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Tomus XIV

Akadémiai Kiadó, Budapest
1966

ACTA MORPHOLOGICA

Tomus XIV

INDEX

INDEX AUTORUM

Acta Morphologica Academiae Scientiarum Hungaricae Tomus 14 (1), pp. 1—6 (1966)

First Department of Gynaecology (Director: Prof. B. Horn), University Medical School, Budapest

HEPATIC AND RENAL CHANGES INDUCED BY SYNTHETIC PROGESTOGENS AND NATURAL PROGESTERONES

F. Tóth

(Received May 15, 1964)

Two hundred female rats of 110—130 g body weight have been treated with progesterone and synthetic progestogens. Normal doses induced no changes in the internal organs. Large doses induced diffuse fatty degeneration in liver and kidney; the lesion was reversible and disappeared after 6 to 7 days.

Progesterone, the most frequently used female sex hormone, was prescribed in two and a quarter million instances in the United States in 1960, and its sale has since increased. The prevention of threatened abortions has much contributed to this increase. According to widerange statistics, progesterone preparations are employed in 45 per cent of threatened abortions. Besides, progesterone is routinely used for the treatment of genital hypoplasia and the disturbances of menstruation due to relative and absolute progesterone deficiency. The popularity of progesterone preparations and their administration in large doses was promoted by the fact that no side effects had been reported. The natural substance, if administered orally, is practically without effect; in view of the difficulties involved by the parenteral administration of progesterone in oil, numerous attempts have been made to produce efficacious oral progestogen preparations. Synthetic products of this kind, marketed in increasing numbers during the last 6 to 7 years, have been satisfactorily applied in the treatment of diverse menorrhoeal disturbances, genital hypoplasia, dysmenorrhoea, endometriosis and in cases of threatened abortion. They have moreover proved highly useful as biological contraceptives. Alarming reports have been published in recent years regarding the effect of high doses of synthetic progestogens prescribed for the prevention of abortions. Administered in the first trimester of gestation, they are stated to have induced foetal masculinization in more than a hundred cases. Dietel [1], by administering 10 mg of progesterone in oil daily for 8 days, induced grave focal hepatic necrosis in pregnant rats.

Appropriate doses of both natural progesterone and synthetic progestogen preparations are frequently administered in this Department for the treatment of the said disorders, and the animal experiments reported in the following were devised in order to supplement our clinical observations with the morphological examination of parenchymal organs.

Material and method

Two hundred white female rats with body weights between 110 and 130 g were divided into 20 equal groups. Three of these groups contained animals in the second half of pregnancy. The rats received the same drugs as employed in clinical practice, namely natural progesterones No. 1, No. 2, and No. 3., further tablets of synthetic progestogens No. 1, No. 2, and No. 3. Natural progesterone No. 3 contains 10 mg progesterone, natural progesterone, No. 2 injection contains 125 and 250 mg of 17-α-methyl-19-nortestosterone, synthetic progestogen No. 2 contains 17-α-ethynyl-17-β-hydroxy oestr-4-en + 0.15 mg methoxy-ethynyl oestradiol, synthetic progestogen No. 3 contains 5 mg of Δ-4-17-α-allyl-(o)-oestren-β-ol. Dosage of the drugs and the consequent hepatic and renal lesions are shown in Table I.

Table I

Effect of progesterone preparations on liver and kidney

Group No	Drug	Mode of treatment mg/100 g	Hepatic lesion	Renal lesion	Notes
1.	Synthetic progestogen (No. 1.)	2 × 0.10 mg weekly for 15 weeks			
2.	Synthetic progestogen (No. 1.)	0.10 mg daily for 60 days			
3.	Synthetic progestogen (No. 2.)	2 × 0.10 mg weekly for 15 weeks			
4.	Synthetic progestogen (No. 2.)	0.10 mg daily for 60 days			
5.	Synthetic progestogen (No. 3.)	2 × 0.10 mg weekly for 15 weeks			
6.	Synthetic progestogen (No. 3.)	0.10 mg daily for 60 days			
7.	Natural progesterone (No. 1.)	0.12 mg daily for 10 days			
8.	Natural progesterone (No. 1.)	2.0 mg daily for 10 days	Lipoid in Kupffer cells		
9.	Natural progesterone (No. 1.)	2.0 mg daily for 10 days	Parench. degener. Lipoid in Kupffer cells	Parench. degener.	Pregnant animals
10.	Natural progesterone (No. 1.)	6.0 mg daily for 10 days	Fatty degener.	Fatty degener.	Pregnant animals

Group No	Drug	Mode of treatment mg/100 g	Hepatic lesion	Renal lesion	Notes
11.	Natural progesterone (No. 1.)	6.0 mg daily for 10 days	Fatty degener.	Fatty degener.	
12.	Natural progesterone (No. 2.)	0.25 mg daily for 10 days			
13.	Natural progesterone (No. 2.)	5.0 mg daily for 10 days	Fatty degener.	Fatty degener.	Pregnant animals
14.	Natural progesterone (No. 2.)	5.0 mg daily for 10 days	Fatty degener.	Fatty degener.	
15.	Natural progesterone (No. 3.)	0.2 mg daily for 10 days			
16.	Natural progesterone (No. 3.)	2.0 mg daily for 10 days	Lipoid in Kupffer cells		
17.	Natural progesterone (No. 3.)	2.0 mg daily for 10 days	Parench. degener. Lipoid in Kupffer cells	Parench. degener.	Pregnant animals
18.	Natural progesterone (No. 3.)	5.0 mg daily for 10 days	Fatty degener.	Fatty degener.	Pregnant animals
19.	Natural progesterone (No. 3.)	5.0 mg daily for 10 days	Fatty degener.	Fatty degener.	Killed at different times
20.	Olive oil	0.1 ml. daily for 10 days	Fat in Kupffer cells.		

The tablets, dissolved in milk, were introduced through a gastric tube; the injections were given intramuscularly. Doses indicated in Table I. refer to 100 g body weight and were given daily. The animals received about the two- to fourfold of the doses of synthetic progestogen employed in clinical practice. The smaller dose of progesterone in oil was approximately the same as usual in cases of threatened abortion; the higher doses are not applied in clinical practice and have a theoretical significance only. If the doses received by the animals are referred to a human subject of 60 kg body weight, the following values result: the single dose of synthetic progestogens No. 1, No. 2, No. 3 corresponds to 60 mg; 0.12 mg of natural progesterone No. 1 to 72 mg, 2 mg to 1.2 g, 6 mg to 3.6 g, 12.5 mg to 7.5 g; 0.25 mg of natural progesterone No. 2 to 150 mg, 5 mg to 3 g, 0.2 mg of natural progesterone No. 3 to 120 mg, 2 mg to 1.2 g, 5 mg to 3 g.

With the exception of Group No. 19, the animals were killed by a blow on the nape at the termination of the experiment, their organs were removed, fixed, and then stained with haematoxylin-eosin, oil red, PAS, methylgreen pyronine, subjected to the Feulgen, Schultz and phenylhydrazine reactions; alkaline and acid phosphatase and non-specific esterase reactions with azo dyes were performed by the "Azo-Kupplung" method with naphthol-As-phosphate substrate.

Results

The applied doses of synthetic progestogens induced no change in the examined organs, neither did 0.12 mg of natural progesterone No. 1, 0.2 mg of natural progesterone No. 3, or 1.25 mg of natural progesterone No. 2. Two mg of natural progesterone No. 2. and natural progesterone No. 3 daily for 10 days induced parenchymal degeneration in the liver and kidney of pregnant animals. The Kupffer cells contained a great number of birefringent lipoid granules. No degeneration was induced by these doses in nonpregnant animals, although the Kupffer cells contained lipoids (Fig. 1). Six mg natural progesterone No. 1, 5 mg natural progesterone No. 3 and 5 mg natural progesterone No. 2 administered daily for 10 days, induced grave fatty degeneration in the liver and kidney of both pregnant and nonpregnant animals. The hepatic lesion was diffuse, mostly centrilobular, but appeared sometimes also at the periphery of the lobules (Fig. 2). In the kidney it was mostly in Henle's loops (Fig. 3) and sometimes in the basal part of the epithelium of the proximal convoluted tubules that fat deposits were found. They were birefringent and gave a positive Schultz and phenylhydrazine reaction. No lipoids were observed in lungs and heart. Administration of olive oil induced no degenerative changes in the control animals, only the Kupffer cells contained finely dispersed oil droplets. Small doses gave rise to intensive alkaline and acid phosphatase and PAS reactions in both the liver and the kidney (Fig. 4); with large doses the reactions were weaker (Fig. 5).

The animals of Group No. 19 were killed at stated intervals after termination of the treatment. The degenerative lesions were found to have disappeared, and the phosphatase and PAS reactions to have returned to normal after 6 to 7 days (Fig. 6).

Five animals (not included in Table I) received 10 mg of natural progesterone but even these doses failed to elicit the hepatic necrosis described by DIETEL. There occurred no intrauterine necrosis or pathologic foetal change. In another group of experiments the administration of 4 times 2.5 mg of oestrogen (without progesterone) to pregnant rats elicited all the phenomena described by DIETEL, namely hepatic necrosis, intrauterine foetal necrosis and partial resorption of the ova.

