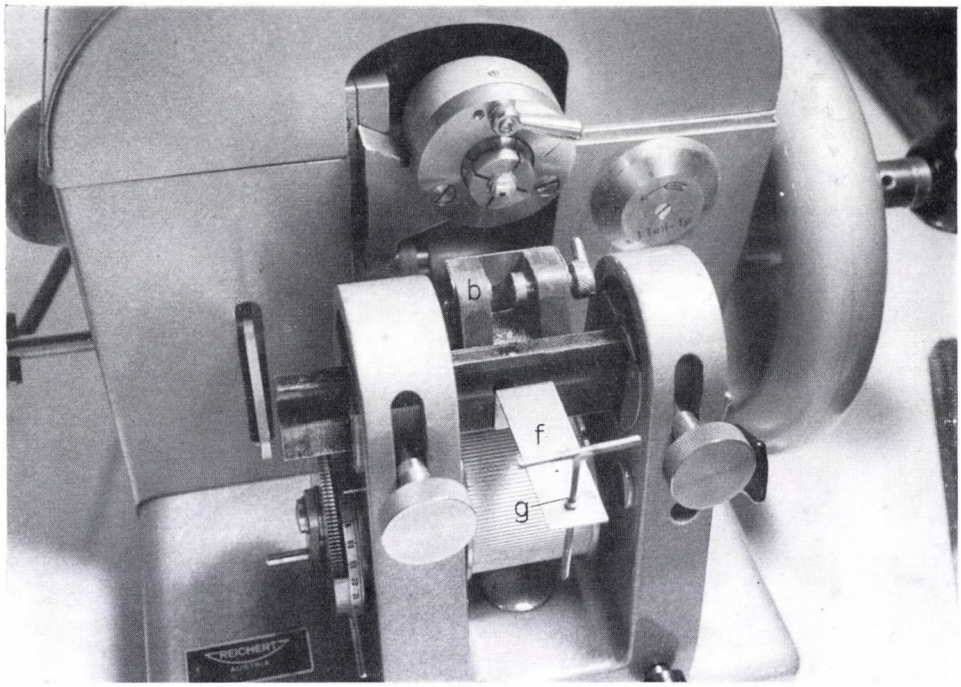

Fig. 1. Glass-knife holder *(b)* and the adjustable supporting device *(f* and *g)* for holding the trough

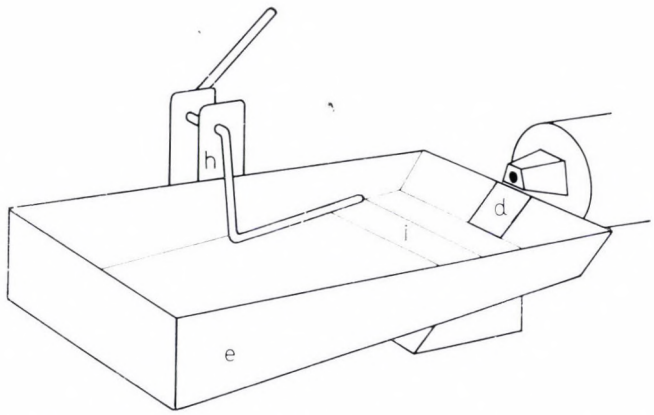

Fig. 2. Trough *(e)*, glass knife *(d)*, lever arm *(i)* with hinges *(h)*

can be cut in series, and ribbons 30—60 mm in length can easily be mounted on the slide. The procedure consists in the following.

The Reichert rotary microtome is adapted to prepare semithin serial sections (Figs 1 to 3). A flat steel bar *(a)* of the approximate size of a Reichert

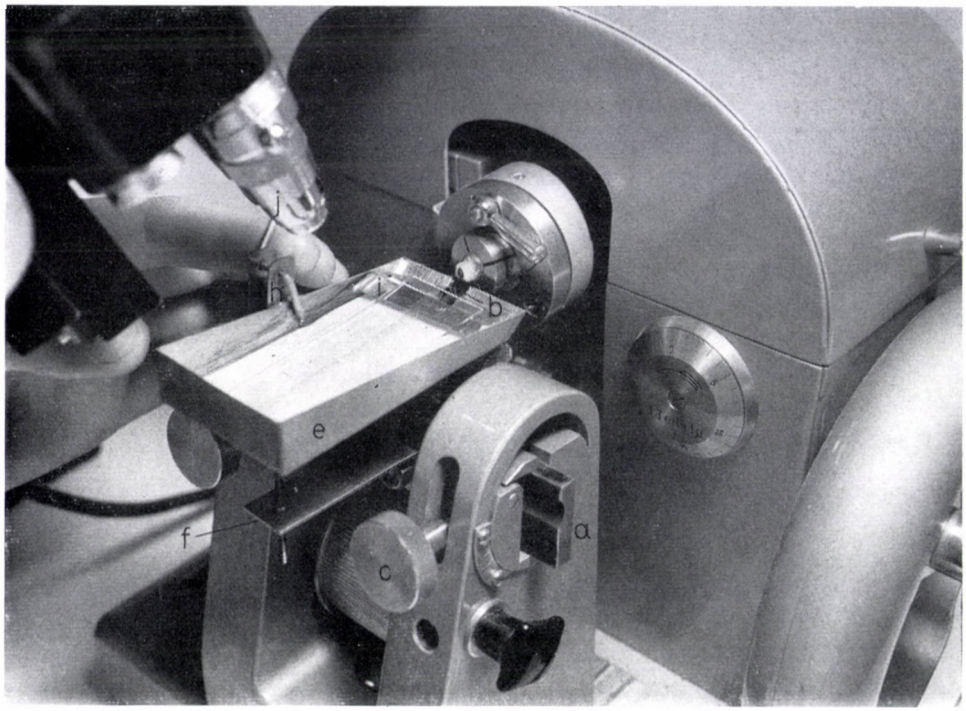

Fig. 3. Reichert rotary microtome adapted for cutting semithin serial sections. The slide with a ribbon of sections is elevated half-way from the trough *(e)* by the lever arm *(i)*. For further details, see text

microtome knife is placed in the knife holder, and a conventional glass knife holder *(b)* is fastened to the middle of the steel bar. The clearance angle of the glass knife can be altered with the original adjusting device of the microtome *(c)*. The advantage of the easily replacable glass knife *(d)* is that it always provides a fresh cutting edge so that the special sharpening of metal knives for semithin sections can be avoided. On the other hand, the use of a glass knife sets the limit to the size of the specimen block. In order to float sections on the surface of water, a large brass trough *(e)* is attached to the angle of the knife by means of dental wax in a manner commonly used in ultramicrotomy, and the other end of the trough rests on an adjustable supporting device (Fig. 1). This device consists of a flat metal plate *(f)* with one end fixed to the steel bar *a*, and a T shaped rod *(g)* stands out at its other end. The height of the rod can be regulated with a fitting screw. The trough should be as long as to hold conveniently a microscopic slide, and about twice as wide as the width of a slide, so that any part of the slide could be placed under the ribbon being cut. To the side of the trough a pair of brass plates *(h)* are soldered, and they serve as hinges to the axle of a level arm *(i)* which rests on the bottom of the trough (Figs 2 and 3).

The maximum size of the tissue block allowing to cut long ribbons of serial sections is about 5×5 mm. If the tissue is embedded in celloidin-paraffin or in epoxy resin, the block should be coated with paraffin which sticks the subsequent sections together. The block is to be trimmed to the form of a tapering square pyramid. Special care must be taken that trimming is made with a sharp razor blade to obtain clean and smooth pyramid sides and parallel wedges, otherwise any little irregularity, especially on the sides lying parallel to the cutting edge, interferes with the serial sectioning.

Cutting is done under the dissecting microscope *(j)*. The slide, thinly coated with a diluted eggwhite-glycerol mixture, lays on the lever arm. The trough is to be filled with 10% ethylene, adding the last drops carefully under the control of the microscope, to bring the water level right under the edge of the knife. On the clean glass surface of the knife the water surface forms a concave film reaching the cutting edge. The speed of cutting depends on the size of the specimen block and on the embedding material. If everthing is arranged correctly, 30—40 sections can be made in a minute. In the case of any trouble with cutting, if sections would not adhere to form a ribbon, or the block face would pick up the last section, one has to make sure that the sides of the pyramid are really smooth, the wedges of the block face are really parallel to another and to the edge of the knife, and the water level is properly set. If the ribbon is long enough, the last section is freed from the knife with a fine steel needle. The ribbon can easily be pushed on the water surface to the appropriate place over the slide. While keeping the ribbon in place, the slide is carefully elevated with the aid of the lever arm up to the point of contact with the last section. After making once more sure that the ribbon is in correct position, the slide is lifted up from the trough by pushing down smoothly the handle of the lever arm, and the ribbon stretches out on the slide (Fig. 3). The water traces are removed with filter paper and the slide is dried for a few minutes, at 56°C if the tissue is embedded in paraffin or in celloidin-paraffin, or 85°C if it is embedded in epoxy resin, to facilitate the adhesion of sections to the slide. Then the slide can be placed back into the trough to mount the next ribbon. When manipulating with the slide, one has to observe that the block-holder arm should stay in the downward position, otherwise the warmth of the microscope light dilates the specimen block and the first section will be too thick.

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EINE EINFACHE METHODE ZUR ANFERTIGUNG VON SERIENSCHNITTEN MITTLERER DICKE

GY. SÉTÁLÓ

Eine einfache Methode zur Anfertigung von Serienschnitten mittlerer Dicke wird beschrieben. Statt dem Metallmesser wird an das Reichertsche Rotationsmikrotom ein Glasmesser angebracht, an das ein Schiffchen zum Sammeln der Schnitte befestigt wird, das auch zur Aufnahme des Objektträgers geeignet ist. Auf der großen Flüssigkeitsoberfläche des Schiffchens lassen sich lange Schittserien herstellen, deren Übertragung auf den Objektträger mit Hilfe der in das Schiffchen eingebauten Hebevorrichtung leicht zu bewerkstelligen ist.

СООБЩАЕТСЯ ПРОСТОЙ МЕТОД ДЛЯ ИЗГОТОВЛЕНИЯ ПОЛУТОНКИХ СЕРИЙНЫХ СРЕЗОВ

Г. ШЕТАЛО

Металлический нож ротационного микротома Рейхерта заменяется стекланным ножом, к которому прикрепляется лодочка для собирания срезов, пригодная для помещения предметного стекла. На большой поверхности жидкости в лодочке можно изготовлять длинные ленты срезов, перенос которых на предметное стекло легко проводимо с помощью подъемного устройства, встроенного в лодочку.

Dr. György SÉTÁLÓ: Pécs, Anatómiai Intézet, Hungary

PROCEEDINGS OF THE ANNUAL MEETING OF HUNGARIAN ANATOMISTS

BUDAPEST 1968

It is for the first time that the Society of Hungarian Anatomists, Histologists and Embryologists has organized a Conference of its own. With the foundation of the Hungarian Association of Medical Societies the investigators of normal morphology have formed their own group and separated themselves from the pathologists, with whom they had cooperated in perfect harmony in all the years ever since the end of World War II. I hope that our fruitful cooperation with the pathologists who now have also formed their independent Society, will continue in the same friendly way and that in the organization of our Congresses we shall proceed according to a common plan.

Our present meeting is closely linked with the Semmelweis Festival and with all the papers given at this meeting, the Hungarian morphologists wish to render homage to the memory of the great Hungarian obstetrician. In the name of the Society of the Hungarian Anatomists, Histologists and Embryologists I welcome the members of our Society and all our guests.

At our first session we have to commemorate those of our Hungarian predecessors who have done outstanding work in our scientific fields: Mihalkovich, Thanhoffer, József and Mihály Lenhossék, Huzella, L. Nagy, Very, Péterfy, Ö. Bartha and F. Kiss. They paved the way for us, they enabled us to reach the present achievements. The future of our newly founded Society is based on the memory of these great scientists.

I think that at our first meeting, today's morphology should be discussed in a way to offer our successors a basis for comparison and also for the evaluation of the progresses achieved. The main subject, the connection between structure and function, is a fundamental question of biology. It is not the simple linkage of two different problems, but the common essence of the phenomena of life. If we meditate whether it is the structure which determines the function, or vice versa, the function which determines the structure, then it becomes clear that these two factors are inseparable and by itself neither of them will be of assistance in the understanding of life.

I. Törő

The Anatomical Substrates of Nervous Inhibitory Functions

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A survey is made of the elementary structure and of neuron arrangement in a number of synapses or synaptic systems that have so far been identified in vertebrates by physiological means as being of inhibitory nature. The general structural features of synapses taken into account were: (1) localization of the synaptic contact (axo-somatic, axo-dendritic, axon-spine, axon-hillock, axo-axonic); (2) ultrastructure of the so called contact regions of the synaptic membranes (membrane thickenings, hexagonal structures of the presynaptic membrane, subsynaptic cisterns or granulae, subsynaptic webs); (3) size, shape and visible contents of the synaptic vesicles; (4) density of terminal axoplasm surrounding the synaptic vesicles.