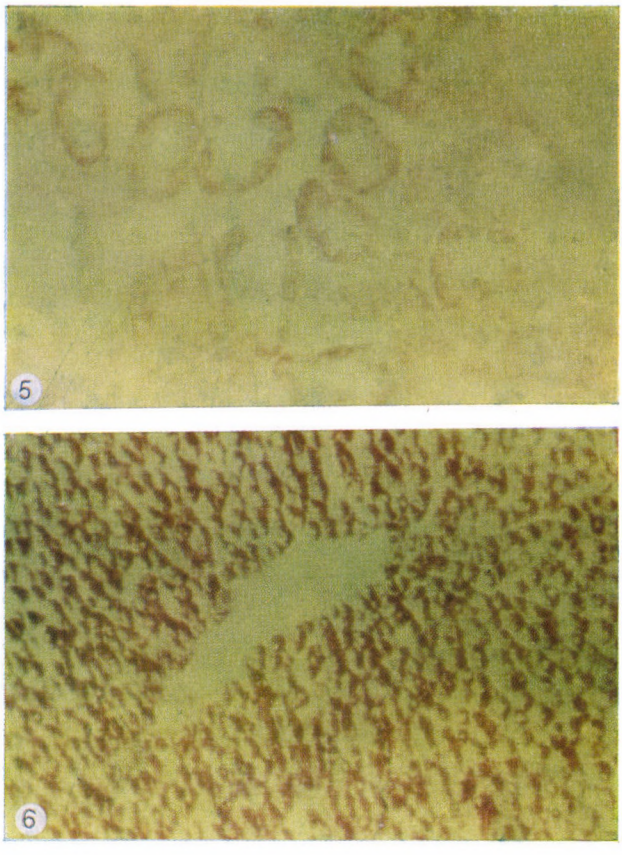

Fig. 5. Weak alkaline phosphatase reaction in the kidney. "Azo-Kupplung" method with naphthol-As-phosphate substrate, × 128
Fig. 6. Great amounts of finely dispersed glycogen in liver cells. PAS, × 80

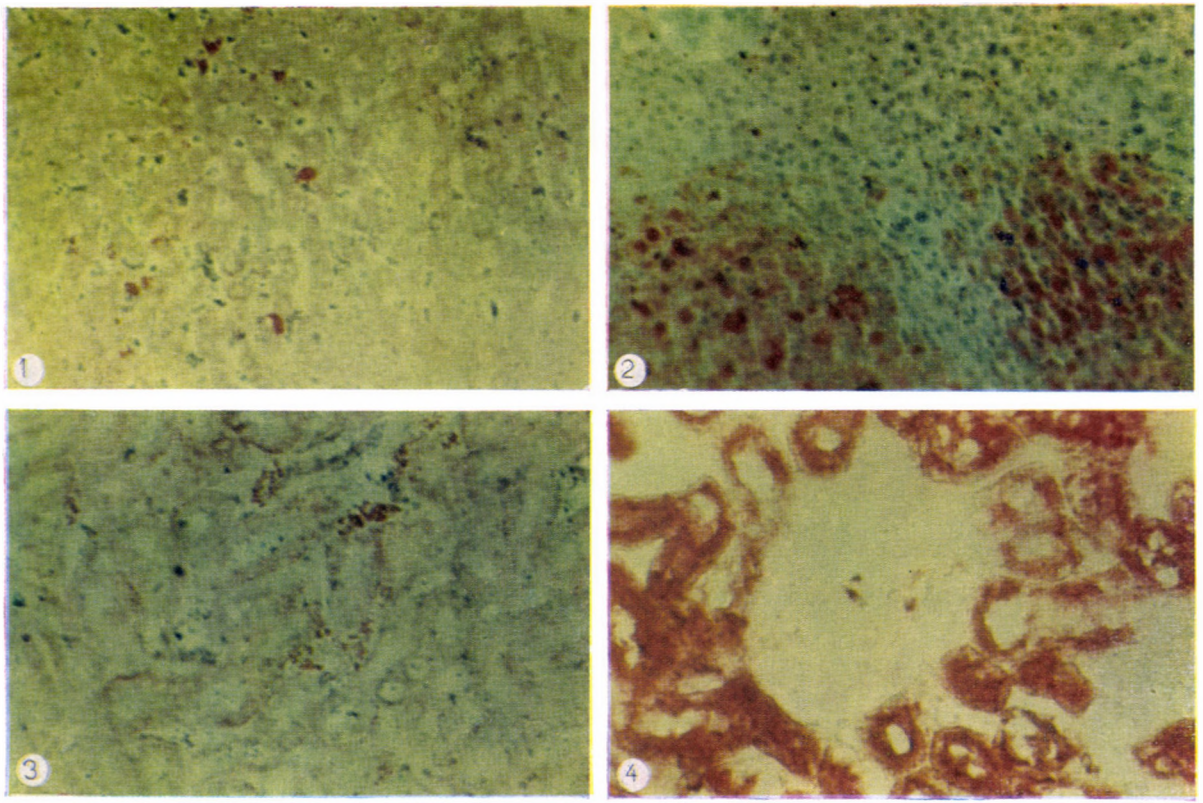

Fig. 1. Kupffer cells filled with birefringent lipoid. "Fettrot" staining, × 128; *Fig. 2.* Dark spots, indicating fatty degeneration in the liver. Oil red staining, × 128; *Fig. 3.* Dark spherules representing lipides, in epithelium of Henle's loops. Oil red staining, × 128; *Fig. 4.* Increased alkaline phosphatase reaction in the kidney. "Azo-Kupplung" method with naphthol-As-phosphate substrate, × 128

Discussion

Literature contains hardly any report on the side effects of progesterone. The gravest effects have been observed by DIETEL [1] in pregnant rats. In nonpregnant animals the lesions were less serious. Therapeutic doses of natural progesterones induced no degeneration in the internal organs, while the alkaline and acid phosphatase reactions became more pronounced in the liver and the kidney. Large doses induced fatty degeneration in these two organs. Histochemical reactions showed that the major part of the lipoids were steroid hormones. These results are in good agreement with the known fact that the liver is the chief organ of steroid conversion, and that the greatest part of progesterone is excreted with urine in the form of pregnanediol. SANDBERG and SLAUNWHITE [3] injected 14 C-labelled progesterone intravenously and demonstrated that, linked to glucuronic acid, only 50 per cent thereof was excreted after 24 to 48 hrs. Approximately 30 per cent passed into the bile without being bound by glucuronic acid and gained then access to the intestines whence it was partly absorbed and partly excreted. The process is known as hepatoenteral hormone circulation. Small doses are broken down and excreted by hepatic and renal activity, the intensification of which is indicated by a pronounced phosphatase reaction. Large doses may, however, exhaust the available stock of enzymes so that the hormone accumulates in the hepatic and renal cells. We have found that nonpregnant animals are able to convert and excrete larger amounts of exogenous hormone than pregnant ones without developing grave hepatic and renal degeneration. This may be due to the fact that conversion of already present steroids taxes the liver and kidney of pregnant animals to such an extent as to be unable to cope with freshly introduced doses of hormone. Nonpregnant animals are, on the other hand, capable of converting high exogenous doses of hormone without degenerative lesions. DIETEL did not discuss the problem as to whether the oil in which the hormone is dissolved, played a role in the observed changes. Observations made in connection with Group No. 19 and the progesterone suspension show that the hormone alone and not the solvent is responsible for the pathologic changes. Hepatic and renal lesions caused by different doses of oestrogens will be discussed in another paper. It should, however, be noted that they are capable of inducing fatty degeneration in considerably lower doses than progesterone. This is in contradiction to the observations of KOVÁCS and DÁVID [2] who found no significant change in the kidney of female rats which had received 1.0 mg of oestrogen daily for 10 days. On the other hand, extensive renal cortical necrosis resulted if the oestrogen was administered in combination with posterior pituitary preparations. The present experiments have reliably shown that not even the larger of the current doses of progesterone and progesteroid preparations causes degenerative lesions in the internal organs of animals.

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AUF WIRKUNG DER GESTAGENE ZUSTANDEKOMMENDE LEBER- UND NIERENVERÄNDERUNGEN

F. TÓTH

Die Untersuchungen wurden bei 200, 110—130 g wiegenden weiblichen Ratten vorgenommen. Die in der üblichen Dosierung verabfolgten Progesteron- und Progesteroidpräparate verursachten in den inneren Organen der Versuchstiere keine degenerativen Veränderungen. Das in großen Dosen verabreichte Progesteron führte diffuse, jedoch reversible, fettige Leber- und Nierendegeneration herbei, die innerhalb 6—7 Tagen spurlos verschwand.

ИЗМЕНЕНИЯ В ПЕЧЕНИ И В ПОЧКАХ ПОД ВЛИЯНИЕМ ГЕСТАГЕНОВ

Ф. ТОТ

Автор проводил исследования на 200 крысах-самках весом в 110—130 г. По его наблюдению при применяемой на практике дозировке прогестероны и прогестероиды не вызывают дегенеративных изменений в внутренних органах подопытных животных. Применяемый в больших дозах прогестерон вызывает диффузное жировое перерождение печени и почек, которое обратимое и за 6—7 дней бесследно исчезает.

Dr. Ferenc TÓTH: Budapest VIII. Baross u. 27., Hungary

Acta Morphologica Academiae Scientiarum Hungaricae Tomus 14 (1), pp. 7—24 (1966)

Second Department of Surgery (Director: Prof. T. KARLINGER) and Department of Anatomy (Director: Prof. J. SZENTÁGOTHAI), University Medical School, Pécs

PRESENT STATE OF TENDON REGENERATION. LIGHT AND ELECTRON MICROSCOPIC STUDIES OF THE REGENERATING TENDON OF THE RAT

A. SALAMON and J. HÁMORI

(Received May 20, 1964)

After a brief review of the literature on tendon regeneration a report is given of light microscopic, histochemical and electron microscopic studies of the regeneration process of the cut rat tendon.

In contrast to some data in the literature it has been found that the pluripotent immature mesenchymal cells capable of differentiation and present in the surrounding connective tissue as well as in the tendon stumps, have a common role in the formation of regenerating tissue between the tendon stumps.

Synthesis of collagen fibers is performed by the fibroblasts (tenoblasts); the mechanism of this process is still a problem wich we can as yet only approach with our actual investigating methods. The fibroblasts of the surrounding scar tissue are distributed in longitudinal orientation on the fibrin net, as well as the precollagenous substances secreted by the fibroblasts in the tendon stumps. The collagen fibrils appearing on and around the cells, thickening gradually and oriented longitudinally under the effect of functional factors in the first place are responsible for the formation of the new tendon structure. Even in the case of smooth healing microstructural changes (cell activity, thick collagen fibers, metachromasia) persist for a long time, and the new structure will never be quite analogous with that of a normal tendon. Reorganization of the original structure — if at all possible — probably takes a much longer time.