In *spinal motoneurons* a considerable fraction of synaptic contacts has to be assumed inhibitory. Unfortunately the number of distinctly different terminal knobs in contact either with motoneuron soma surfaces or what can be assumed being motoneuron dendrites, is much more than two, so that a simple division into excitatory and inhibitory categories appears to be impossible for the time being. D. Bodian distinguishes at least five types of motoneuron synapses based on No (3) alone of the above listed criteria. Obviously more types could be distinguished if the other criteria would be taken into account too. Nevertheless close to one third, or depending on the fixation technique used, perhaps one half of the synaptic contacts appears to contain vesicles that tend to become flattened. This might give support to the Uchizono concept of flattened vesicles being characteristic of inhibitory synapses. — An EM study of chronically isolated ventral horn preparations that contain virtually only motoneurons and some Renshaw cells (Szentágothai, 1958) showed that only one type of relatively small synaptic contacts survive on the motoneuron that are deeply embedded in impressions of the motoneuron body surface. The synaptic vesicles are small but their shape is too irregular to be judged either flattened or spheroid. This might, of course, be due to the unusual circumstances under which the neurons survive in these preparations. Whenever the isolated ventral horn contains more than a small part of the motor nucleus region — *i.e.* a substantial number

of interneurons survives — numerous intact synapses of various kinds are found in contact with the motoneurons.

In the *cerebellum*, where seven types of synapses can be relatively easily identified, of which three are known to be excitatory (mossy terminals, climbing fibers, parallel fibers) and four are known to be inhibitory (Golgi terminals in the glomeruli, basket terminals, stellate terminals on Purkinje cell dendrites, Purkinje axon collateral synapses on Golgi and basket neurons), all of these synapses conform with the UCHIZONO concept; the inhibitory terminals having smaller, usually flattened vesicles and the excitatory ones having larger, spheroid vesicles.

The *glomerular synapses of thalamic and geniculate body relay nuclei* offer ample opportunity to study both Golgi 2nd type neuron synapses — generally assumed to be inhibitory — and axo-axonic synapses that might be considered as the structural bases of presynaptic inhibition. The synaptic terminals of Golgi 2nd type neurons appear to belong almost invariably to the inhibitory type of UCHIZONO (1965) having small synaptic vesicles with a clear tendency to become flattened. The specific sensory afferents (lemniscal afferents in the VPL, optic afferents in the LGB, and inferior collicular afferents in the MGB) and also descending cortico-thalamic (and cortico-geniculate) fibers have synaptic terminals of the excitatory type. — Axo-axonic contacts are found in abundance, mainly between the specific sensory afferents and Golgi 2nd axon terminals. In these synapses the specific sensory afferent is invariably presynaptic, by structural standards — *i.e.* accumulation of synaptic vesicles and subsynaptic web — to the Golgi 2nd axon terminals. This arrangement leads logically to the assumption of a presynaptic inhibition of inhibitory terminals, hence to the hypothesis of a presynaptic disinhibition. Presynaptic disinhibition in the spinal neuronal network has been postulated on the basis of physiological observations so that its existence in the thalamic relay nuclei would be likewise conceivable.

Glomerular synaptic complexes in the substantia gelatinosa of the spinal cord have been described earlier (RALSTON, 1965 and RÉTHELYI and SZENTÁGOTHAI, 1965). More recent analysis of neuron arrangement in these complexes leads to the assumption that they might be the structural basis of primary afferent depolarization and presynaptic inhibition of impulse transmission from cutaneous afferents to dorsal horn ascending relay neurons (RÉTHELYI and SZENTÁGOTHAI, 1969). The presynaptic terminal of this synaptic system, which is of local spinal origin, contains unusually large synaptic vesicles that, although spheroid in shape, cannot be classified as either of the two main types of Uchizono.

In the *cerebral cortex* the basket interneurons described already by RAMÓN Y CAJAL (1911) are the most likely candidates for being inhibitory interneurons. These are cells that resemble Golgi 2nd type neurons but with

a horizontal axon giving rise to basket terminals on pyramid cell bodies. It has been shown earlier (SZENTÁGOTHAI, 1962) that most pericellular basket synapses are of local origin while a considerable number of the synapses established with dendritic spines originate from axons of extraneous origin. Most recently COLONNIER (1968) in Canada and HÁMORI in this Department have shown independently that most synaptic terminals contacting pyramid cell bodies or the initial smooth parts of the pyramid cell dendrites have small flattened vesicles, while the numerous synapses established with the spines of the pyramid cell dendrites contain larger spheroid synaptic vesicles. This might be used in favour of the assumption that the basket neurons are of inhibitory nature. Neuron circuit models for surround inhibition in the cerebral cortex have been proposed on the basis of this assumption already earlier (SZENTÁGOTHAI, 1967). The basket type interneuron being, however, only one of the numerous types of Golgi 2nd type interneurons, the question arises, whether all or some at least of the other interneurons are also inhibitory. This seems unlikely especially in the case of the large Golgi cells giving rise to so called "horse tail" axons that establish synapses mainly with the apical dendrites of pyramid cells. — A considerable amount of work is required, mainly in mapping the synapses established by various kinds of afferents using experimental degeneration methods on the electron microscope level, before the neuronal network of the cerebral cortex will be understood in principle. But in dialogue with the modern unit level neurophysiological methods and observations the histological methods available are offering possibilities today that even five years ago one could not have dreamt of.

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Perspectives of Ultrastructural Research by Topooptical Reactions

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The characteristics, conditions of development, optical effect and use in ultrastructural research of topooptical reactions are discussed. Such reactions may develop on micellar structures and without altering the basic structure they produce typical anisotropic effects. Amorphous objects do not show such reactions. Thus the demonstration of topooptical reactions of biological objects is suggestive of a micellar structure even if it cannot be demonstrated by other ultrastructural methods (electron-microscopy, X-ray interference, etc.). This underlines the important role of topooptical reactions in the study of ultrastructure. The reactions arise on micellar structures by oriented linkages of molecules from coloured or colourless materials and cause either a marked increase, or an inversion, of the structures' original birefringence. Demonstrating the different types of reactions observed on biological structures, the possibility of analyzing cytomembrane structures (ergastoplasm, nuclear membrane, cell membrane) ultrastructurally by topooptical staining reactions is discussed.

Considerations on the Unity of Tissular Structure and Metabolic Activity

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Structure and function form an inseparable unity in all living matter.

The different forms of metabolic activity are related to certain well-defined structures such as the oxybiotic metabolism to a capillary net carrying oxygen-rich blood. In the same tissue, metabolic processes take place also by lactic acid fermentation and by mucopolysaccharide formation.

In poorly vascularized tissues the main form of metabolism is lactic acid fermentation.

In avascular tissues the mucopolysaccharide-type metabolic activity predominates.

Examinations in the fields of phylogenesis, ontogenesis, regeneration and neodifferentiation have supported the above findings. The same has been found under experimental conditions and during pathologic processes which for different reasons resulted in a deficiency of vascular supply to certain tissues.

Ultrastructural, enzymological and biochemical studies have disclosed further details of the above correlations.

The apparent alterability of the components of the unity of structure and function by certain functional influences may offer some information as to the structural modification of certain tissues, as the changes of either of the two closely related components (form and function) will result in a corresponding alteration of the other component.

Ultrastructural Study of Carotid Bodies

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An ultrastructural study of glomus cells, endothelial cells and synapses has been made. The glomus cells are of two types, chemoreceptor cells and capsule cells. The former are polygonal in shape and have roundish nuclei; in their cytoplasm a pronounced endoplasmic reticulum composed of parallel tubes, a juxtanuclear Golgi complex, mitochondria with cristae, osmiophilic bodies varying in shape and size and polymorphous lysosomes are seen. The typical components of the chemoreceptor cells are cilia of 9 + 2 type, microvilli and protrusions. The nuclear membrane is a double layer, the nuclear pores are clearly defined and there are many nuclear membrane invaginations into the cytoplasm. The capsular cells are elongated, their nuclei are dark, and in their hem-like cytoplasm nerve fibres surrounded by mesaxon membranes are passing. They resemble the Schwann cells. The body of the endothelial cells is roundish, some with long processes and many small vesicles in their cytoplasm. The nucleus is homogeneous, with many indentations. In the Schwann-type capsule cells the nerve fibres form intricate mesaxon systems. The overwhelming majority of the synapses is of the afferent type, but desmosomal thickenings and the typical synaptic vesicle aggregations are absent. These afferent synapses serve as the stimulus receptors of the chemoreceptor cells. Synapses structurally identical with those of the central nervous system are rare. They are efferent synapses which form the motor terminals of the reflex arc whose sensory terminals are formed by the pressoreceptors located in the glomus vessels.

Propriétés biologiques des vaisseaux sanguins de l'embryon de poulet en culture organo-typique

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Par la présente communication, nous voulons exposer certains résultats qui ressortent d'une étude systématique portant sur les vaisseaux sanguins de l'embryon de poulet selon la méthode de la culture organo-typique de Wolff. On a explanté, in vitro, des vaisseaux sanguins de type élastique et musculaire, l'aorte et les artères vitellines et chorioallantoïdiennes de l'embryon de poulet du 4^{e}—5^{e} jour d'incubation jusqu'à l'éclosion. Les artères de type élastique vivent mieux in vitro que les artères de type musculaire. L'aorte montre in vitro un comportement différent dans sa partie craniale élastique et dans sa partie caudale musculaire. Toutefois, les vaisseaux sanguins de poulet ne manifestent pas, in vitro, la capacité de se développer et de croître comme les autres organes embryonnaires de poulet. L'auteur discute les raisons éventuelles de ce comportement.

Dans un autre groupe d'expériences, on a voulu observer les comportements de la paroi vasculaire vis-à-vis de certaines stimulations experimentales: certaines d'entre elles (coupures partielles ou totales) détruisant la paroi vasculaire, d'autres (corps étrangers enfilés dans la lumière) excitant certains composants de cette paroi. Les résultats acquis révèlent des propriétés insoupçonnées de la paroi vasculaire de l'embryon de poulet. Cette paroi, portée en culture organo-typique dans des conditions expérimentales adéquates, montre un haut degré de malléabilité et d'adaptation qui se traduit soit par une propriété remarquable de régénération tissulaire, soit par une possibilité de véritable néoformation vasculaire, soit enfin par une capacité d'adaptation aboutissant au dédoublement de la lumière.

Neuere Untersuchungen über Fettgewebe

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Elektronenmikroskopische Untersuchungen ergaben, daß das lange Zeit hindurch vernachlässigte Fettgewebe komplizierter strukturiert ist, als bisher angenommen wurde, und in sehr verschiedenen Typen auftritt. Der Vortrag beschäftigte sich zunächst mit der Abgrenzung des weißen vom braunen Fettgewebe und wendete sich den strukturellen Besonderheiten des letzteren zu, die mit seiner Rolle bei der Wärmeregulation in Zusammenhang stehen. Aufmerksamkeit wird vor allem der Innervation des braunen Fettgewebes geschenkt. Als dritte Form von Fettgewebe wird das sog. epithelioretikuläre Fettgewebe geschildert, das die Fettorgane der Amphibien aufbaut.

Ultrastructure of Cell Nuclei in Tissue Cultures

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The effect of various fixatives, freeze drying and drying on the cell nuclei of HeLa cells, as well as of primary and subcultivationable monkey kidney cultures prepared by the "flying glass" method, was studied with polarization microscopy. During subcultivation, freeze drying and drying caused anisotropy in all cell nuclei, after settlement only the mitotic nuclei were found to be anisotropic. The polarization microscopic structure of the cell nuclei varied after using various chemical fixatives.

Ultrastructural Changes of Interscapular Brown Fat Tissue in Different Functional States in the Newborn Rabbit

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In some rodents and, as recently shown, also in newborn infants brown adipose tissue plays an important role in thermoregulation. The ultrastructural relations of this function have been studied in starved newborn rabbits in cold and warm environment. Electron microscopic examinations revealed that in warm environment the brown fat tissue of the starved animals was inactive and seemed to play the role of a reserve, whereas in cold environment it became markedly active. Activation was indicated by the disappearance of lipid droplets, increase of the cytoplasm of lipid cells, hypertrophy of the smooth endoplasmic reticulum, increase in the number of mitochondria and the disappearance of the normally numerous intramitochondrial granules. Changes of temperature caused no structural alteration of white adipose tissue, suggesting that in thermoregulation exclusively the brown fat was involved, owing supposedly to the marked adaptability of its oxidation processes as indicated by the mitochondrial changes among the given experimental conditions.

Generation Time of Cultured Bone Marrow Cells

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Examinations have shown that in cultures of bone marrow cells usually the fibroblast-like (FL) cells proliferate.

The generation time of FL cells was examined by microcinematography and autoradiography, using ^3H thymidine.

The generation time of FL cells was found to take 24—30 hours, including an S-phase of approximately 10 hours. The knowledge of the generation time failed to disclose any information concerning cell origin.

Cell Differentiation and Granulogenesis in Rat Bone Marrow

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The ultrastructural and cytochemical process of differentiation was examined in bone marrow cells and peripheral granuloid cells in the rat.

Cellular pattern and mitotic index were assessed from myelo- and haemograms stained according to Pappenheim. First of all the differentiation of the neutrophilic series from myeloblast to mature granulocyte was examined. A description is presented of the ultrastructural changes of cell organelles (mitochondria, Golgi apparatus, endoplasmic reticulum, ribo- and polysomes, nuclear membrane, heterochromatin and nucleolus). It was demonstrated that the ring-shaped nucleus typical of the rodents' mature granulocytes originated through a perforation of the nuclei of promyelocytes and myelocytes, and the Golgi apparatus and the centrioles usually played a part in this process. In the perforated area alkaline phosphatase and peroxidase activity was increased. The types of granules and their distribution during the differentiation were determined and correlated with enzyme histochemical and ultrastructural data. Deviations between the differentiation of eosinophilic and neutrophilic cells is discussed and some types of basophilic granulocytes are described.

Fluoreszenzmikroskopische Befunde an Gefäßkanälen der knorpeligen Handwurzel

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Für die Untersuchungen standen formalinfixierte Handwurzeln von Früh- und Neugeborenen beiderlei Geschlechts zur Verfügung. Neben makroskopischen Aufhellungsverfahren kamen zur Darstellung des Inhaltes und der Umgebung der Knorpelkanäle histologische Färbungen, Fluorochromierungen und histochemische Reaktionen auf Mucopolysaccharide in Anwendung.

In jeden Knorpel dringt, ausgehend vom anliegenden Perichondrium, Mesenchym zusammen mit Blutgefäßen unterschiedlichen Kalibers ein. Die dabei entstehende Kanälchen können verzweigt sein. Größere Blutgefäße werden von perivaskulären Kapillarschlingen umsponnen. Fluorochromierungen zeigen, daß die chemische Zusammensetzung der Grundsubstanz in der Umgebung der Kanäle im Gegensatz zu der restlichen Knorpelgebiete verschieden ist. Nach histochemischen Reaktionen ist sie reicher an sauren Mucopolysacchariden und PAS-positiven Substanzen. Von innen nach außen schließt sich einer schmalen Zone saurer Mucopolysaccharide ein PAS-positiver Hof an.

Diese Veränderungen im Aufbau der Grundsubstanz in der Umgebung von Gefäßkanälen embryonaler Handwurzelknorpel werden im Zusammenhang mit der späteren Verknöcherung gesehen.

Electron Microscopic Study of Megakaryocytes

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A young and a mature form of megakaryocytes was differentiated in human bone marrow and mouse spleen by electron microscopy. The young cells were characterized by the presence of many free ribosomes in the cytoplasm and groups of smooth endoplasmic reticulum located near the nuclear parts. In the cisterns of the endoplasmic reticulum a medium electron dense substance was demonstrated. In the young cells, two nuclear lobules presenting different chromatin structures were seen; this phenomenon seems to be related to the maturation of megakaryocytes.

The mature megakaryocytes contain granules in the endoplasm which is likely the site of origin of thrombocytes. The ectoplasm is poor in cell organelles. This area may play a role in the further development of the megakaryocyte.

In some cells with apparently mature cytoplasm, mitoses were seen.

Histochemical and Electron Microscopic Study of the Granules of Eosinophilic Leukocytes from Mammals

A. Németh and **G. Kelényi**

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Previous studies on certain histochemical and electron microscopic properties of the granules of eosinophils from fishes, amphibia, reptiles and birds have been reported earlier. In the present study the staining properties of the granules of mammalian (rodent, predator, ungulate) eosinophilic leukocytes were examined using special dyes, polarisation and fluorescence microscopy, and their IEP-dependent acidophilic staining and neutral and alkaline benzidine-peroxidase reactions were studied. Electron microscopy was carried out after aldehyde or osmium tetroxide fixation; aldehyde-fixed preparations were treated with phosphotungstic acid. Based on the appearance of matrix and crystalloid and certain structural characteristics various granule forms were differentiated. A phylogenetic explanation is offered for their origin.

Submicroscopic Morphology of Eosinophilic Leukocytes with Special Regard to Electron Microscopic Staining Methods

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The electron microscopic morphology — matrix and crystalloid — of the eosinophil granules is well known. Histochemically, the presence of certain enzymes and basic protein was demonstrated; electron microscopic histochemistry revealed benzidine peroxidase and, in some species, acid phosphatase activity. The matrix of the granules stains with uranyl acetate and phosphotungstic acid, whereas the crystalloid with osmium tetroxide and potassium permanganate.

In earlier studies a granular or granularvesicular transformation of the matrix of eosinophil granules was observed in specimens obtained from humans and albino rats. Transformation of the matrix was associated with certain changes of the crystalloid and of the density relationship between matrix and crystalloid. Granular transformation allows the application of various staining methods permitting a further characterization of the fine structures in the matrix. From the structure and the reactivity of structural elements of the matrix it is possible to draw conclusions concerning the functional role of the granules.

Mechanism of Mast Cell Granule Formation

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Previous experiments have shown that in the tissue culture model of mast cell formation, heparin uptake by the cells did not whereas the serotonin or histamine uptake did result in the formation of true mast cell granules. It could not however, be disclosed, whether serotonin and histamine induced synthesis of heparin in the cells or promoted its uptake from the nutrient medium. Other experiments revealed that the glucocorticoids induced mast cell formation and subsequently were incorporated into the granules of mast cells. The present experiments were undertaken to clarify the mechanism of mast cell formation on the basis of the operon scheme. Thymus cultures were treated with actinomycin D or chloramphenicol and subsequently serotonin, heparin or histamine were added to the nutrient medium. The antibiotics did not inhibited the uptake of heparin and serotonin, but these brought about the appearance of metachromatic granules in the cells. This implies that heparin appeared in the cells as a result of active synthesis induced by serotonin or histamine. On this basis, it is supposed that the glucocorticoids suppress the operon involved in serotonin production through binding to arginine rich histones. The serotonin formed induces the synthesis of heparin and histamine. The glucocorticoid-histon complex is, together with histamine and serotonin, incorporated into the mast cell granule.

Zur hormonellen Beeinflussung des MPS-Stoffwechsels der Blutgefäßwand

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Störungen im metabolischen Verhalten der sulfatierten Gefäßwandmukopolysaccharide stellen einen frühzeitigen Indikator für die Entwicklung von Angiopathien dar. Es wurden daher anhand von Schnitt- und Flächenpräparaten autoradiographisch mit ^{35}S-Sulfat die zellulären Bildungsorte der sauren MPS von Aorta und Coronarien untersucht; mit Hilfe der Säulenchromatographie wurden die sulfatierten MPS aus der Aorta isoliert und ihre spezifische Aktivität nach Variation der radioaktiven Versuchszeit gemessen. Diese Untersuchungen wurden durch photometrische Bestimmungen der Schwärzungsintensität von Autoradiogrammen ergänzt. So konnten Befunde über die relative Bildungs- und Abbaugeschwindigkeit der Gefäßgrundsubstanz erhoben werden. Als Modell zur Erzeugung von Abweichungen im MPS-Stoffwechsel der Gefäßwand dienten Eingriffe in das endokrine System, wie Alloxandiabetes, Radiothyreoidektomie und STH-Applikation. Die Bedeutung der dabei erzielten Ergebnisse wird im Zusammenhang mit den heutigen Vorstellungen über die Entstehung von Gefäßerkrankungen diskutiert.

Structural Alterations of Liver Vessels After Subtotal Hepatectomy in the Albino Rat

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Data are scarce as to the changes taking place in the hepatic vessels in the course of regeneration. The present experiments were undertaken to clarify, whether the changes of organ weight during regeneration are associated with changes in the capacity of blood vessels; and whether regeneration takes place through a hypertrophy of pre-existing lobules or new complex lobules are formed.

The rate of regeneration following subtotal hepatectomy was followed by a quantitative injection-corrosion method. The procedure proved suitable for the assessment of the extent and quality of vascular regeneration and for the determination of vascular capacity. Repeated partial hepatectomy allowed a clear demonstration of the changes taking place in the liver lobules.

During the first week, vascular regeneration in both the portal and the vena hepatica system appeared to lag behind the rate of organ regeneration. There was no parallelism between the regeneration rates of the portal and hepatic vessel systems. No qualitative alteration occurred in the typical hepatic vessel structure. The examinations offered convincing proof of the regeneration of liver lobules taking place partly by a hypertrophy of pre-existing lobules and partly by the formation of new lobules.

Storing Capacity of Mesenteric Lymph Vessels

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The caliber of mesenteric lymph vessels was examined in experimentally induced mechanical insufficiency. In the mesentery, lymph congestion spread retrogradely toward the intestinal wall. The beginning of compensatory activity toward the reserve lymph vessels and adjacent areas is shown in a graph. An attempt was made to demonstrate a correlation between the caliber of lymph vessels, the number of valves and the rate of lymph flow. The importance of the frequency of valves in mesenteric lymph circulation is pointed out.

The Structure of the Lymph Vessel Valves

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The structure of the lymph vessel valves was studied after impregnation and by electron microscopy. The linking of endothelial cells coating the valvular surfaces and the relationship between them and the elements of the lymph vessel wall and the elastic and collagenous fibres at the base of the valve are reported.

Adenosintriphosphatase Activity of Cerebral Capillaries and the Blood-Brain Barrier

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According to Torack and Barnett, the ATPase activity of rat brain capillaries is localized not so much in the pinocytotic vesicles of the endothelium and cytoplasm as in the basement membrane of the capillary wall and in the surface membranes of glial feet, in contrast to other capillaries of the organism.

To clarify the eventual relationship of that particular enzymic localization with the physiological function of the blood-brain barrier, the barrier's permeability and the fine structure of cerebral capillaries were examined after blocking of ATPase activity. Examining the histochemically demonstrable ATPase activity of brain capillaries after intraperitoneal administration of 0.25 g/kg nickel chloride, ATPase inhibition was complete in 3—6 hours. Subsequently, trypan blue injected intravenously was found to have extravasated and, giving a marked perivascular fluorescence, diffused deeply into the brain matter. Electron microscopy revealed that on treatment with nickel chloride, the basement membrane became undulating and thickened considerably.

The present findings suggest that the ATPase activity localized in the basement membrane of brain capillaries is closely related to the function of the blood-brain barrier, and that the organization of the basement membrane needs an undisturbed ATPase activity.

Substructural and Functional Properties of Ribosomes in the Rat and Mouse Placenta Cells

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Electron microscopic studies revealed on the one hand an ultrastructural and dynamic relationship between ribosomes and the so-called glomerular bodies first described by the present author and, on the other hand, a supposedly primary role of ribosomes in the appearance of acid phosphatase activity. The findings are summarized as follows:

1. Observing the pictures made with high power resolution in appropriate planes of the sections, 12—15 Å filaments were seen within the ribosomes, spirated in different degree.

2. Beside the known polysomes, other types of ribosomal aggregations were seen.

3. On the application of actinomycin D, ribosome-like particles of less than normal size appeared.

4. The glomerular bodies consisted of helical bundles 400—500 Å in diameter, whose density, staining properties and ultrastructure resembled those of the ribosomes. It is postulated that the glomerular bodies are formed by the aggregation of several hundreds of ribosomes, very likely through a polymerisation of ribosomes which then assume a complicated steric structure inside the bundles of glomerular bodies.

5. Under the influence of actinomycin D, most of the glomerular bodies disappeared from the cytoplasm; it is supposed that they fell again to ribosomes.

6. The cytoplasm of the protein-synthetic trophospongial cells, at the end of pregnancy, shows a very high activity of acid phosphatase, associated partly to rough-surfaced membranes, and, partly to the free ribosomes. In the surroundings of the enzyme active sites, the secretion products disappeared from the cisterns of the endoplasmic reticulum, while in the ergastoplasm increasing autolysis took place, without the presence of preformed lysosomes or autophagic vacuoles. Extensive studies indicated that the appearance of acid phosphatase activity was due primarily to ribosomal function. The newly developed enzyme activity seemed to be in no morphological relation to the Golgi apparatus.