Tendon, being a tissue particulary poor in cells and rich in fibers, differs essentially from the other tissues. Its blood supply is restricted, its metabolism is low (PEACOCK, 24). Beside these morphological and physiological peculiarities the tendon possesses a specific function which is difficult to restore in case of injury. Between the tendon stumps, the tendons and the surrounding tissues, scar tissue frequently develops, causing serious functional damage.

The chain of events during the course of regeneration of the damaged tendon was repeatedly studied in the last decades and a vast literature has been published on this subject. Most authors seem to hold that the cells of the connective tissue surrounding the tendons are mainly responsible for the development of regenerating tissues between the tendon stumps, i.e. that the injured part of the tendon is replaced mainly by the proliferation of cells of the paratenon or the injured tendon sheath. According to them the cells of the tendon stumps play a passive part in the course of regeneration, they have no role in the formation of the "tendon callus" (HAUCK, 12, LANGE, 17, BUCK, 4, SKOOG and PERSSON, 30, DAVIDSSON, 5, POTENZA, 26).

A view which seems inadmissible is that of IMAYOSHI (13), according to whom the healing of tendon defects would be due solely to the proliferation of

the tendon cells. In his opinion tendon cells would never arise from other connective tissue cells or by way of metaplasia from other cells. A similar role of tendon cells was maintained by MIGLIAVACCA.

Another conclusion is that the surrounding proliferating cells as well as the cells in the tendon have an equal part in the regeneration, independently of the degree of maturity of the latter (ENDERLEN, 6, BORST, 3, SEGGEL, 28). In this connection we quote MASON and SHEARON's (20) conclusion: "From this study it seems logical to assume that these two tissues (connective tissues and tendon tissue itself) have each a definite role or function in the healing process." The recent papers of LINDSAY and THOMPSON (18) and FLYNN and GRAHAM (8) share this opinion.

As in every regenerative process, it is evident that in the tendon regeneration, too, a decisive role has to be attributed to the so-called indifferent mesenchymal basic tissue. This applies first of all to cells which remain in their embryonic condition, the undifferentiated cells. These immature mesenchymal cells are ubiquitary, they are found in the whole organism as "medium" tissues capable of pluripotent differentation. This means that the cells originating from the mesenchyma in the course of embryonic evolution may develop into various cells, i.e. types of tissues during the process of regeneration. In the extensive literature concerning tendon regeneration we hardly find any such data, with the exception of some references in the papers of PEACOCK and of LINDSAY and THOMPSON. This important role of the mesenchymal basic tissue in other fields of the regenerative process has been emphasized by several authors; its importance in bone callus formation has been discussed by KROMPECHER (16), BLOCK (2), FROMME (7), KARLINGER (14) and confirmed by HARANGHY (11).

In close relation to this question stands the problem of the origin, synthesis, growth and orientation of the collagen fibers. As a tissue rich in collagen fibers, the tendon constitutes an excellent model for such investigations. Based on the promising results of the last decades the study of submicroscopic structures has allowed recent investigations to go a step further. Today the majority of workers seems to agree that collagen synthesis is a function of the fibroblasts and not of the intercellular substance. WASSERMANN (31) found in the regenerating rat tendons that the collagen fibers are secreted by the fibroblasts. Other workers made similar morphologic and biologic studies in regenerative connective tissues and tissue cultures. The electron microscopic observations of WASSERMANN and later of GIESEKING (9) revealed filaments of around 100 Å in diameter in the fibroblasts; others hold that only precollagenous substances can be formed in the cell plasma — ("tropocollagenous molecules") — and their composition into collagen fibrils is an extracellular process [GROSS (10), YARDLEY et al. (32), MERKER (21), SCHWARZ et al. (29)]. PORTER (25) observed that the fibrils developed on the protoplasmic membrane of the

fibroblast. The orientation of the newly formed collagen fibers, in other words, their union between the stump and the newly formed tissue, further the factors influencing this process are problems still discussed. Some authors believe that in the early stages of regeneration there is no specific orientation and that the longitudinal arrangement of the cellular and fibrous elements occurs only as a reaction to the function. BUCK (4) on the other hand, observed the longitudinal "stress line" orientation of these elements even with fibroblasts appearing on the very first days.

The metachromatic staining of regenerating tissue is a well-known phenomenon. MEYER and RAPPORT (22) stated that even the injured tendon contains some hyaluronic acid and somewhat more chondroitin sulphate, consequently it shows some slight metachromatic staining. In the regenerating tendon this phenomenon persists for a surprisingly long time. According to *Buck*'s data, metachromasia is still present a year after the tenotomy, although in the healing skin it soon disappears.

The above discussed controversial data have made us to study the role in the regeneration process of the cellular elements of the tendon stump and the surrounding tissues, further the formation, secretion and orientation of the collagen fibers.

Method

Sixty adult white rats were used in the experiments. The animals were kept under similar conditions and received the same diet throughout the experiments. Under ether anaesthesia and aseptic conditions a small incision was made on one of the hind limbs through which the Achilles tendon was exposed, cut transversely (Fig. 1). As a result a gap of 4—5 mm had formed between the tendon ends, due to the retraction of the proximal parts. After closing the skin wound, the legs were immobilized for 2 weeks in plaster casts, unless specimens were

Fig. 1. Schematic representation of conditions after division of tendon. The dark square fields mark the place where sections were excised for electron microscopic investigation

required for earlier studies. This simple operative technique (tendon division without suture) was chosen in order to spare as much as possible the tendon ends, as with the usual tendon suture the tissue reaction around the thread may influence the pure regenerative conditions. The animals were sacrificed at various intervals, the hind legs were fixed in formalin and allowed to harden in the deep freeze, then the Achilles tendon was removed together with the newly formed tissues. The shortest observation time was 3 days, the longest 100 days after operation. For light microscopic studies the histologic sections were stained with haematoxylin-eosin and van Gieson's stain. To demonstrate acid mucopolysaccharides, pH 4,6 toluidine blue was used.

Electron microscopic studies were made on 1 sq. mm, portions of the proximal tendon stump and the newly formed tissues, each excised from 3 animals on the 7th, 21th, and 56th days after tenotomy. The specimens were fixed for two hours in a 1 per cent solution of osmium tetroxide buffered to pH 7,4 at +4° C, then embedded in araldite according to LUFT (19). Contrasting was done in blocks and it was stained with a 1 per cent solution of phosphotungstic acid in alcohol for 60 minutes. Ultrathin sections were cut by an LKB Ultratome and the sections were examined by Zeiss El.-Mi. D 2 (Jena) or Tesla table electron microscopes.

Results

Gross examination. On the third day after tenotomy a very soft vascular tissue is seen between the tendon ends. By the end of the second week the tissue is decidedly stronger and holds the tendon ends loosely together. The newly formed tissue, which is of a dull greyish colour, continues to strenghten through the third and fourth weeks. Its close connection with the environment lessens, only loose tissues surround it. Still later there is hardly any difference from the normal tendon.

Light microscopy. On the third day after tenotomy marked cell proliferation is seen around the tendon ends. Between the stumps, among the precipitated fibrin threads there are erythrocytes, white blood cells and fibroblasts. The tendon ends are in a resting state. After 7 days the picture changes considerably, the fibroblasts arising from the paratenon on the newly formed fibrin net are oriented, more or less longitudinally, there are many capillaries, but less white blood cells. In the peripheral parts of the tendon stumps there appear swollen cells with ovoid or round nuclei rich in chromatin and with basophilic granules in the cytoplasm. Vacuoles are present in most tendon cells in the centre of the stumps, the nuclei are pycnotic and shifted aside. At the end of the second week the fibroblasts in the gap between the tendon ends continue to multiply, their arrangement is in general longitudinal with fine collagenous fibers between them. The newly formed tissue is still hyperaemic, the connection of the tendon ends with their surroundings is still strong. Cell activity in the stumps is invariably present.

In three to four weeks the collagen fiber bundles become more and more interwoven with the organising granulation tissue, the cellular elements diminish in number. The newly formed tissue is vascular, the hitherto firm connection with the paratenon is looser. The limit between stumps and organizing granulation tissue is still visible. Cells abound in the peripheral parts of

the tendon ends in contrast to the lack of cells in the centre. By the sixth week the limit between the tendon ends and the organizing granulation tissue is hardly noticeable. There are much more cells in this region than in normal tendon. The nuclei of the new tendocytes are still oval and the arrangement of the cells, although the longitudinal orientation dominates, is irregular, they

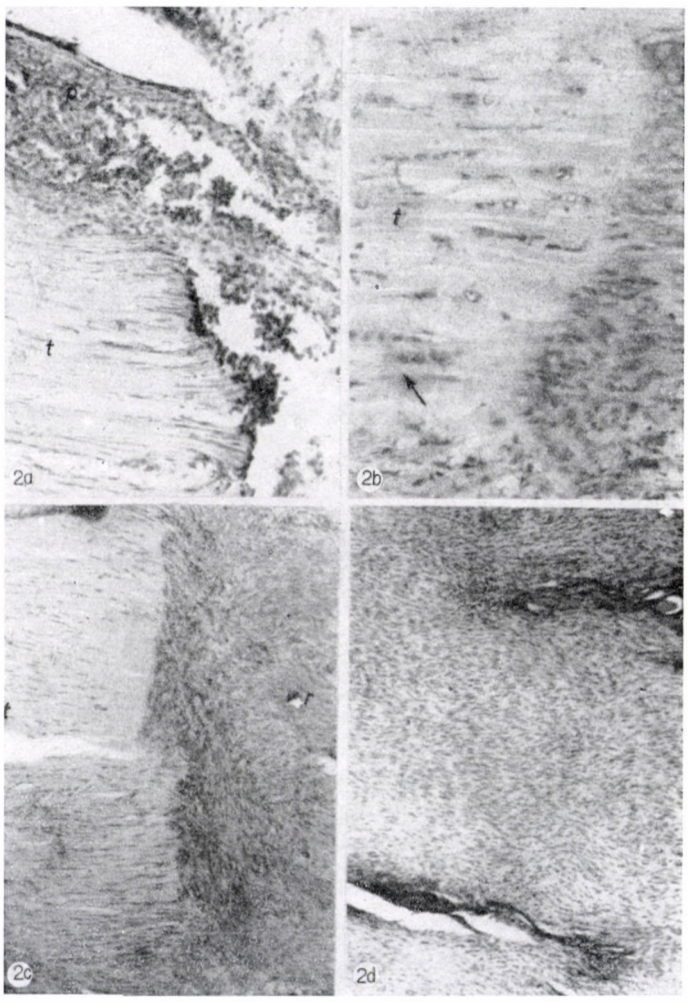

Fig. 2. A. — *third day:* Cellular proliferation from paratenon; t — proximal tendon stump. p — paratenon (× — 125). B. — *seventh day:* Tissue rich in cells and vessels between stumps. Immature cells in the peripheral parts of proximal stump; in the central part signs of cell necrosis; t — proximal tendon stump, r — regenerating tissue; arrow — immature cells (× — 200). C. — *three weeks:* The bord between stump and newly formed tissue is clearly visible. t — proximal stump, r — newly formed tissue; arrows — cell proliferation in peripheral parts of the stump; arrow-acellular region (× — 100). D. — *six weeks:* New tendon cells in region of the stump and repair tissue, orientated in general longitudinally, arranged densly and randomly (× — 100)