Electron Microscopic Structure of the Palatine Tonsil

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Light and electron microscopic studies of reticular area (the epithelium infiltrated by lymphocytes) in rabbit tonsils reveal a connection between the intraepithelial passages and subepithelial lymphoid tissue, through the discontinuities of the basal membrane. Two peculiar cell types were found in the reticular area. One of them similar to the special cell was observed in the medulla of the rat thymus. The other cell contains some dense core vesicles as the enterochromaffin cells in the gut epithelium.

The subepithelial lymphoid part of the tonsils is divided into intercommunicating lacunae by septae, coming from the capsule. The surface of these septae is covered by fibrocytes. It is surmised that the subepithelial lymphoid space turns to be an efferent lymph vessel of the tonsils.

Mucopolysaccharide Matrix Surrounding the Photoreceptor Structures in Vertebrates and Insects

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Electron microscopy of the vertebrate retina revealed a moderately electron dense substance located between the pigment epithelium and the photoreceptor inner and outer segments. In some species (frog, monkey, man) the matrix occurs in large amounts. A similar substance was found in the so-called open rhabdoms of certain insects, where it surrounded and separated the rhabdomeres of the individual retinula cells.

In both cases, alcian blue and Hale's stain showed the ground substance to contain acid mucopolysaccharides. The role and the site of formation of matrix remains to be clarified. Its close topographic connection with the photoreceptor structures (the outer segments, rhabdomeres) seems to point to its auxiliary role in the photoreception process.

Osmiophilic and Osmiophobic Degeneration Patterns of Postganglionic Autonomic Nerve Fibres

Elizabeth Knyihár, Á. Párdutz and **B. Csillik**

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Ultrastructural investigations revealed two kinds of secondary axon degeneration of the postganglionic adrenergic nerve fibres. In most cases, intraaxonal osmiophilic cytolysosomes are formed in the degenerating axons, taken up later by the enveloping Schwann cell processes. In striking contrast to this osmiophilic degeneration pattern, an osmiophobic degeneration is characteristic of the postganglionic adrenergic nerve fibres of the rat iris. This form is characterized by the appearance of large vacuoles, and a complete disappearance of axoplasmic material. 6-HODA administration (chemical sympathectomy) induces similar differences in the electron microscopic structure. Osmiophilic degeneration is accompanied by a strong light microscopic (lysosomal?) acid phosphatase activity, whereas no considerable acid phosphatase staining could be seen during osmiophobic degeneration.

Mesenteric Nerve Terminals

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The different types of mesenteric nerve were studied after silver impregnation. In contrast to the Vater—Pacini bodies, encased, semi-encased and free terminals were encountered in the "mute", less motile areas of the mesentery. The terminals were located either along the blood and lymph vessels or in completely avascular areas. Free terminal(s) were often seen either along, or in close junction with, the Vater—Pacini body. The structure of the Vater—Pacini body's internal cylinder was compared to that of the encased terminal. It was concluded that in the mesentery such terminals are not specific of one or another sensory quality.

Innervation of Intestinal Wall and Villi

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The location and relative position of nerve elements and reticula in the intestinal villi and the various layers of the intestinal wall have been studied after silver impregnation and histochemical examination. The nerve elements found in the villi and below the internal circular muscle layer were of cholinergic nature. Adrenergic fibres were demonstrable exclusively in perivascular location in some of the layers. Morphologic and steric differences between the nerve cells of the myenteric plexus and the submucous plexus, and interconnections between certain cell groups are described. The extension and overlapping of the areas supplied by the individual nerve cell types is discussed.

Analysis of the Renal Monoaminergic Mechanism by Fluorescence and Electron Microscopy

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The intraparenchymal topography of monoaminergic nerves has been examined by the modified Falck method in cryostate sections from cat and rat kidneys.

The larger arteries of the renal parenchyma are surrounded by a rich monoaminergic nerve net located at the border between adventitia and media. The terminals do not enter the media. The specialized periarterial fibres can be followed along the afferent arteriole up to the glomerule. At the vascular pole, the reaction becomes more pronounced owing to the presence of juxtaglomerular cells. Some fibres bend over the Bowman capsule, while others continue along the efferent arterioles. In the venous system, monoaminergic nerves were found exclusively along the large collecting branches.

The monoaminergic nerve fibres located between, and apparently connected with, the contorted tubules showed a relationship with the pericanalicular vessels in electron micrographs. Thus, the catecholamine nervous control of tubular secretion seems to be questionable.

In the medulla, monoaminergic nerve terminals were found around the narrow part of Henle's loop.

The electron microscopic counterparts of these terminals were axoterminals containing dense-core vesicles but lacking Schwann sheath; they were closely connected with the basement membrane of the fenestrated capillaries surrounding the portio conducens. It is suggested that the catecholamines demonstrated at this site by fluorescence and electron microscopy play a role in the control of capillary permeability.

Functional and Structural Relations of the Monoaminergic Innervation of the Iris

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In whole rat iris preparations considerable aggregations of monoaminergic nerve fibres were found in the area corresponding to the pupillary sphincter. By means of Falck's paraformaldehyde method and Bielschowsky's silver impregnation, a close relationship of the specific nerve fibres to the peripupillary vessels was demonstrated, indicating their role in the control of vascular supply.

In pharmaco-morphological experiments, the uptake of endogenous and exogenous catecholamine and of its precursors by vegetative nerve terminals and post-synaptic structures has been examined.

In animals pretreated with reserpine, the uptake of exogenous monoamine in the presence of ATP was particularly pronounced in the pupillary region of the iris, suggesting a close relationship between noradrenaline and the vascular wall.

The above pharmacological data imply that the circulation in the vessels supplying the pupillary margin is controlled by adrenergic fibres located in the area of the pupillary sphincter.

Reabsorption of Monoamines in the Adrenergic Nerve Fibres of the Rat Iris

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Adrenergic nerve fibres of the iris show a fluorescence under the effect of formaldehyde gas. It is well known (Falck; Falck, Carlsson and Hillarp) that different chemical agents

applied *in vivo* cause catecholamine depletion in these nerve fibres. In the present study the reabsorption of some biogenic amines (adrenaline, noradrenaline, dopamine), was investigated in vitro after depletion induced by chemical agents. The experiments were performed on the rat iris. Twenty-four hours after intraperitoneal reserpine administration and removal of the superior cervical ganglion, the iris was removed and immersed in different solutions containing monoamines. The solutions were oxygenated and different kinds of macroergic phosphates were added. After 20 minutes treatment the catecholamines were estimated by the method of Falck.

The results showed that noradrenaline and dopamine are fixed in the tissues of iris. Dopamine is bound to the cytoplasm and nucleus of endothelial cells while noradrenaline reabsorption takes place in the axons of adrenergic nerve fibres. When using different types of macroergic phosphates, different patterns of noradrenaline reabsorption were observed. When ATP-ase was inhibited by strophantine, no ATP-enhanced reabsorption could be observed.

Origin of Axolemmal Acetylcholinesterase

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Electron microscopic histochemical investigations have shown that end-product of the acetylcholinesterase reaction is localized on the outer surfaces of the unit membranes of cholinergic nerve cells.

Histochemical observations of developing nerve cells revealed that enzyme synthesis takes place inside of the endoplasmic reticulum of the cholinergic neurones. It was attempted to solve the problem how the enzyme passes from the inside of the cell body to the outer surfaces of nerve cell processes.

We found some tubules exerting acetylcholinesterase activity inside the axon. At some places enzyme active tubules were connected with the axolemma. These enzyme active tubules seemed to "extrude" acetylcholinesterase into the space between the axons and dendrites. From these results it may be concluded that the enzyme is in a soluble form while being transported through the tubules from the nerve cell body and becomes structurally fixed only on the outer surface of the membranes.

The Neurosecretory Nucleus Preopticus as Member of the Liquor Contacting Neuronal System

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In our earlier studies it has been shown that the paraventricular organ which is one of the ependymal organs of the 3rd ventricle, contains monoamine producing neurons. The dendrites of the neurons form free ciliated terminals which extend into the cerebrospinal fluid. On the basis of the morphological picture it has been supposed that the monoamine producing neurons of the paraventricular organ have a receptor function in the cerebrospinal fluid.

In further studies an area resembling the paraventricular organ has been detected in the preoptic recess. This so-called "preoptic recess organ" also contains monoamine producing neurons whose ventricular processes form club-shaped terminals in the 3rd ventricle. The terminals carry cilia. Since the structure of the two areas is similar, it has been assumed that they form a common system, "liquor contacting neuronal system" whose main function appears to be to maintain a special informative connection between cerebrospinal fluid and central nervous system.

In the present study the neurosecretory nucleus preopticus was examined in different species. The dendrites of the neurons which are known to pass toward the ventricular wall do not terminate at the ependymal level but enter the ventricle where they form club-shaped terminals resembling those of the paraventricular and recessus preopticus organs. The nerve endings carry cilia. This finding indicates that the preoptic nucleus, too, belongs to the liquor contacting neuronal system, and like its other members it may have a receptor function. It is supposed that one of the main functions of the neurosecretory ADH system is to maintain the homeostasis of cerebrospinal fluid.

Comparative Study of the Area Postrema's Monoamine System in Various Vertebrates

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The area postrema, classified as a circumventricular organ, is located at the caudal end of the fourth ventricle, at the origin of the central canal. The area is clearly distinguishable from the surrounding structures on the basis of its typical vascular system and special glial and nerve elements. According to comparative studies, the area postrema is found exclusively in high vertebrates (reptiles, birds, mammals). In the present study the area postrema of the latter classes was examined by Falck and Owman's fluorescence microscopic method to demonstrate similar and dissimilar features of the monoamine systems in these areas.

A great variation was found in the location, number and shape of monoamine containing nerve cells and fibres even in the different vertebrate species. A relatively uniform response was observed to MAO inhibitors and to reserpine, which causes monoamine depletion. The presence of monoamines in the cells of the area postrema allows to conclude that these cells are nerve cells although their morphology may be often reminiscent of glia cells.

Kreislaufversagen und Herzinsuffizienz beim Haifischkeimling als Modell für die Pathogenese des Fruchttodes nach Einwirkung von Histamin und Allylformiat

H. Fischer

(Humboldt Univ., Frauenklinik, Berlin)

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Morphology of Synaptic Transmission in the Central Nervous System of Lower Vertebrates

G. D. Smirnov

(Severcov Institute, Moscow)

Paper not received

Contribution à l'étude de l'histophysiologie de l'organe sous-commissural

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On a étudié le comportement de l'organe sous-commissural (OSC) sous conditions du stress aigu.

Les rats males adultes ont été soumis a la contrainte par immobilisation totale pendant 25 heures (immobilisation dans un grillage suspendu horizontalement), sans recevoir aucune boisson ni nourriture.

Les épendymocytes et leurs noyaux sont hypertrophiés, la zone périnucléaire du cytoplasme est hyperbasophile. Les grains de sécrétion aldéhyde-fuchsine positifs varient en quantité et en chromophilie d'une cellule à l'autre.

La morphodynamique corrélative des cysternes du réticulum endoplasmique et de l'espace perinucléaire est plus accentuée que chez les rats témoins. Le pourcentage de la

disposition des cysternes ergastoplasmiques est le même dans les deux poles des cellules épendymaires. C'est surtout le complexe morpho-fonctionnel formé de pieds des épendymocytes, de cellules hypéndymaires et de capillaires, qui est affecté. Les corps à structure périodique sont plus nombreux et plus vastes. Les cellules endothéliales présentent des protrusions notables dans la lumière capillaire. Les terminaisons axonales intraépendymaires sont bourrées de microvésicules synaptique («hypersynapsie fonctionnelle»).

Les résultats obtenus plaident en faveur d'une activité stimulée de l'OSC sous l'influence des facteurs agressifs associés (immobilisation, soif et faim) et d'une fonction bipolaire des épendymocytes. Les corps à structure périodique faisant part d'une barrière d'échange et de transport spécialisée de l'OSC, seraient doués d'une perméabilité séléctive entre les épendymocytes et le sang.

Transmission Mechanisms in Motoneuronal Boutons

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According to electron microscopic studies, motoneuronal boutons contain a neurofilamentous ring, mitochondria and synaptic vesicles. After aldehyde fixation, boutons in the rat spinal cord can be divided into four groups on the basis of shape and size of their vesicles. Boutons containing large amounts of ovoid vesicles undergo marked structural alterations after strychnine convulsion, suggesting their involvement in post-synaptic inhibition. Autoradiographic studies performed by means of ^{14}C thiosemicarbazide suggest that the formation of gamma-amino-butyric acid takes place in glial cytoplasms, both in the white and in the gray matter; thus, inhibitory neurons exerting their action by means of this substance, appear to utilize GABA-stores of glial cells.