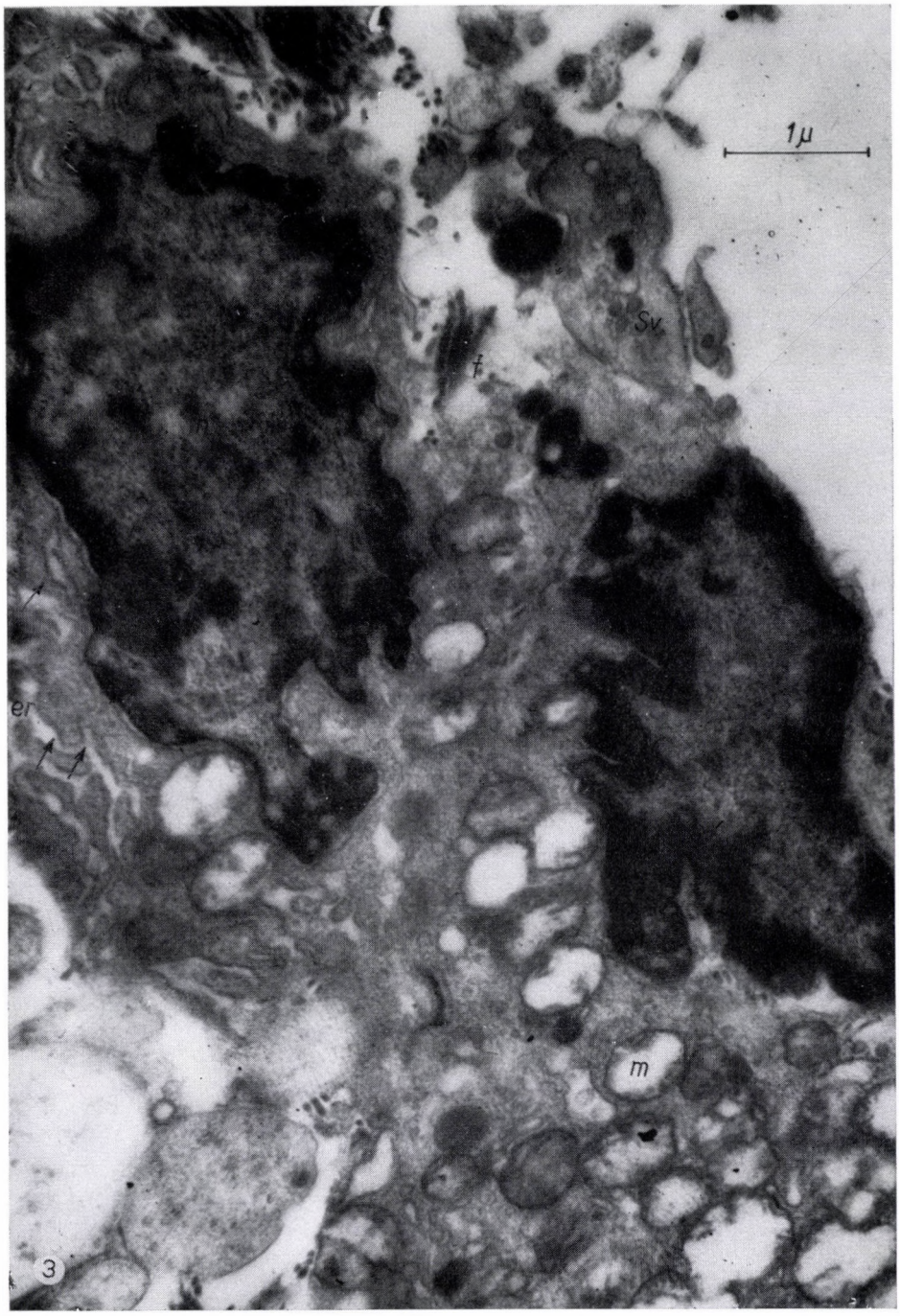

Fig. 3. Seven days: Electron microscopic picture of fibroblasts from proximal stump. n — nucleus, er — endoplasmic reticulum, sv — secretory vesicle, m — mitochondrium, arrows — RNS granules, f — collagen fibers (× — 20,000)

are not yet formed in lines. The collagen fiber bundles increase considerably in number. The histologic picture of a section excised after 100 days, the longest interval after operation in the present observations, reveals a decrease in the number of cells and an increase in that of fibers; there was no other essential

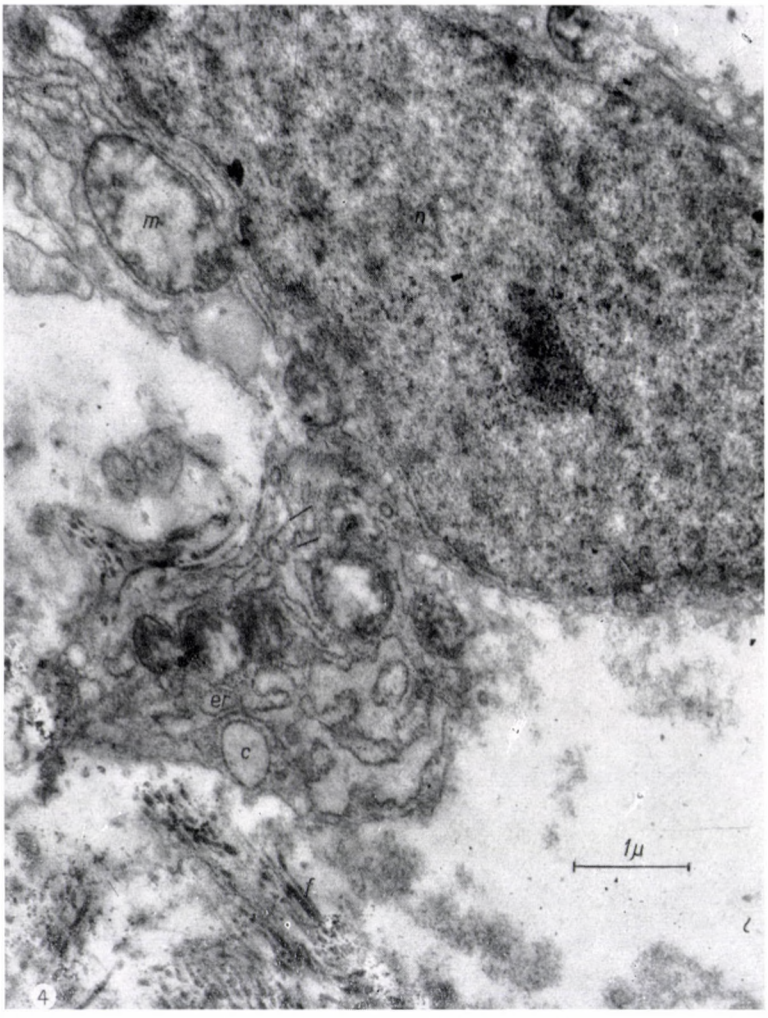

Fig. 4. Seven days: Electron microscopic picture of fibroblasts from the newly formed tissue between the tendon stumps. The collagen fibrils are in close contact with the cell surface. n — nucleus, er — endoplasmic reticulum, c — cistern, m — mitochondrium, arrows — RNS granules, nc — nucleolus, f — collagen fibrils (× — 20,000)

change as compared to the end of the sixth week *(Fig. 2 a, b, c, d)*.