Development of Synaptic Organization in Partially Agranular Cerebellar Cortex

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X-ray irradiation of the newborn cat's cerebellum with a 200 r dose is apt to destroy selectively, without damaging the prenatally differentiated Purkinje and Golgi cells, 70—80% of the postnatally differentiating granule cells. In the partially agranular cortex, owing probably to the numerical reduction of parallel fibres, development of the dendritic tree of Purkinje cells is retarded. This is shown by the fact that tertiary dendritic spines which normally form synapses with the parallel fibres, develop in a much lower number than in the non-irradiated controls. In contrast with the dendritic spine system of the Purkinje cells, the two inhibitory interneurons of the molecular layer, the basket and stellate cells do not seem to be affected by the deficiency in parallel fibres or, indirectly, in the mossy fibre afferentation. In comparison to the controls their differentiation is even accelerated. This and other data presented in the paper suggest that the climbing fibres of the cerebellum play a role in the differentiation of stellate and basket cells.

Vesicle Population of Excitatory and Inhibitory Cerebellar Synapses under Normal and Experimental Conditions

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According to Uchinozo and Bodian, excitatory and inhibitory synapses differ from each other with respect to the size and shape of vesicles within the terminal. After perfusion fixation with Karnovsky's aldehyde mixture, three types of excitatory and four types of

inhibitory synapses were studied in the rat cerebellar cortex. Except for the synapses on primary and secondary Purkinje dendrites, a close correlation was found between the large-spheroid vesicles in excitatory junctions and small-ovoid vesicles in inhibitory synapses. The ratio of spheroid versus ovoid vesicles (Q) is characteristic of each type of junction. Q values did not undergo considerable changes after blocking acetylcholine synthesis by means of hemicholinium, or after blocking gamma-aminobutyric-acid-synthesis by means of thiosemicarbazide.

Histological Analysis of the Cerebellar Granule Cell Layer in the Cat

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The number of granule cells per 1 cu. mm of the cerebellar granule cell layer was determined by the modified Chalkley method. The studies of karyometric homogeneity have shown a diffuse homogeneous distribution of granule cells over this area. Their number as related to one Purkinje cell and the dimensions of the area occupied by them in the layer were calculated. By means of Palkovits—Hajtmann's dot diagram method, the other cellular components of the granule cell layer (Golgi cells, astrocytes) were differentiated and their absolute numbers as well as their counts related to each other and the Purkinje cells were determined. The above method allowed to differentiate between the Golgi cells (large, fusiform). The Golgi cell to Purkinje cell ratio was 1 : 3—4 on the average. The density of Golgi cells was greatest below the Purkinje cell layer and tended to decrease, whereas the number of astrocytes to increase, with the depth. The above quantitative and stereoscopic data should serve to obtain more information on the cerebellar neuronal junctions and help to study them in an experimental model.

Efferent Paths of the Frog's Optic Centre

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After destruction of the optic centre on one side three path systems lending to different parts of the central nervous system could be followed.

Fibres emerging basally (laterally) from the 7th layer of the tectum opticum give rise to one rostral and one caudal path. The dorsal part of the rostral path is partly formed by fibres which leave the 7th layer anteriorly. The path provides an ipsilateral connection with the isthmic nucleus, the mesencephalic tegmentum, pre-tectal region and thalamus, then, after crossing in the postoptic commissure, some fibres are sent to the retina through the optic nerve. After crossing, the path turns caudally and terminates in the contralateral thalamus, pre-tectal region and mesencephalic tegmentum.

The uncrossed part of the caudal path, as well as its part crossed in the commissura ansulata send fibres to the small cells surrounding the motor nuclei, to the intermediary zone of the grey matter, and to the dorsal part of the anterior horn.

The third path leaves the 7th layer medially and connects the two tecta through the posterior and tectal commissures. Its fibres terminate in the 2nd—6th layers of the tectum.

Electron Microscopic Study of Optic Fibre Terminals

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According to electrophysiological studies of the optic centres of amphibia, the optic nerve fibres terminate in three layers of the superficial zone. Traditional neurohistological methods are not suitable for the reliable demonstration of optic terminals, but five days

after removal of the eye the degenerated terminals are readily identifiable in the electron micrograph. They are located in three not sharply demarcated layers of the superficial zone; a thin fourth layer is seen along the border between the superficial and medial zone. The superficial fibres terminate in glomerule-like groups formed by dendritic protrusions and other axon endings; the deeper fibres are seen along the apical dendrites of the deep cells of the optic centre. In addition to the optic terminals, three other axon terminals can be differentiated on the basis of the electron density, size and density of vesicles, and the shape of mitochondria; the origin of these axon terminals remains to be clarified.

Reaction of the Auditory Pathway Ganglia to Pure Tone Stimulation

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Stimulation of guinea pigs with pure sound was found to induce an increase of nuclear volume in the outer hair cells of the organ of Corti whose structure and density depends on the tone quality. The nuclei of the cochlear ganglion spiral respond to the stimulation only by a small increase of nuclear volume in relatively extensive sections. All the cochlear nuclei react with a steady and significant increase in nuclear volume but the cell size is not influenced by the frequency. The cell nuclei in the inferior colliculi also respond to tone stimulation by a small increase in size, but this increase as well as the position of the swollen nuclei is again determined by the tone quality.

Cortical and Subcortical Connections of the Pulvinar Thalami

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The morphological structure of the pulvinar complex has drawn attention to the origin of the nerve fibres which help to form the synaptic glomerules.

The present experiments were undertaken to obtain direct anatomical evidence (selective axon degeneration method, Fink—Heimer, Nauta—Gygax) of the cortical and subcortical connections of the pulvinar which have been assumed on the basis of electrophysiologic and retrograde degeneration studies.

The origin of the cortical afferent system of the pulvinar was demonstrable morphologically in more cortical areas than previously supposed. The sites of origin of the cortical fibres correspond to the area of efferent fibre terminals. The subcortical connections of the pulvinar are formed by fibres originating from the tectum and spinothalamic tract.

The multiple cortical and subcortical connections of the pulvinar are suggestive of its probable role in stimulus transformation.

Golgi Analysis of Anterior Thalamic Nuclei

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In the course of the Golgi analysis of the thalamic relay nuclei, in the anterior nuclei the cell and fibre structures and the internal connections were studied. In the present experiments, interconnections of the anterior nuclei were examined by the degeneration method, with special regard to the AD connections. The Golgi analysis revealed that the three members of the group of nuclei differed in respect of neurons, dendritic tree and collaterals. Tufted

type relay cells occurred exclusively in the ventral nucleus, whereas the cells of the other two relay nuclei were larger and showed a transitory or radial distribution of dendrites.

The proportion of Golgi II type cells varied. The AD contained less Golgi-type neurons than the two other nuclei, but the connection between relay cells and Golgi II interneurons was conspicuous in the anterior nuclei, too.

The terminals of the specific afferent fibres are characterized by a moss-like branching. Such terminals occur chiefly at the division of the relay cells' principal dendrites.

Degeneration studies have shown intensive connections of AD with AV and AM. The connections as well as the distribution of the main cell types of AD are reminiscent of those of elaborative nuclei.

Golgi Analysis of the Ventral Lateral and Medial Nuclei of the Thalamus

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Hodological examinations do not justify the independent discussion of the lateral ventral and medial thalamic nuclei. The medial nucleus, too, receives the majority of its afferent fibres from the cerebellum and pallidum. Similar observations have been made in Golgi preparations. In the cortical projections of the two nuclei, located in the motor and premotor areas, a certain regularity of order was observed.

According to physiological investigations, on stimulation of the brachium conjunctivum, the excitatory phase was followed by postsynaptic inhibition in the lateral ventral nucleus. An explanation of this phenomenon was sought for by Golgi analysis, which revealed the presence of Golgi II type neurons in addition to principal (relay) cells. The connection between the latter two cell types offers a morphological explanation of the physiological phenomena.

The Synaptic Structures of the Dorsomedial Nucleus

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In Golgi preparations, four cell types are demonstrable in the dorsomedial nucleus: relay or principal cells, type Golgi II/a and II/b neurons and integrator cells belonging to the reticular system. The afferent fibres arrive from several directions and form glomerule-like synaptic junctions both along the relay cells and the dendrites of Golgi II neurons, as indicated also in Golgi preparations. Electron microscopic examinations were carried out to identify the various cells and dendrites and also to differentiate the fibres and examine the structures of synapses. The glomerular type of synapsis was apparent also in the electron micrographs; a form not fully encased in glial capsule was seen chiefly in junction with the dendrites of relay cells.

Quantitative Analysis of Synaptic Organization in Non-sensory Relay Nuclei of the Thalamus

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The Golgi-analysis of non-sensory thalamic relay nuclei — dorsomedial, ventrolateral, anterior medial, dorsal and ventral — yielded results similar to those obtained with the sensory nuclei. The two main cell types, the relay (principal) cells and the interneurons (Golgi II type) were distinguishable also on the basis of their nuclear volumes. The further subgroups represent cell types known from Golgi preparations which seem to play different functional roles. Correlating the proportions of cell types with quantitative data related to the area of the dendritic tree and axon branchings in Golgi preparations, conclusions have been drawn as to the functional morphology of synaptic organization.

Quantitative Analyses of Synaptic Organization in Sensory Relay Nuclei of the Thalamus

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The sensory relay nuclei of the thalamus — posterolateral ventral nucleus and the lateral and medial geniculate bodies — have in spite of their different tasks in many respects similar synaptic structures owing to the identical level of their integrative activities. The main cell types and cellular structures of similar functional role are to be found in all the three nuclei. Of the cellular structures, the initial collateral of the relay or principal cells and the axon branching of the interneuron are important. The cells' dendritic arborization which represents their stimulus perception, the area traversed by the terminal parts of the specific afferent fibres, as well as the number of relay cells present in the latter area have been studied.

Data obtained with Golgi-preparations have completed and confirmed the results of nuclear volume estimation.

The quantitative data on the synaptic organization of sensory relay nuclei contribute some new information concerning the transmission activity taking place in these nuclei.

Golgi Analysis of the Cytoarchitectonics of the Nucleus Caudatus

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A Golgi analysis of the nucleus caudatus was carried out with regard to elements important for nuclear afferent fibre endings. The number of dendrites, their length and type of branching as well as their spike patterns and the axon's morphological properties were examined. Cells differing in size and dendritic arborization could be divided into two groups on the basis of axon behaviour: cells with long and short axons, *viz.* projective and interneuron-type neurons. Within these groups several subtypes were differentiated by other properties.

New Possibilities of Golgi Analysis

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Golgi sections were analyzed by superprinting of selected axon branchings and correlating them to data obtained by electrophysiological examinations. The method was found to disclose information about axon endings and areas.

The working principle is that 5—20 axons, selected on the basis of predetermined morphological criteria are drafted in one and the same scheme and on the drawing thus obtained further common properties of the branching are sought for. Thus certain regularities may be revealed in the endings or collateral systems of the axons which may remain inconspicuous if axons are studied one by one.

From the electrophysiological findings, monosynaptic connections may be conclusive in neurohistological analysis, as they indicate the location of a group of nerve cells at the termination of an axon. As an example the localisation of the ventral neurons of the spinocerebellar tract is demonstrated.

Afferentation and Intranuclear Structure of Brain Stem Viscero-Sensory Nuclei

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The afferent fibres which are sent to the brain stem by the glossopharyngeal and vagal nerves terminate in three nuclei which are closely related to, but topographically well dis-

tinguishable from, the fibre bundle of the solitary tract. The distribution of the afferent fibres shows the following characteristics.

(i) Two thirds of the nucleus alae cinerea lateralis oralis are supplied exclusively by terminals of ipsilateral n. IX. and n. X. fibers, while (ii) its caudal third receives bilateral afferentation from both brain nerves. (iii) The latter applies also to the commissural nucleus. (iv) In the nucleus tractus solitarii exclusively ipsilateral, mainly vagal fibres have their endings.

Following nerve transfixion, Fink—Heimer's method revealed coarse terminal elements in the tractus solitarii and commissuralis nuclei whereas very delicate ones in the nucleus alae cinerea lateralis. This correlates well with the data obtained by the Kopsch perfusion method. These types of terminals characterize exclusively the nuclei and are unrelated to the origin of the fibres.

Electron microscopically, the visceral afferents of the brain nerves in question appeared to form primarily axo-dendritic synapses with the neurons of the above areas while on a smaller, fusiform cell type chiefly axo-somatic synapses were apparent. In the latter case, however, the axons originated from nerves other than the examined ones.

Corpus Luteum Formation Induced by Ovariectomy in Rats without Spontaneous Ovulation

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In rats with lesions in the anterior hypothalamus showing permanent cornification of vaginal epithelium, corpus luteum formation started in 3—8 weeks following the operative removal of one and a half ovary. Ten weeks after surgery luteinisation was no longer observed. Apparently, the reduction of the amount of oestrogen-producing tissue diminished the negative oestrogen feedback and resulted in luteinisation of the animals in whose ovaries not a single corpus luteum had developed for two months prior to partial ovariectomy. However, the ovarian tissue left in situ increased in 2.5—3 months to such an extent that no corpus luteum formation could be elicited by removing again half the ovary.

Ovulation Elicited in Anovulatory Rats by Injection of Autogenous Pituitary Extract

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Rats rendered anovulatory by various methods were treated with autogenous pituitary extract. Forty-eight hours after treatment more than 50% of the animals had ova in their oviducts. Inconsistently with the literary data this has shown that rats rendered anovulatory by electrolytic lesion or frontal division of the anterior hypothalamus, or by prolonged light or perinatal androgen treatment, had still enough luteinizing hormone in their pituitary gland to elicit ovulation.

The Mechanism of Androgen Sterilization

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The preoptic and anterior hypothalamic neurons which are sensitive to oestrogens are indispensable in the neuro-hormonal feedback processes which ensure the cyclic secretion of gonadotrophic hormones. Immediate postnatal androgenic influence resulted in a reduc-

tion of the oestrogenic reactivity of these hypothalamic structures. In recent studies, the oestradiol-binding capacity of the anterior pituitary, uterus and anterior hypothalamic region of androgen-sterilized female rats was found to be reduced as compared to the controls. This suggests that an immediate postnatal androgenic effect impairs the specific receptor mechanism responsible for the binding of oestradiol molecules to the above hypothalamic structures; the impairment of the receptor mechanism necessary for the development of full oestradiol action seems to account for the absence of cyclic gonadotrophin secretion in male or androgen-sterilized female rats.

Histoenzymatic Reactivity of the Thyroid in Tissue Culture

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In cultures of normal and pathological thyroid tissue the activity of aerobic and anaerobic glycolytic enzymes, pentose shunt enzymes, diaphorases, lysosomic enzymes, and ATPase has been studied in tissue specimens obtained from patients with exophthalamic goitre, thyroid cancer, adenoma, and diffuse goitre. In all cases the activity of enzymes belonging to the Krebs and Embden-Mayerhof cycles was prevailing, both at the site of the lesions and in normal thyroid tissue. As regards the type of lesion, the highest enzymatic activity was noted in exophthalamic goitre and the lowest degree in embryonal adenoma. The DNA content, determined histophotometrically, was highest in carcinoma and lowest in embryonal adenoma.

Swelling of Nerve Cell Nuclei Following Surgical Interruption of Neural Connections to the Medial Basal Hypothalamus

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(Institute of Anatomy, University Medical School, Pécs)

Partial or complete interruption of neural connections to the medial basal hypothalamus of 70 adult rats was carried out stereotaxically. Three kinds of intervention were undertaken: 1. Complete deafferentation; 2. frontal cut to disconnect the anterior connections; 3. partial deafferentation by cutting all except the anterior paths. 30—120 days after the intervention nuclear volume was determined in the cells of the arcuate and ventromedial nuclei. In every case, 200 nuclei were measured. All the three types of intervention induced a significant enlargement of the nuclei: in cases where the intervention was unilateral, the change occurred on the deafferented side. In some cases there was a conspicuous nerve cell hypertrophy in the deafferented area. As a control, a temporal cortical segment was tsolated; swelling of the nerve cell nuclei occurred also in the isolated area. It is supposed ihat the nuclear enlargement was due to a transneuronal degeneration.

Einschwingvorgänge nach einmaligen Eingriffen in das Hypophysenvorderlappen-Nebennierenrindensystem

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Einmalige hochdosierte ACTH-Zufuhr löst beim Meerschweinchen ein charakteristisches Verhalten der 17-OHCS-Ausscheidung im Harn und der Kernvolumina der Zona fasciculata der Nebennierenrinde aus. Dem bekannten initialen Anstieg folgt innerhalb 48 Stunden ein Abfall auf subnormale Werte, die über einen längeren Zeitraum bestehen bleiben. Die Normalisierung setzt mit einem Rebound um den 10. Tag ein. Die Verfolgung der Kernvolumina in Leber und Schilddrüse zeigt, daß beide Organe von diesem Ereignis beeinflußt

werden. So lassen die Leberparenchymzellen einen sofortigen Abfall des Kernvolumens auf subnormale Werte erkennen. Erst nach dem 7. Versuchstag erfolgt eine Annäherung an die Norm. Die Schilddrüsenepithelzellen reagieren auf die ACTH-Zufuhr mit einer kurzfristigen Zunahme des Kernvolumens. Der folgende Abfall auf subnormale Werte endet am 5. Versuchstag. Danach finden sich Kernvolumina im oberen Normbereich.

Zusätzliche ACTH-Gaben in der Phase der niedrigen 17-OHCS-Ausscheidung und der subnormalen Kernvolumina zeigen keine Auswirkung auf die beiden Meßgrößen. Ebenso bleiben Streßreaktionen auf Diphtherietoxinvergiftung aus. Dieser Zeitraum wurde deshalb als Depressionsphase bezeichnet.

Daß diese zusätzlichen ACTH-Gaben jedoch nicht ganz ohne Auswirkung bleiben, läßt sich daran erkennen, daß durch eine ACTH-Gabe am 5. Versuchstag der am 10. Tag nach der initialen ACTH-Zufuhr zu erwartende Rebound gelöscht wird. Dafür findet sich am 15. Tag, also 10 Tage nach der zweiten ACTH-Gabe, ein Reboundgipfel.

ACTH Production under the Influence of Metyrapone in the Pituitary Transplanted in Prenatal Age

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Earlier studies have shown that the avian pituitary gland implanted subcutaneously in prenatal age not only maintained TSH production but, under the influence of goitrogenic agents, even elevated it above the basic level. The problem investigated at present was whether the transplanted pituitary deprived of its hypothalamic connection was, under external influences, able to increase the production exclusively of TSH or also of the other trophic hormones.

Anterior pituitary was implanted on the 6th day of incubation under the abdominal skin of chick embryos decapitated on the 2nd day of hatching, and after treatment with 3×0.1 mg of metyrapone, adrenal weight, nuclear volume of cortical cells and the ascorbic acid content of the adrenals were examined every hour for 3 hours. The results were correlated with those obtained in 18-day-old intact chick embryos. The operated embryos responded to metyrapone in a similar way as the non-operated controls.

It appeared that, in contrast to adult birds, the pituitary gland transplanted in embryonic age is capable not only of basic ACTH secretion but, on adequate stimulation, may even increase it above the basic level.

Role of the Medial Habenular Nucleus in the Control of Salt and Water Household

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Several data have pointed to the role of the epithalamic region in the control of salt and water metabolism. The present experiments were undertaken to clarify whether the medial habenular nucleus (NHM) is involved in that function. The problem was approached from two angles and the following observations were made: 1. The NHM was destroyed bilaterally and nuclear volume of the adrenocortical cells was assessed. Two weeks after the intervention there was a significant decrease in nuclear volume of the zona glomerulosa cells, while no change occurred in the fasciculate zone. 2. A disturbance of salt and water metabolism was induced and nuclear volume of the NHM cells was estimated. Salt load (2×4 ml of 3% NaCl through a gastric tube) for 3—5 days caused a significant decrease in the nuclear volume of NHM cells. Treatment with 2×10 ml of physiological saline daily for 3 days had a similar effect, but after 5 days the effect ceased. Five or eleven days after adrenalectomy, the NHM nuclei increased in volume. Based on the above observations it is assumed that the NHM plays a role in the regulation of salt and water household.

Cyto-histometric Studies of Sarcoma

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Sarcomas of different cyto-histological types are often unsuitable for bio-micrometric investigation, as their cells do not always show clear cut cytoplasmic borders, because they keep the usual architectonics of connective tissue, with occasional cytoplasmic processes forming bridges between the cells. Moreover, if dense enough, hyaloplasmic structures are seen under the phase contrast microscope. More favourable for study are the sarcomas of lymphatic and myeloid tissues, but even in these the reticular elements, as well as histiocytes and their polyblastic forms are often difficult to differentiate.

Thus, we found it convenient to apply only karyointerkaryometry for most of the gliosarcomas and common sarcomas, and to use cytometry and intercytometry only when the cytoplasm has clearly defined outlines.

Nuclear volume (Puff formula) and cellular volume (based on the previous one) were measured in 50—1000 cells, tabulated as log-values, and presented in graphs, together with the internuclear and intercellular distances.

When such objective findings are compared during follow up examinations, the biological conditions can be expressed well for each patient and it becomes possible objectively to evaluate the prognosis and the therapeutic method.

The method is especially suitable for routine work in the hospital laboratory and all what is needed is a micrometric device.

Catecholamine Synthesis and Storage by the Intermediate Pituitary Lobe

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On treatment with paraformaldehyde, an intensive yellowish-green fluorescence of the rat's intermediate pituitary lobe was noted. Neuropharmacological studies have shown that the cells of the intermediate lobe contained primary catecholamines in high concentration, in the form of small granules. The cellular catecholamines formed with paraformaldehyde a condensate which showed a specific fluorescence, was instable in UV light, resisted reserpine and was not influenced by MAO inhibitors. This would mean that the catecholamines in the pituitary are linked with bonds other than those in the nerve terminals.

On treatment with L-Dopa, the cellular fluorescence increased markedly and the cells incorporated the L-Dopa and transformed it to dopamine. The increase in intensity could be prevented by blocking the decarboxylase activity. This indicates that the cells of the intermediate pituitary lobe contain large amounts of decarboxylase and accordingly play a role not only in the storage, but also in the biosynthesis of catecholamines.

In the area bordering the posterior pituitary, many monoaminergic nerve terminals sensitive to the appropriate drugs were demonstrated. Their close relationship with the cells suggested the role of a monoaminergic mechanism in the intermediate lobe's endocrine function which is the control of the secretion of the melanocyte-stimulating hormone.

RNA Production by Thyroid Epithelium of Normal and Decapitated Chick Embryos

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According to earlier studies, the DNA production of thyroid epithelium is markedly influenced by the onset of pituitary TSH secretion. TSH causes an abrupt decrease of the intense DNA production characteristic of young embryos and simultaneously the secretion of

thyroid hormone begins. The question was to what an extent RNA synthesis followed the change in DNA synthesis, *viz.* how far it was influenced by TSH.

Uptake of ^{3}H cytidine by the thyroid epithelium of normal and decapitated chick embryos of similar age was examined by autoradiography between the 8th and 14th days of incubation. In the case of decapitated embryos, *viz.* those developing without TSH, both RNA nnd DNA synthesis tended to decrease with age. In contrast, in normal embryos, at the onset of TSH secretion on the 11th day, there was a critical fall of ^{3}H-cytidine uptake by the thyroid gland, showing only a minor part of the activity observed in 8-day embryos.

Histochemical and Electron Microscopic Study of Parafollicular "C" Cells in the Rat Thyroid Following Nervous Injury

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Though the parafollicular thyroid cells have been known for several decades, their origin, structure and function have been clarified only recently by electron microscopic, histochemical and fluorescence examinations, pharmacological experiments and clinical and pathological observations. It has been shown that these cells are related to the monoaminergic mechanism and secrete some additional hormones, such as thyrocalcitonine, bradiquinine and protaglandine. An electron microscopic study of the rat thyroid revealed in the parafollicular cells secretion granules which were the counterparts of the fine phase of the argyrophilic granules shown by light microscopy. The electron microscopic counterparts of the coarse phase were residual bodies which showed lipofuscin properties. In the parafollicular thyroid cells and CNS of neurotraumatized rats a moderate increase and coarser granulation of lipofuscin granules was seen. It appears that lesions of this type develop by a similar mechanism in both the central and the peripheral nerve structures.

Ultrastructural Study of the Human Adrenal Medulla

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For the study of the ultrastructural organization of the human adrenal medulla practically only surgical specimens removed from patients suffering from various diseases are available. In the present study, the ultrastructural properties of adrenal medulla from patients with Buerger's disease and inoperable breast cancer were examined. Pre-fixation in glutaraldehyde allowed the differentiation of adrenaline and noradrenaline producing cell types. The adrenaline and noradrenaline granules were found to measure 3000 and 2000 Å in diameter, respectively.

No notable ultrastructural difference was found in the nuclei and cytoplasmic organelles of adrenal medullary cells between patients with Buerger's disease and those with breast cancer. The individual cell organelles showed no pathological alterations. The only conspicuous difference was that in Buerger's disease the cells contained many lysosomes and lipofuscin granules, whereas in breast cancer there was a marked increase in the number of lipid droplets. At the border between adrenal medulla and cortex, special transitional cells of corticomedullary architecture were seen, which contained tubulovesicular mitochondria typical of cortical cells, and catecholamine granules, in adjacent location.

Since no significant difference has been found in the ultrastructural organization of adrenal medulla between patients with Buerger's disease and those with breast cancer, it is assumed, that the submicroscopic appearance of the examined specimens essentially corresponded to that of the normal adrenal medulla.