Histochemical studies. 3 days after tenotomy, the paratenon and the loose tissues between the tendon ends stain somewhat metachromatically with toluidine blue. At the end of the first week the metachromatic staining of the

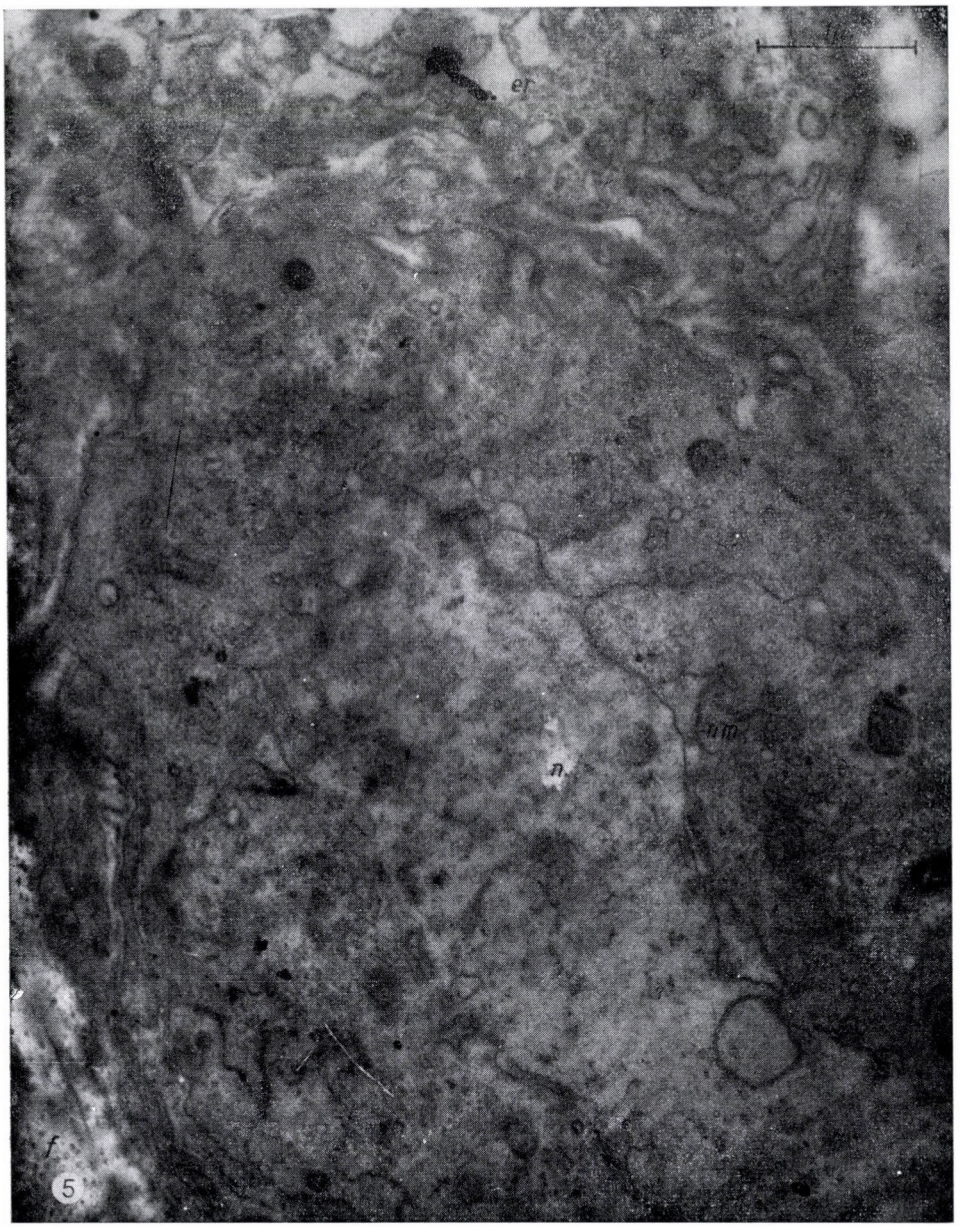

Fig. 5. Three weeks: Cell division in the proximal stump. Burst nuclear membrane, one cytocentre is well visible; n — nucleus, nm — nuclear membrane, cy — cytocentre, f — collagen fibers (× — 20,000)

tendon ends was increased, and that of the newly formed tissue quite marked. This staining is unchanged at 100 days.

Electron microscopy. On the seventh day after tenotomy we could observe

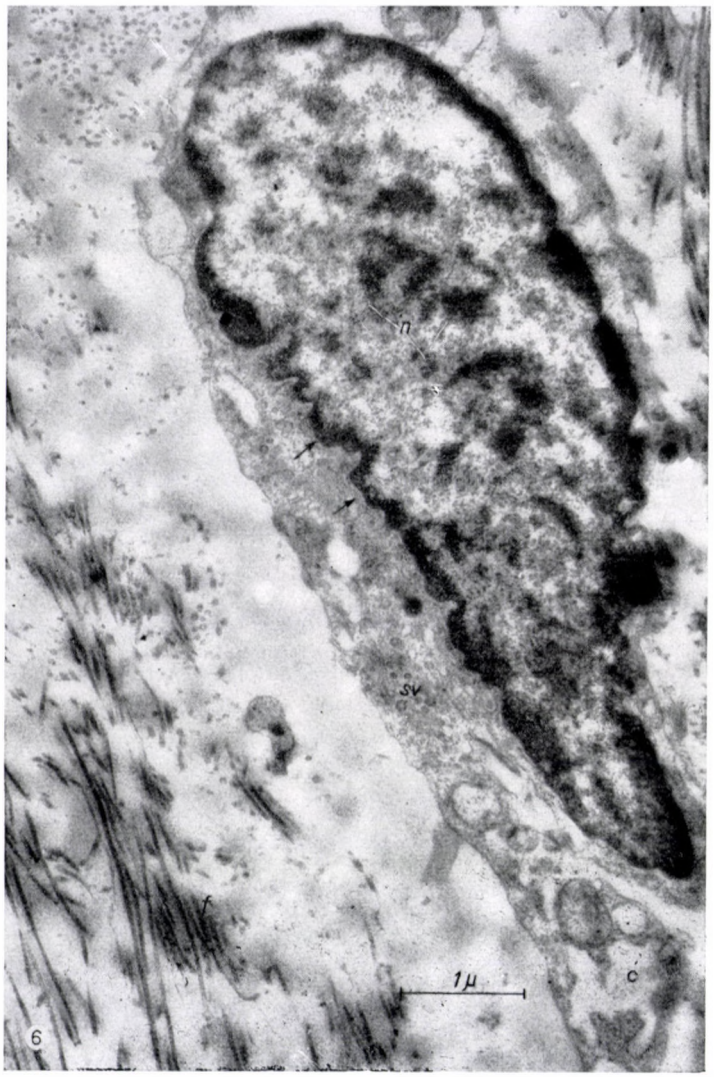

Fig. 6. Eight weeks: Active fibroblasts in proximal stump; n — nucleus, sv — secretory vesicle, er — endoplasmic reticulum, c — cistern, arrows — RNS granules, f — collagen fibers (× — 20,000)

in the proximal stump beside sporadic spindle-shaped tendon cells with a narrow rim of cytoplasm, also fibroblasts showing decided signs of activity. The endoplasmic reticulum is pronounced, with intensively staining RNS gran-

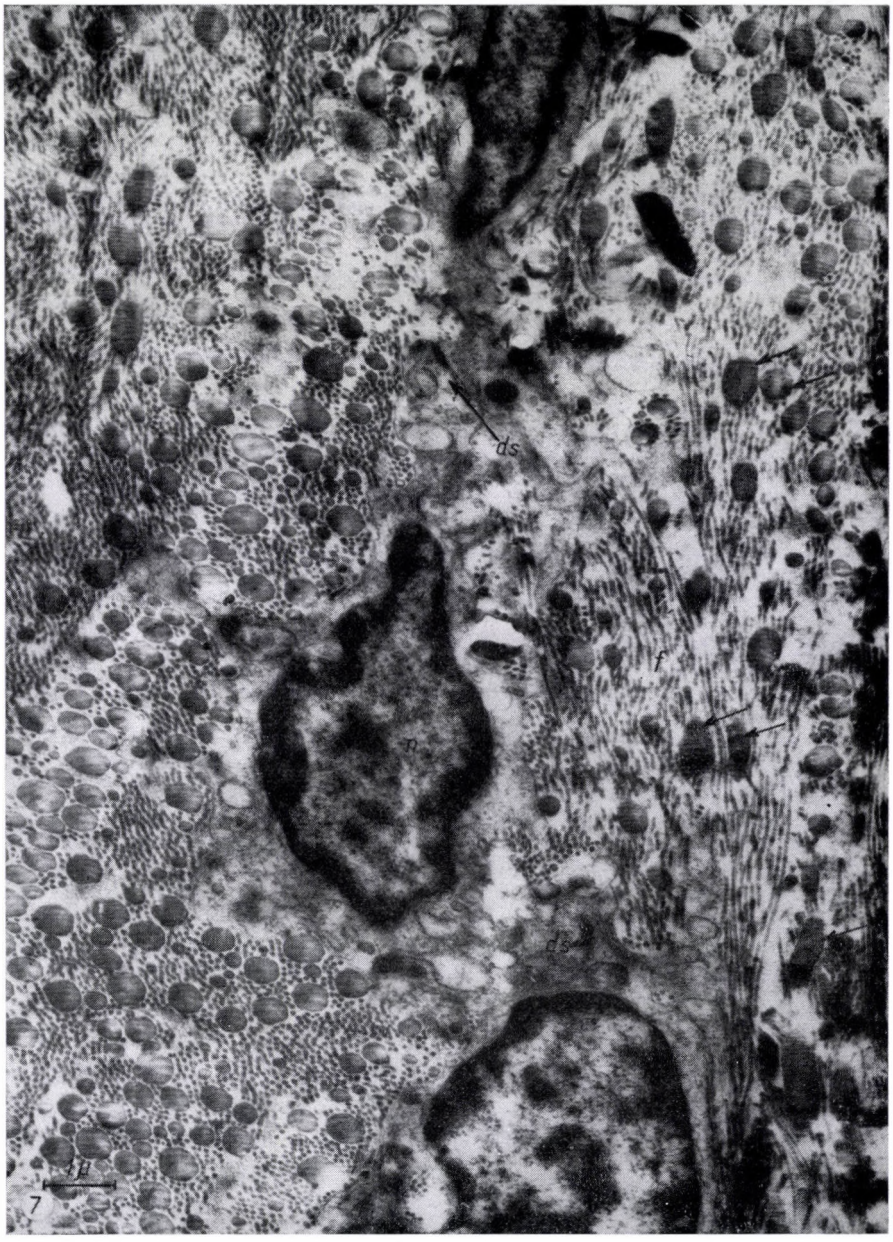

Fig. 7. Eight weeks: Semiactive cells from regenerating tissue between the stumps. Longitudinally oriented cells and fibers; n — nucleus, ds — thickening of desmosomal character, f — collagen fibers, arrows — thick collagen fibers (× — 11,000)