Secretory Activity of the Adrenomedullary Cell Nuclei in the Rat

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Ultrastructural cytomorphologic studies yielded extensive evidence of the role in secretory function of the different cytoplasmic organelles. However, data are scarce as to the secretory activity of the cell nucleus. In the present studies vigorous adrenaline secretion was induced by excess insulin treatment in albino rats. Prior to the phase of hormone resynthesis 5-fluorouracyl or actinomycin D was given with the aim to disclose an eventual correlation between inhibition of nuclear DNA and RNA synthesis by these drugs and the synthesis of catecholamine by the glandular cells. 5-fluorouracyl treatment was followed by a reduction of nuclear electron density in the phase of resynthesis, whereas actinomycin D treatment induced a segregation of the nucleoli. The disturbance of granulogenesis manifested itself with the appearance of empty secretion granules of low electron density. It is assumed that nuclear DNA and RNA synthesis has a direct influence on intracytoplasmic granulogenesis, through storage and transport of genetic informations.

Ultrastructure of the Adrenal Cortex of Rat Embryos from Hypophysectomized Mothers

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Normally, the endoplasmic reticulum and mitochondria of embryonic adrenal cells tend to transform after the 15th day of foetal life into the vesicular type organelles reminiscent of the zona fasciculata cells of adult animals. Earlier studies by others and ourselves have shown that ACTH accelerates the transformation to vesicular organelles. In the present studies an attempt was made to clarify whether an ACTH deficiency caused by maternal hypophysectomy would influence the ultrastructure of embryonic adrenal cell organelles. Maternal hypophysectomy was performed on the 15th day of pregnancy and the foetal adrenals were processed on the 21st day. It appeared that post-hypophysectomy ACTH-deficiency did not inhibit either qualitatively or quantitatively the vesicular-type transformation of endoplasmic reticulum and mitochondria, but rather enhanced it slightly. This implies that maternal ACTH has no direct influence on the development of the typical ultrastructure of embryonic adrenal cells.

Cycloheximide-induced Ultrastructural Changes in the Rat Adrenal Cortex

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Cycloheximide (CHI) rapidly decreases corticoid synthesis and increases the free cholesterol content of the adrenal cortex. In animals treated with CHI, $\triangle$ 5 pregnenolone and ACTH enhanced the synthesis of corticoids and simultaneously increased the amount of free cholesterol in the adrenal cortex. It was examined whether the biochemical changes following CHI treatment had caused ultrastructural alterations involving the organelles of cortical cells. In the cells of the fasciculate zone, aggregates of granules were seen between the cell organelle which formed often double concentric membranes, or sometimes a net-like structure. Parallel to corticoid synthesis, the endoplasmic reticulum varied in appearance: in the cells of the fasciculate zone of the adrenal cortex from animals treated with CHI alone, only cross-sections of a few vesicles were seen in addition to the granular substance, while on treatment with CHI + ACTH vesicular endoplasmic reticulum, reminiscent of that of the controls, was apparent.

It is believed that the granular substance represented one form of free cholesterol, which may give rise — under the influence of the technical procedure — to thick conspicuous membrane systems.

Carbohydrate Metabolism in the Regenerating Joint Surface

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The early proliferative tissue layer formed on joint surfaces after Krompecher—Puky's semi-joint surgery develops to a cartilaginous tissue. Parallel to this morphologically well defineable change, biochemical changes are also taking place, of which the increase of mucopolysaccharides appears to be the most conspicuous. The mucopolysaccharide components are supposed to form in the course of carbohydrate metabolism. A study of the carbohydrate metabolism of the proliferative tissue showed that in the 70 postoperative days the hexosamine content decreased, whereas O_2 uptake and lactic acid production (glycolytic activity) decreased after an initial rise. However, not all glycolytic enzymes followed the changes of glycolytic activity.

Phosphoglucomutase activity decreased, while phosphohexose-isomerase activity increased. Prior to the 33-day period of gradual avascularization and cartilaginous transformation, phosphofructokinase activity showed first maximum then tended to decrease. Simultaneously, there was a sharp rise of hexosamine-synthetase (hexose-6-phosphate transaminose) activity.

Histochemical and Biochemical Examination of Intact and Regenerating Joint Cartilage Following Prolonged Prednisolone Treatment

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The effect of 70-day prednisolone treatment on intact and regenerating joint cartilages of dogs was examined histochemically and biochemically with special regard to the behaviour of the mucopolysaccharide component.

In the intact cartilage, the treatment did not seem to cause histological and histochemical changes.

Biochemical assay showed no alteration of the total hexosamine content and an about 10% decrease of the galactosamine content. The uronic acid content decreased significantly as compared to the controls, as assessed by Dische's carbazol method, while its decrease was lesser when estimated by Brown's orcinol method.

The effect of prednisolone on cartilage regeneration was examined after Krompecher—Puky's semi-joint operation. In this case the regenerating tissue contained chiefly young cartilage cells of fibrous appearance, and vessels narrower than normally.

In the newly formed cartilage of the articular surface there was an increase in total hexosamine content resulting from an increase of galactosamine, and both methods of assay showed a significant increase of uronic acid content. This implies that under the influence of prednisolone the mucopolysaccharide components behave in a different way in normal and in regenerating joint surfaces.

Total Adrenal Extract Enhancing Vascularization and Ossification

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The vascularization promoting active substance in total adrenal extract has been studied. Extinction coefficients of UV and visible light spectra and absorption maxima of

extinction curves of the extracted aqueous phases indicated the presence of proteins, proteids, nucleotides, lipids, haemoglobin derivatives, and compounds with a porphyrine base. The curve of the infrared absorption spectrum of ether, petroleum benzene, and chloroform extracts of the rest substance indicated the presence of glycerates of various saturated and unsaturated fatty acids, and in the ether extract the presence of lipids. According to the spectrophotometric findings, vascularization of umbilical cord pieces transplanted onto chorioallantoic membrane was best promoted by the test substance's ether extract and its aqueous residue, though even in this case vascularization did not exceed 58 and 53%, respectively, as related to results with the crude extract.

The vascularization promoting action of total adrenal extract may be regarded as a complex effect of the adrenal medulla and cortex, with a thermolabile, non-dialysable large molecular protein-like (enzyme?) substance as its chief component.

Study of the Osteo-hematopoietic Unity

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The interaction between bone and bone marrow has been studied on the basis of the morphological and physiological relationship described by Vereby—Röhlich, and the pathologic and therapeutical relationship described by Krompecher who termed these relations as osteo-haematopoietic unity. On a phylogenetic basis, this unity can be extended also to regeneration and its stimulation.

Studying the regeneration of bone and bone marrow in bone cavities and after fractures, it was found to take place in a phylogenetic sequence. Stimulation of bone regeneration enhanced also the regeneration of the bone marrow. Alteration of bone regeneration by different hormonal and rickets-inducing processes resulted in a pathologic change in the bone marrow. The pathological unity acts also during regeneration. The present experiments have supported the existence of the osteo-haematopoietic unity and its multiple and extensive realization.

A Histochemical Study of Mineralization During Ossification

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The deposition of minerals is an important phase of ossification. The follow-up of this process requires highly sensitive and specific methods. In the present experiments we correlated the results obtained with traditional (Kossa, Gomori, alizarin red S) and new methods (tetracyclin, fluorexon and morine fluorescence; N, N-naphtalyl-hydroxylamine, polarisation optical and chloraniline acid techniques). The specificity of the histochemical procedures was assessed by control tests (^{45}Ca autoradiography in vitro; determination of C and P by chemical analysis). The comparison of the results yielded by the different methods seems to allow a differentiation of the calcification and mineralization processes. In the present study it was used to follow up the postembryonic chondral bone development of the albino rat at 10, 30, 40, 60 and 90 days of age. The deposition of mineral salts was correlated with the changes of the organic matrix (MPSs). The close relationship between mucopolysaccharides and chalk compounds was confirmed by the process of calcification induced in the kidney. Combination of fluorochrome stains in vivo allowed a study of mineralization dynamics under both normal and experimental conditions.

Correlation between Specificity and Chemical Structure in the Histochemistry of Acid Mucopolysaccharides

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The histochemical value of 120 diverse cation dyes has been examined in acid mucopolysaccharide rich tissues (cartilage, mast cells, cornea). The results obtained under identica staining conditions have been compared with regard to specificity. The selectivity of the individual reactions was checked by digestion, blocking, autoradiography and other methods. An attempt was made to demonstrate a correlation between the quality, number and location of auxochromic and chromophoric cation dyes and the histochemical results. Under the given conditions, selectivity depended on the number and position of free auxochromes. The results were confirmed by model experiments, paper chromatography and spectrophotometric measurements. Histochemical specificity could be increased by the selection of fluorochromes with favourable cationic properties; also, it appeared that the histophysical and histochemical relations of fluorescence had advantageously completed each other. Some new histochemical methods are described of which the two-phase fluorescent metachromatic flavophosphin reaction appeared to be most promising, as it allowed the differentiation by UV spectrophotometry of the two acid mucopolysaccharides heparin and chondroitin sulphate.

Immunological and Immunohistochemical Study of Contractile Proteins of the Calf Carotid

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Tonoactomyosin was isolated from the common carotid artery of the calf by Laszt and Hamoir's method. By fractionation of this protein with ammonium sulphate, tonomyosine and F-actin were obtained. The purity and immunological properties of the isolated proteins were examined with the double agar gel diffusion precipitation test, using antisera specific to tonoactomyosin and tonomyosin. It was found that tonoactomyosin contains at least 4, whereas tonomyosin 2 components of antigenic nature. The origin of these components was studied by biochemical and immunological methods. Using antisera labelled with fluorescein isothyocyanate, the intracellular localisation of contractile proteins was examined immunohistochemically. The morphologically different muscle cells of the media contained both tonoactomyosin and tonomyosin.

Serum Mucopolysaccharide Fractions in Experimental Hypo- and Hyperthyroidism in the Rat

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In clinical and experimental studies, Krompecher et al. have demonstrated a close relationship between thyroid function and the serum mucopolysaccharide level. In the relevant experiments chiefly the changes of serum hexosamine level were studied. The dissimilar behaviour of serum mucopolysaccharide fractions has been extensively reported in the literature. The present experiments were performed on 58 albino rats. The hexosamine, glycoprotein, sialic acid and fucose contents of the serum were examined after treatment for 30 and 44 days with thyroxine and methylthiouracyl, respectively. Thyroxine caused a decrease, while methylthiouracyl an increase, in the quantity of all examined fractions.

Influence of Constitutional Factors on the Tensile Strength of Human Skin

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The behaviour of human skin on stretching, its elasticity and tensile strength have been studied primarily from the surgical (Jansen, Rottier, Gibson, Kenedi, Craik), geriatric (Árvai, Takács, Verzár), and pathophysiological (Wöhlisch et al., Wenzel, Rollhauser, Zink) angles. Literary data are, however, scanty and equivocal concerning the resistance to stretching of the skin in the different body regions, in spite of the knowledge of the mechanical properties of human skin being of importance in forensic medicine as they may be conclusive of the force of impacts.

The skin of different body regions (scalp, neck, anterior thorax, cardiac region, abdomen, back, buttocks, upper and lower arm, thigh, legs) from 121 corpses (71 men, 50 women) was examined with an electric tensile meter provided with an automatic recorder. Tensile strength was expressed in terms of kg/1 cm skin width. The results were studied according to age groups, body regions and sex distribution. It was found that the tensile strength of the human skin varies significantly with sex, body region and age. Statistical analysis of the results revealed a correlation between the constitution and the tensile strength of the skin in the different body regions.

Experimental Hypoxia in Rat Skin

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Krompecher et al. have repeatedly described the role of tissue hypoxia in mucopolysaccharide formation. In the present experiments the changes induced in rat skin and serum by abruptly blocking the local capillary supply by freezing have been examined. Freezing was carried out with carbon dioxide ice on the dorsal skin for 2 minutes. The material was processed one half hour, 3 days, 5 days, and 14 days after cold exposure.

According to the findings, the hexosamine contents in serum and tissue reached the maximum on the 3rd day after freezing and fell to close the control value by the 14th day. In further experiments, acid and neutral mucopolysaccharides were isolated quantitatively, from the exposed areas and the percentual distribution of some of their typical components was determined. Consistent results were obtained concerning tissular hexuronic acid, sialic acid and hexose contents as well as serum hexuronic acid, sialic acid, fucose and seromucoid levels. The local hexosamine production of the hypoxic skin was examined in vitro in the Warburg apparatus, under aerobic and anaerobic conditions, with glutamic acid added.

Owing to the disturbance of tissue metabolism, a considerable increase of mucopolysaccharides took place in the impaired skin area as well as in the serum, throughout the period of capillary occlusion.