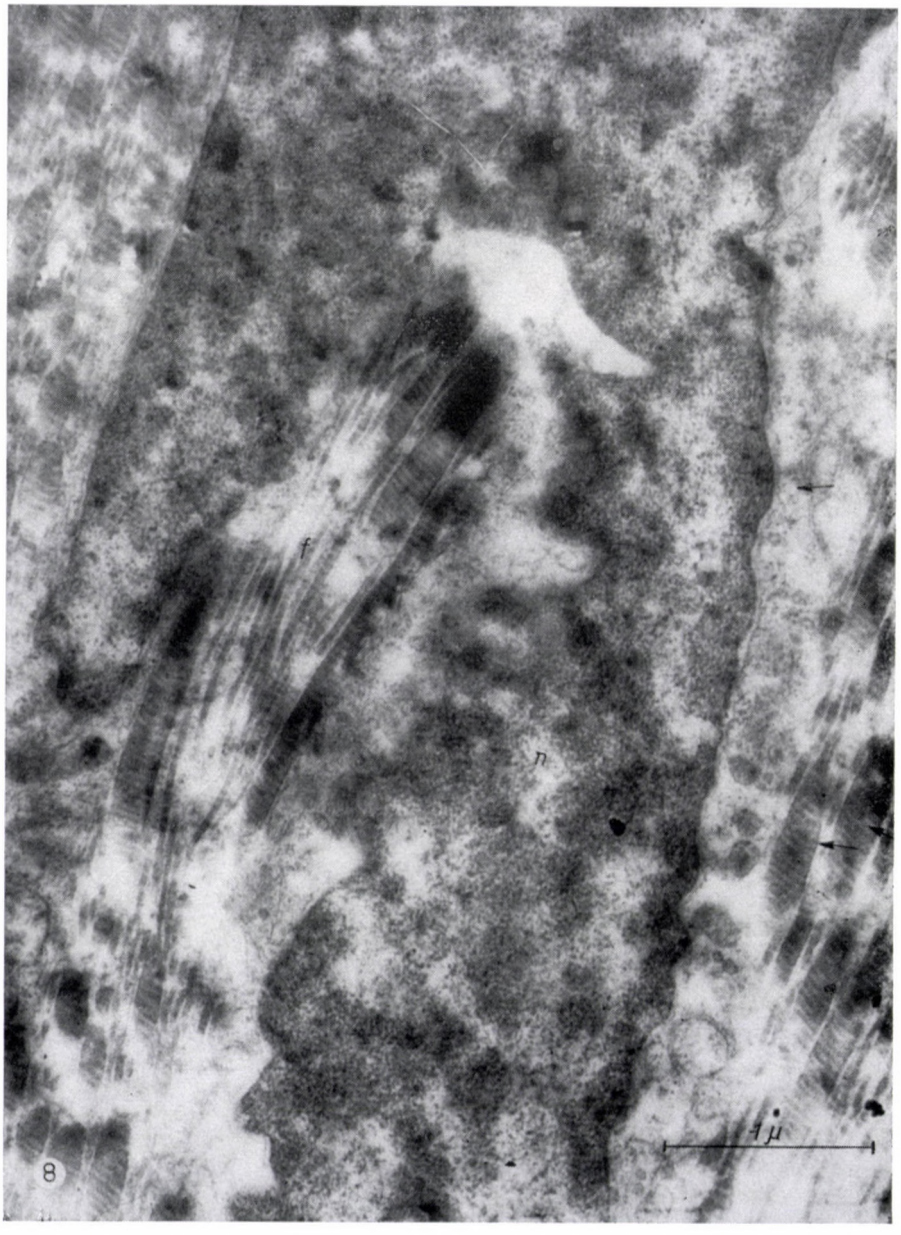

Fig. 8. Eight weeks: Semiactive cells between oriented fibers varying in thickness from regenerating tissue; n — nucleus, f — collagen fibers, arrows — thick collagen fibers (× — 29,000)

ules in the walls. The plasma contains many secreting vesicles and mitochondria. The nucleus is rich in chromatin, the nuclear membrane shows a wavy arrangement. On and around the cells collagen fibrils about 200 Å thick are found *(Fig. 3)*.

On the seventh day, in the newly formed tissue between the tendon stumps there is a great number of fibroblasts displaying strong activity,

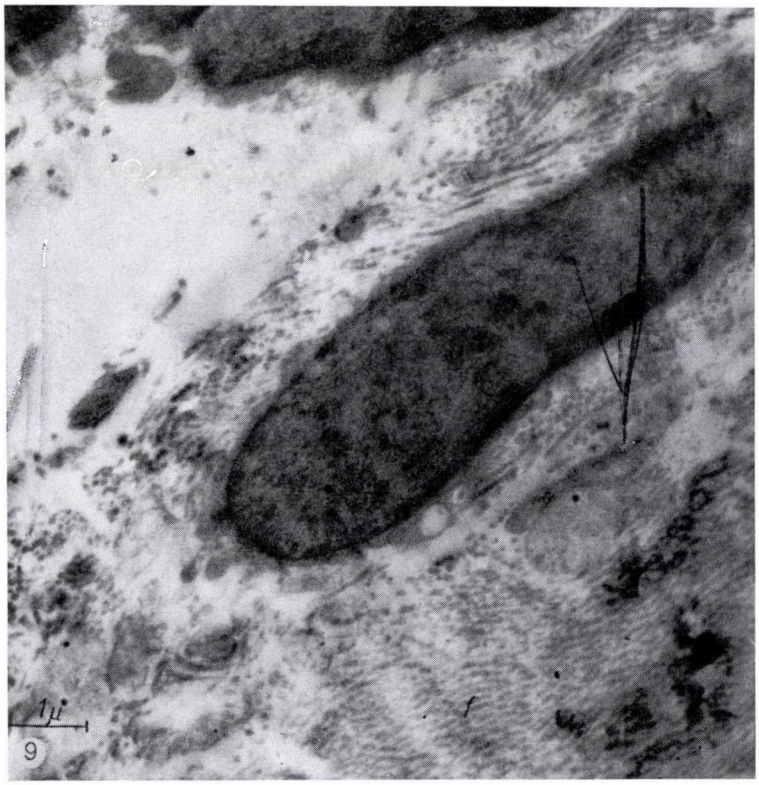

Fig. 9. Control: Electron microscopic picture of normal control tendon cell; n — nucleus, f — collagen fibers (× — 11,000)

presenting the characteristic appearance of endoplasmic reticulum, with occasional large cisterns. These cells correspond to PEACH and CHAPMAN's (23) type B, i.e. "synthesizing" fibroblasts. Occasionally it seems as if the cisterns would open towards the border of the cell and empty their content into the intercellular space. Their nucleus is ovoid, rich in chromatin, the cell membrane shows a wavy arrangement. On the surface of the fibroblasts and surrounding them we see transversely striated collagen fibrils measuring 200—300 Å in diameter *(Fig. 4)*. The presence of filaments in the cell plasma,

as described by WASSERMANN, could not be corroborated. After 21 days we observed in the proximal stump practically resting tendon cells and some cells with moderate activity. Other cells were distinctly active, even dividing cells could be observed *(Fig. 5)*. Active fibroblasts are invariably present at this stage in the newly formed tissue, with increased numbers of thickened collagen fibers between them.

After 56 days, there were still signs of tissue activity in the proximal stump although on gross examination the new tendon seemed to be fully developed. Beside completely resting tendon cells there were again fibroblasts showing signs of activity *(Fig. 6)*. After 8 weeks the differentiating immature cells of the newly formed tissue had not yet completely transformed into tendon cells — at least not all of them *(Fig. 7, 8)*. The most striking feature in these pictures is the great difference in size of the collagen fibers. Beside thin collagen fibers of 300—400 Å there are extremely thick cross striated ones of 2000— 4000Å in the dense fibrous tissue, while in the normal control tendon as well as in the region of an 8 weeks old tendon stump the fibers are 200— 300 Å in diameter. We obtained these data by measuring the diameter of 4000 fibers (Fig. 9 showes the normal control).

Discussion

The different opinions about the tendon repair process are far from convincing. Our experiments did not support the first of these views, that the tendon stumps are quite inert in the regenerative process, -- and even less can we accept the second view which attributes an almost exclusive role to the differentiated tendon cells. There remains the third school of thought with the theory that the proliferating cells of the surrounding connective tissues and the cells of the tendon stumps in different stages of maturity, have an equal share in the formation of the new regenerative tissue. This last opinion can be accepted only by assuming that the mature tendon cells are capable of division and differentiation.

It is evident from our findings that proliferation of the immature cells in the paratenon starts in the first days after tenotomy and they soon fill the gap between the tendon ends. After the first week, however, cell activity is manifest also in the tendon stumps, in particular in the epitenon and the peripheral parts of the stump, while in the central parts the majority of tendon cells shows signs of necrosis. We observed these immature cells in both our light and electron microscopic sections. Their nucleus is round or oval, rich in chromatin, their cytoplasm is full of basophil granules. In their submicroscopic structure there are definite signs of activity. These cells are densely and irregularly arranged and appear after two or three weeks. Concerning their

origin they seem to arise from proliferating capillaries of the tendon stumps, mainly from the mesenchymal cells in the wall of capillaries in the epitenon and endotenon. The supposition that the mature tendon cells should have changed into young cells seems far less probable.

As soon as the end of the first week the fibroblasts appearing in the tendon stumps and in the regenerating tissue between them begin to secrete the collagen precursors. According to present knowledge, the mucopolysaccharide-protein complex building up the collagen develops on the RNS granules in the endoplasmic reticulum of fibroblasts, but the mechanism of its release from the cells is still a controversial point, being it not clear whether the cisterns open up and empty their contents, or else the cell membranes burst. We could never notice collagen filaments in the cytoplasm of fibroblasts. By the seventh day after tenotomy transversely striated collagen fibrils were clearly visible close to the cell membrane or in the proximity of the cells; their average thickness was 200 Å.

The role of mucopolysaccharides in collagen production is an interesting problem. Our own observations agree with the data of other workers in that metachromasia of the regenerating tendon persists for a remarkably long time, in other words there is a prolonged production of "tenomucoid" substance. These findings are supported by electron microscopic data. It has been shown that in the course of regeneration (wound healing) the fibroblasts are secreting mucopolysaccharides (BERNSON and DALFERES, 1), (KENNEDY, 15), but their role in collagen formation is still discussed. ROMHÁNYI (27) found the mucopolysaccharide components to be embedded longitudinally in the submicroscopic structure of collagen fibers and that the anisotropic structural component built in here is responsible for the metachromatic reaction.

Taking into account the histochemical, the light and electron microscopical findings, the changes appearing during the first days in the microstructure of the regenerating tendon seem to persist for a surprisingly long time in spite of the fact that on gross examination the regenerative process may appear to be complete.

On the basis of the above data, regeneration of the injured tendon seems to proceed as follows. In the first week immature cells of mesenchymal origin, arising from the surrounding tissues, begin to proliferate in the space between the tendon stumps. In this vascular loose tissue the fibroblasts invade the fibrin net which had developed during these first days and begin to secrete the collagenous substance. At this time the fibroblasts are already oriented more or less longitudinally. The collagen fibrils appearing on and around the cells, increase in thickness and are also oriented longitudinally. At the same time the cells of mesenchymal origin in the tendon stumps, mainly in their peripheral parts, are also multiplying, differentiating, changing into fibroblasts, and later into tendon cells. Meanwhile they continue to secrete collagen fibers which

after due orientation meet with the longitudinally oriented fibers arising from the surrounding regenerating tissue. The underlying cause of this differentiation of the mesenchymal cells is not known, but in addition to the chemical and biological factors arising in the tissues following injury the role of functional factors has also to be considered. The regenerating tissue is richly vascularized in the first week and it adheres closely to its surroundings. By the fourth week, provided there is no scar formation, the close connection with the paratenon decreases and the tendon can assume its independent function. The changes in the finer structures may persist for a remarkably long time. Although the cellular elements in the tendon stumps and the newly formed tissue decrease in number and that of the fibers increases, cellular activity is still present at 8 weeks. The regenerating tissue contains thick collagen fibers with regular cross striation. In addition to these, the metachromatic staining reveals a still significant mucopolysaccharide synthesis at 100 days.

To finish we have to remind that with our morphological methods we can at most only follow the process of regeneration as a series of independent pictures waiting still to be grouped. Even the electron microscopic data must be interpreted with adequate critical care. Howewer, the combined use of morphological and biological methods will certainly help to bring more light into the subtle process operating at the microstructural level.

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DER GEGENWÄRTIGE STAND DES PROBLEMS DER SEHNENREGENERATION. LICHT- UND ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN AN REGENERIERENDEN RATTENSEHNEN

A. SALAMON und J. HÁMORI

Die Regeneration der durchschnittenen Achillessehne von Ratten wurde mittels dem Licht- und Elektronenmikroskop sowie mit histochemischen Methoden untersucht.

Gegenüber zahlreichen Literaturangaben wird die Ansicht vertreten, daß an der Herausbildung des Regenerats zwischen den Sehnenstümpfen die im umgebenden Bindegewebe und in den Sehnenstümpfen enthaltenen pluripotenten, unreifen, d. h. über eine große Differenzierungsfähigkeit verfügenden mesenchymalen Zellen gemeinsam beteiligt sind.

Die Synthese der kollagenen Fasern erfolgt durch die Fibroblasten. Mit den heutigen Untersuchungsmethoden ist nur eine annähernde Klärung des Mechanismus ihrer Ausscheidung möglich. Die sich am Fibringerüst orientiert anordnenden Fibroblasten des Regenerats und die in den Sehnenstümpfen erscheinenden, von den Fibroblasten sezernierten kollagenen Fasern werden allmählich dicker, nehmen — in erster Linie unter der Wirkung funktioneller Faktoren — eine Längsorientierung an, vereinigen sich und bilden auf diese Weise die Struktur der neuen Sehne heraus.

In der Mikrostruktur lassen sich selbst nach komplikationsfreier makroskopischer Heilung der Sehne noch lange Zeit hindurch Veränderungen (Zellaktivität, extrem dicke kollagene Fasern, lange Zeit nachweisbare metachromatische Färbung) nachweisen. Eine der Gewebestruktur der intakten Sehne völlig entsprechende Struktur ist auch später nicht vorhanden. Die Herausbildung der ursprünglichen Struktur, sofern dies überhaupt möglich ist, erfordert scheinbar eine viel längere Zeit.

СОВРЕМЕННОЕ ПОЛОЖЕНИЕ ВОПРОСА О РЕГЕНЕРАЦИИ СУХОЖИЛИЯ. ИССЛЕДОВАНИЕ РЕГЕНЕРИРУЮЩЕГОСЯ СУХОЖИЛИЯ КРЫС СВЕТОВЫМ И ЭЛЕКТРОННЫМ МИКРОСКОПАМИ

А. ШАЛАМОН и Й. ХАМОРИ

После обзора литературных данных авторы сообщают о результатах своих исследований, относящихся к регенерации сухожилий. Они изучали регенерацию перерезанного Ахиллова сухожилия крысы. Оценка проводилась при помощи методов исследования под световым микроскопом, электронным микроскопом, а также гистохимическими методами.

В противоположность многочисленным литературным данным авторы придерживаются того мнения, что в образовании регенерирующейся ткани между культями сухожилия совместно участвуют плурипотентные, незрелые, значит, обладающие значительной способностью к дифференциации мезенхимальные клетки, имеющиеся в окружающей соединительной ткани, а также в сухожильных культях.

Коллагенные волокна синтезируются фибробластами. При помощи современных методов исследования нельзя точно выяснить механизм их выделения. Фибробласты, ориентированно располагающиеся на фибриновом остове регенерирующейся ткани, и коллагенные волокна, выделяемые фибробластами, появляющимися в сухожильных культях, постепенно утолщаются и — прежде всего под влиянием функциональных факторов — ориентируются в продольном направлении, соединяются, и образуют структуру нового сухожилия.

В микроструктуре даже в случае макроскопического неосложенного излечения сухожилия долгое время наблюдаются изменения (клеточная активность, крайне толстые коллагенные волокна, метахроматическое окрашивание в течение продолжительного времени), и в последствии также не наблюдается структуры, совершенно соответствующей гистологической структуре неповрежденного сухожилия. Для образования первоначальной структуры, поскольку это вообще возможно, по-видимому, требуется гораздо более длительного времени.

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CYTOCHEMICAL OBSERVATIONS DURING THE SPERMATOGENESIS OF TWO INDIAN SQUIRRELS, FUNAMBULUS PENNANTI AND FUNAMBULUS PALMARUM

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Cytochemically, spermatogenesis in the two species of squirrels, *F. pennanti* and *F. palmarum*, is basically similar. The Golgi bodies are made up of lipoid-polysaccharide materials. The "externum" consists of acidic lipids while the "internum" contains simple polysaccharides, mainly glycogen. Proteins are completely absent from this inclusion. The Golgi bodies secrete Nile-blue positive proacrosomic granules which fuse to form the proacrosome. The latter is then transformed into the final acrosome. The acid lipids which persist through the proacrosome, form the outer membrane of the acrosome while the inner core is made up of PAS positive material.

The nucleus is rich in DNA and has a lipoid-polysaccharide-protein complex. The nuclear membrane towards the posterior aspect is interspread with lipoprotein positive granules, the postnuclear granules, which fuse to form the postnuclear cap of late spermatids. The postnuclear granules and the postnuclear cap are of nuclear origin.

The granular mitochondria contain acidic lipids, RNA and proteins. Small amounts of cystine are also present in this inclusion. The centriole does not divide at any stage and remains a single body, developing a flagellum in late spermatids. Both the centriole and the flagellum contain proteins. The lipids present in the earlier stages are lost in the final sperm.

The mode of sperm formation in various mammals has been studied by many workers. Recently, NATH (1957), BISHOP and WALTON (1960) and ROOSEN—RUNGE (1962) have published comprehensive reviews. Although the phenomenon of mammalian spermatogenesis has been understood to a greater extent, the chemical nature of the intracellular organelles in the various stages of sperm formation need further elaboration. Recently, the author (1965) has described the cytochemical events in the developing male germ cells of *Meriones* and has been able, by the use of specific techniques and blocking reactions, to resolve to some extent the Golgi controversy and the origin of the postnuclear cap.

The purpose of the present investigations was to perform cytochemical studies in other rodents, to determine the precise nature of the Golgi-acrosome systems and the postnuclear regions and, further, to clear the interrelationships between the various cell inclusions and their ultimate fate in final sperm.

Material and method

F. pennanti and *F. palmarum* were collected locally and at Port Novo (South India). Active testes were dissected in mammalian Ringer and samples of 10 cu. mm were fixed in various fixatives (Table 1). Processing, embedding and sectioning of the tissues were carried out in the usual way. In the case of frozen sections, the tissues were frozen directly without previous fixation and sections 10 μ thick were prepared. The applied cytochemical methods have been listed in Table I.

Table I

Summary of cytochemical methods

Test or Reaction	Fixative	Reference
Saturated solution of Sudan black B in 70% ethanol	Formol calcium; Bouin's; Frozen.	BAKER (1946); MCMANUS (1946).
Nile blue sulphate	Formol calcium; Bouin's; Frozen.	CAIN (1947).
Oil red O	10% formalin; Bouin's; Frozen.	LILLIE, 1944, as given by PEARSE (1960).
Phosphomolybdic acid	Formol calcium; Bouin's; Frozen.	LANDING *et al.*, as given by PEARSE (1960).
Periodic acid Schiff (PAS)	Formol calcium; Bouin's.	HOTCHKISS (1948).
PAS without previous oxidation in periodic acid	Formol calcium; Bouin's.	PEARSE (1960).
Acetylation at room temperature for 24 hours followed by PAS	Formol calcium; Bouin's.	LILLIE (1954).
Pyridine extraction of lipids at 60° C for 48 hours followed by PAS	Formol calcium; Bouin's.	LILLIE (1954).
Treatment with 1% solution of diastase to remove glycogen, followed by PAS	Formol calcium; Bouin's.	PEARSE (1960).
Pectinase treatment to remove galactogen, followed by PAS	Formol calcium; Bouin's.	GRAINGER and SHILLITOE (1952).
Carmine stain for glycogen	Formol calcium; Bouin's; Carnoy.	BEST, 1906, as given by PEARSE (1960).
Alcian blue	Formol calcium; Bouin's; Carnoy.	MOWRY (1956).
Dialyzed iron	Formol calcium; Bouin's; Carnoy.	HALE (1946).
Methylation for 24 hours at 60° C followed by alcian blue	Formol calcium; Bouin's; Carnoy.	FISHER and LILLIE (1954).
Methylation followed by Hale's reaction	Formol calcium; Bouin's; Carnoy.	FISHER and LILLIE (1954).
Demethylation following methylation-alcian blue and Hale's	Formol calcium; Bouin's; Carnoy.	SPICER and LILLIE (1959).
Azure A.	Formol calcium; Bouin's.	KRAMER and WINDRUM (1955).