Development of Elastic Fibre under Functional Strain

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The development of elastic fibres has been examined by light and electron microscopy in the rat embryo, the newborn rat, experimental intimal thickening, organization of arterial grafts, and of surgical meshes.

Electronmicroscopically, the elementary units of elastic fibres were found to be granules 70—90 Å in diameter, with free basic groups. These granules partly formed micro-

filaments, partly aggregates 300—2000 Å in diameter. The aggregates joined to form mature elastic lamellae or, according to the functional requirements, an undulating internal elastic lamina in the developing aorta or in experimental intimal proliferations.

In contrast to intimal thickening, in synthetic grafts a few isolated elastic fibres developed exclusively in the segment close to the host aorta and lumen, where pulsation was still felt. In the case of surgical meshes implanted into the abdominal wall, where neither tissue elasticity is required in that sense as in the aorta, nor the supportive role of elastic fibres is needed, no elastic fibre formation whatever was observed.

Role of Golgi Apparatus in Mucopolysaccharide Synthesis

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The precise role of the Golgi apparatus is still unclear. Many findings suggest secretion of lipid (Palay and Bourne), protein (Ferquhar and Welleings, Helander), mucoid and mucoprotein (Petersen and Leblond) by the Golgi complex. Recently, various enzymes such as alkaline and acid phosphatase, thiamine pirophosphatase, etc. have been demonstrated in the Golgi systems of several organs from diverse animal species.

Comparative examinations have been carried out using methods suitable for the demonstration of the Golgi apparatus in cartilage, salivary gland, adrenal, thyroid gland, nerve tissue of the albino rat. The types of Golgi complex typical of certain organs or tissues were correlated with the results of protein, lipid, carbohydrate and enzyme histochemical reactions in the appropriate cells. Results obtained in normal organs were compared to those found in the Golgi systems of cells with altered secretory activity. Evaluation was made with special regard to the relationship between mucopolysaccharides and Golgi apparatus. Based on these and earlier findings, a role in mucopolysaccharide synthesis is ascribed to the Golgi complex.

Cytochrome Oxidase Activity of Various Parts of the Nervous System in the Dog

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It is known that in animals of low order, cytochrome oxidase activity of the different tissues is low owing to the low oxygen supply and the poorly differentiated circulatory and respiratory organs.

Cytochrome oxidase activity was studied in various parts of the nervous system of dogs, using the modified Pearl—Cascarano—Zweifach method. Those parts of the nervous system which occur also in low animals had a low cytochrome oxidase activity, whereas those which are related to higher stages of phylogenetic development showed a gradual increase of the enzyme activity.

Enzyme Histochemical Processes in Ganglial Cells of the River Mussel (Unio pictorum)

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The lipofuscin of ganglial cells from river mussels of various ages was examined with different methods for the presence of hydrolases (lipase, non-specific esterase, alkalin phosphatase, acid phosphatase, 5'nucleotidase, cathepsin, ATPase) and oxidases (succinic dehydrogenase, monoaminoxidase, $NADH_2$ diaphorase, cytochrome oxidase).

Since lipofuscin is a coloured substance with a complex structure, the methods of its enzyme histochemical and light microscopic examination have to be chosen and evaluated with precaution.

Of the enzymes listed above, a positive reaction was obtained for non-specific esterase, acid phosphatase, cathepsin, succinic dehydrogenase, cytochrome oxidase, $NADH_2$ diaphorase. The reaction products were localized in the form of granules on the surface of the lipofuscin, or surrounded it in the form of a letter C or less often O.

The presence of the enzymes was noted in more granules in young than in old animals, but even in the former case not all the granules contained enzyme.

These localizations of enzyme reaction products seem to support the view that the lipofuscin granules consist of a cortical and a medullary part, the former containing enzymes and other substances, the latter chiefly neutral fat. The results suggest that mitochondria and other cell organelles play a role in lipofuscin formation. Their development takes place through the "segregosoma"-like state described by Ericsson et al.

Pre- and Postnatal Cholinesterase Activity in the Chick and Rat Heart

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Cholinesterase activity in the heart from chick and rat embryos and newborn and older rats has been examined histochemically. In both species, myocardial cholinesterase activity was demonstrable prior to the entry of the vagal nerve. Subsequently, the activity tended to increase rapidly, then during ontogenesis to decrease. This reduction was inconspicuous when examined on successive days, whereas it was conspicuous when examined at greater intervals. The decrease of enzyme activity went parallel with the morphological differentiation of the cells, *viz.* with the gradual cessation of their activity. Spontaneous activity and cholinesterase activity of young myocardial cells seems to be related. The supposed quantitative relationships of heart acetylcholine content during embryonic development are discussed.

Histochemical and Electron Microscopic Study of Intestinal Epithelium from Young Ruminants, with Special Regard to Protein Absorption

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Epithelial cells from the intestinal segments of dwarf Kameron goats were examined for morphological changes occurring during the absorption of colostral proteins, *viz.* intact immune material.

Comparing the intestines of lactated newborn goats to those of adult or colostrum-deprived newborn animals, the former had in the jejunal and ileal epithelium eosinophilic, PAS, ninhydrin and Schiff positive droplets varying in size and filling the entire cell. At the margin of the droplets a pronounced annular alkaline phosphatase reaction and a lack of acid phosphatase activity was demonstrated. Electron microscopically, many pinocytotic vesicles were seen among the microvilli, with cloud-like electron dense contents. In the apical parts of the cells vesicles different in size were seen, with contents of varying density but otherwise resembling the contents of pinocytotic vesicles. The vesicular contents entered the intercellular space by reversed pinocytosis at the cell base which appeared markedly broadened as compared to the controls. After the third day the pinocytotic activity as well as the number of vesicles had considerably decreased, then disappeared; the width of the intercellular space became normal and the ultrastructure of the epithelial cells corresponded to that of the adult animals.

The findings indicate that colostral protein uptake by pinocytosis takes place also in ruminants; during their passage through the cell the colostral proteins are enveloped by a membrane. This circumstance may play a role in macromolecular absorption and thus in the passive immunity of the newborn.

Electron Microscopic Observations on Capillary Innervation

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For the investigations tissue samples obtained from the jejunum and ileum of mice were utilized after fixation in glutaraldehyde/OsO_4 or in OsO_4, and following embedding in Vestopal W or Epon. In the lamina propria mucosae blood capillaries were frequently observed. In the neighbourhood often one or sometimes several unmyelinated nerves occurred. The axons forming such a nerve are obviously varying in number. Part of these axons containing numerous vesicles is dilated. The dilatations are frequently localized in that part of the unmyelinated nerve which is turned towards the capillary. The distance of these vesicular segments of the axons from the pericytes and endothelial cells was measured on electron micrographs. The results are discussed with regard to capillary innervation.

Development of the Paneth Cell Population in the Small Intestine of Young Mice

I. Möller

(Institute of Anatomy, Friedrich-Schiller-University, Jena)

The small intestine of mice, from one to twenty days old, has been investigated. The material was analysed qualitatively and statistically, taking into consideration the ratio of Paneth cells to intermedial forms in each part of the small intestine. The experiments have confirmed the observation that the development of the gut occurs from proximal to caudal. There are many intermediate forms in the first few days, whereas the number of Paneth's cells is still relatively small. During the second week, the Paneth cells increase in number, the intermediate forms begin to decrease at the end of the second week. The third week shows a further decrease of intermediate forms with a corresponding increase of Paneth's cells, until the number normal for adult mice has been reached.

The various possibilities of interpreting the results, concerning the development and origin of intermediate forms and Paneth's cells, are discussed.

Morphodynamics of the Cerebral Epiphysis in the Case of Stress

V. Devečerski and **N. Šijački**

(Institute of Histology and Embryology, Novi Sad)

Histochemical changes of the rat pineal gland were studied with special reference to the lipid content of the pinealocytes. The animals were subjected to 24-hour immobilisation during which they were deprived of food and water.

Using the combination of different methods, hypertrophy of pinealocytes and their nuclei, clear differentiation of bright and dark cells as well as the abundance of floxinophilic granules in the pinealocyte nuclei have been observed in the experimental animals.

Sudan black B reaction was less marked in the animals subjected to acute stress. The sudanophilic substance was less than in the controls and occurred in the form of dispersed bright granules of uneven size.

Results were quite different with Sudan III. The reaction as a whole was definitely expressed, and the sudanophilic substance was present in larger amounts in the whole intracellular space where the granule-like and larger formations of uneven colour intensity are dominant.

The Oil Red 0 reaction was more expressed in the experimental rats where dark red and red formations were observed in increased numbers.

Phosphin 3R revealed a considerably wider fluorescent zone of uneven intensity in the experimental than in the control animals.

Non-specific esterase activity was more expressed in the experimental animals, especially in the vicinity of the capillary vessels.

The changes found are of a progressive character. Hypertrophy of the glandular cells and their nuclei supports their stimulated activity. The decrease in the quantity of material after the application of Sudan black B points to the mobilisation of certain lipid classes, according to the supposition of Lison and other workers, especially of the phospholipids. Both this finding and the increased activity of non-specific esterases suggest that in the case of stress the pineal gland releases large amounts of its product from the complex lipid classes.

Assuming that the neutral fats are identified with Sudan III and triglycerides with Oil Red, it may be concluded that the reaction is a reflection of the special phase of the complex lipid metabolism in the epiphyseal glandular cells.

The above results are considered to support the hypothesis that in addition to melatonins the epiphysis elaborates some other active substances of complex chemical structure and biological activity.

Functional Anatomy of Corneal Degeneration

Ildikó Süveges

(Department of Ophthalmology, University Medical School, Debrecen)

Corneal degenerations, particularly its hereditary forms, occur infrequently. Accordingly opportunities for their histological examination are rare and mostly limited to specimens excised during keratoplasty. In the present studies reticular, nodular, spotty familiar degenerations of the cornea and keratoconus were examined histologically. An interesting feature of these types of corneal degeneration is that the partial or complete discoloration of the cornea takes place without vascularisation.

In addition to usual histological methods, carbohydrate assays were also done.

It was found that in reticular, nodular and spotty degeneration, the degeneration product was similar, differing only in localisation; in the reticular form it occurred in the upper third of the parenchyma, in the nodular form beneath the epithelium, and in the spotty form throughout the parenchyma. Accordingly, the reticular and nodular forms call for a lamellar, while the spotty form calls for a perforating keratoplasty.

Tensile Strength of Abdominal Aponeuroses

B. Somogyi, Flora Undi and **M. Kausz**

(Institute of Histology and Embryology, University Medical School, Budapest)

Aponeuroses of the anterolateral abdominal region were tested for resistance to tension. In 280 specimens obtained from fresh cadavers, lengthening was determined in absolute per cent and tensile strength in kg.

The strength of the sheets was greater in the direction of the descent of aponeurotic fibres than in the opposite direction. The linea alba and the rectus sheet were stronger transversally than longitudinally and higher values were obtained above than below the Douglas line. The anterior rectus wall was three times stronger than the posterior on longitudinal stretching, while they behaved identically on transversal stretching. The iliotibial tract was considered an indicator of individual aponeurotic strength; if its tensile strength was above the average 50 kg the tensile strength of the abdominal aponeuroses was also higher. With age, the resistance of abdominal aponeuroses seemed to decrease. Above 70 years of age, the resistance of the iliotibial tract was found to have decreased on the average by 33%, that of the supraumbilical linea by 21%, implying that the intricate tissular fibre system is less liable to lose tensile strength.

It has been concluded that a strong transversal stretching force acts above the Douglas line. Accordingly, surgery in that region should be done from a transversal rather than a longitudinal abdominal incision.

Experimental Disturbances of Carbohydrate Metabolism and Teratogenesis

Cecília Horváth

(Institute of Histology and Embryology, University Medical School, Budapest)

To study the disturbances of carbohydrate metabolism induced by teratogenic substances, the effect of phenothiazine derivates on the morphogenesis of chick and rat embryos was examined. Pharmacologically, the teratogenic properties of phenothiazine derivatives consist in the blocking of oxidative phosphorylation, a reduction of the FAD level, alteration of cell membrane permeability and inhibition of the activity of nicotine amide methylferase.

Chick embryos in different stages of development were treated with various doses of chlorpromazine. 0.5 mg of chlorpromazine given after 48 or 72 hours of incubation, or 1—2 mg given after 96 hours of incubation resulted in the lack or abnormal development of an extremity.

Pregnant Wistar rats were treated with chlorpromazine on the 9th, 10th, 11th and 12th day of pregnancy. Depending on the time and dose of treatment, part of the embryos died, while the survivors showed disturbances of bone development. Abnormalities were restricted chiefly to vertebral ossification, to the occipital bone and the sternum.

The results are indicative of a correlation between the pharmacological effects of phenothiazine derivatives and their toxic or teratogenic effects on embryos.

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