Test or Reaction	Fixative	Reference
Toluidine blue.	Formol calcium; Bouin's.	PEARSE (1960).
Combined dialysed iron and PAS	Helly; Bouin's.	RITTER and OLESON, 1950, as given by PEARSE (1960).
Millon's reaction as modified by Baker.	Carnoy; Bouin's; Helly.	BAKER (1956).
Bromophenol blue.	Carnoy; Bouin's.	MAZIA *et al.* (1953).
Deamination for 20 hours at 40° C, followed by bromophenol blue.	Carnoy; Bouin's.	BURSTONE (1959).
Ninhydrin-Schiff.	Carnoy; Bouin's.	YASUMA and ICHIKAWA (1953).
Deamination followed by ninhydrin-Schiff.	Carnoy; Bouin's.	BURSTONE (1959).
Acetylation followed by ninhydrin-Schiff.	Carnoy; Bouin's.	LILLIE (1954).
Blocking of aldehyde groups followed by ninhydrin-Schiff.	Carnoy; Bouin's.	BURSTONE (1959).
Methylation followed by ninhydrin-Schiff.	Carnoy; Bouin's.	FISHER and LILLIE (1954).
Performic acid-alcian blue.	Formol calcium; Bouin's.	ADAMS and SLOPER, 1955, as given by PEARSE (1960).
Feulgen's reaction.	Formol calcium; Bouin's; Carnoy.	PEARSE (1960).
Methyl green—pyronine G.	Formol calcium; Bouin's; Carnoy.	JORDEN and BAKER (1955).
Digestion of RNA by human saliva followed by methyl green—pyronine G.	Formol calcium; Bouin's; Carnoy.	BRADBURY (1956).
Digestion of DNA by TCA followed by methyl green—pyronine G and Feulgen's.	Formol calcium; Bouin's; Carnoy.	ALFERT and GESCHWIND (1953).

Results

The different spermatogenetic stages in the two squirrel species were clearly defined and hardly different from each other. Application of various cytochemical techniques allowed to obtain definite information on these inclusions in each cell stage.

In fresh frozen and paraffin sections stained with Sudan black B the inclusions gave a positive reaction due to the presence of lipids or lipoproteins. The mitochondria and the nucleus of the spermatocyte were strongly positive, the Golgi bodies showed a weak reaction (Figs. 1 and 2). In the nucleus, the reaction was more intense on the nuclear membrane while the chromatin contents were brownish black suggesting the presence of lipoproteins. In the early spermatid, there was no appreciable change in the intensity of the reaction in the mitochondrial region and the nucleus. A weak reaction was noticed in the post nuclear granules and the centriole, while the proacrosome appeared to be negative. In the late spermatid and final sperm, the acrosome and the nucleus were positive with faint staining in the developing flagellum. Colouring in the mitochondria and the centriole gradually diminished until in the final sperm both were negative (Figs. 1 and 2).

To evaluate the nature of lipids, nile blue sulphate was used which gives a red or pink colour due to neutral and blue for acid lipids. The nucleus of the spermatocyte stained a weak pink which disappeared in the spermatids and reappeared in the sperm nucleus (Figs. 3 and 4). This indicates low contents of neutral lipids in the nucleus. This was further substantiated by the application of oil red O which gave positive reaction in the nucleus (Figs. 5 and 6), while all the other inclusions were negative suggesting the location of neutral lipids being restricted to the nucleus. The mitochondria, on the other hand, gave a strong reaction for acidic lipids throughout the entire course of spermatogenesis. In the spermatids, the postnuclear granules and later the postnuclear cap, the "externum" of the aggregated mass of the Golgi bodies, the proacrosome and later the acrosome showed the blue staining of acidic lipids. The centriole and the flagellum, however, were showing traces of acidic lipids (Figs 3 and 4).

Treatment with phosphomolybdic acid, which identifies choline containing lipids, revealed positiv reactions in the Golgi bodies of the spermatocyte and the centriole of the early spermatid (Figs. 7 and 8). The intensity of the reaction decreased considerably in later stages so that all the cell inclusions were negative.

It may, therefore, be inferred that most of the lipid positive material in the cytoplasm is acidic in nature with little choline containing lipids while the neutral lipids are located in the nucleus.

Identification of polysaccharides, mucopolysaccharides and mucoproteins was carried out by the periodic acid Schiff (PAS) technique. Nucleus and Golgi bodies of the spermatocyte gave positive reactions (Figs. 9 and 10). PAS positive granules in the nucleus appeared as intensely stained bodies and could be differentiated from the rest of the nucleoplasm. With the transformation of the spermatocytes into spermatids, the intensity of the reaction increased until in the final sperm the head was strongly positive (Figs. 9 and 10). The Golgi bodies showed a different behaviour. In the spermatid, the "internum" was

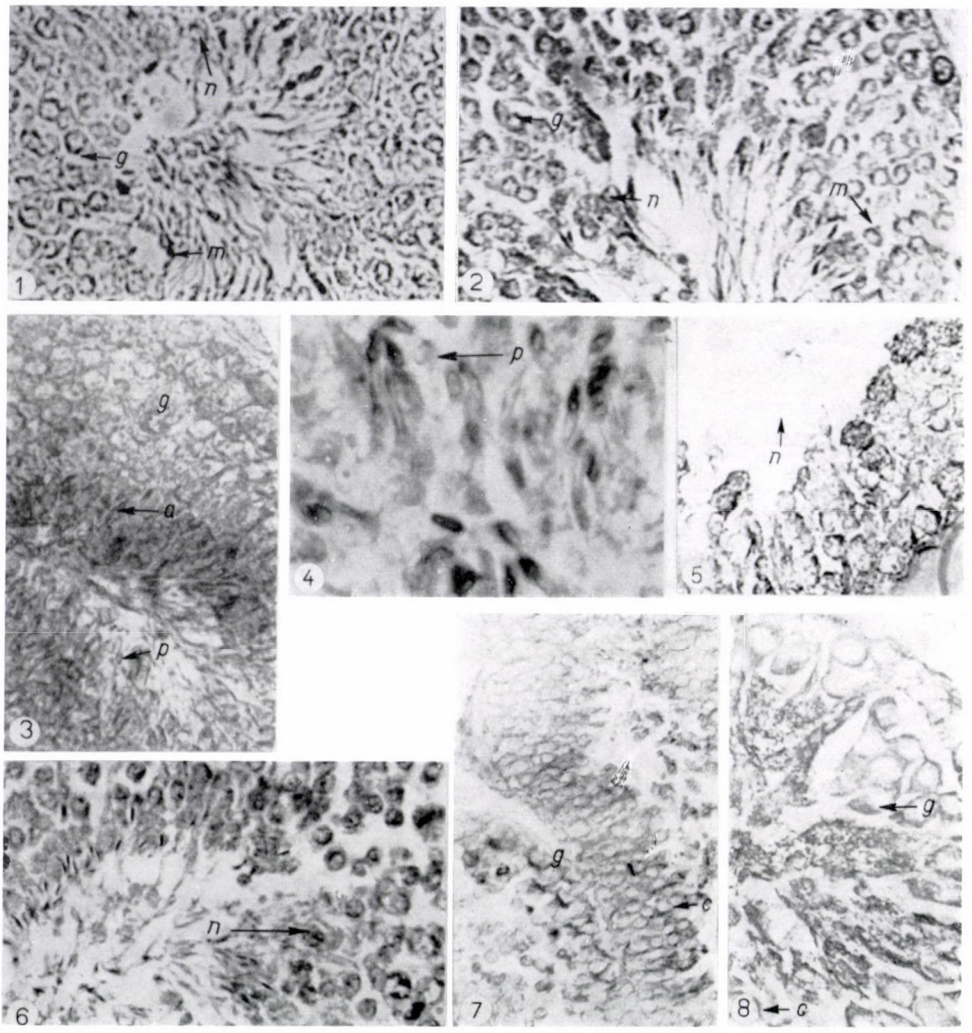

Fig. 1. F. pennanti. Sudan black B staining of the fresh frozen section. Note the lipoid substance in the Golgi bodies (g), the mitochondria (m) and the nuclear membrane (n). × 1000

Fig. 2. F. palmarum. Sudan black B positive sites in the various stages showing the Golgi bodies (g), the mitochondria (m) and the nuclear membrane (n). Bouin's fixed, × 1000

Fig. 3. F. pennanti. Nile blue sulphate reaction showing the presence of acid lipids in the postnuclear region (p), the externum of the Gogi body (g), the acrosome (a) and the mitochondria. Fresh frozen section, × 1000

Fig. 4. F. palmarum. Nile blue sulphate reactive material accumulated in the postnuclear region (p). Bouin's fixed, × 1200

Fig. 5. F. pennanti. Sites of neutral lipids indicated by oil red O. Note the reaction in the nuclei (n) of the spermatids. Fresh frozen section, × 1000

Fig. 6. F. palmarum. Neutral lipids in the nuclei of late spermatide and sperms (n). Bouin's fixed, × 800

Fig. 7. F. pennanti. Phosphomolybdic acid reaction showing traces of choline containing lipids in the Golgi bodies (g) and the centriole (c). Fresh frozen section, × 1000

Fig. 8. F. palmarum. Location of choline containing lipids in the centriole (c) and the Golgi body (g) of the spermatid. Bouin's fixed, × 1500

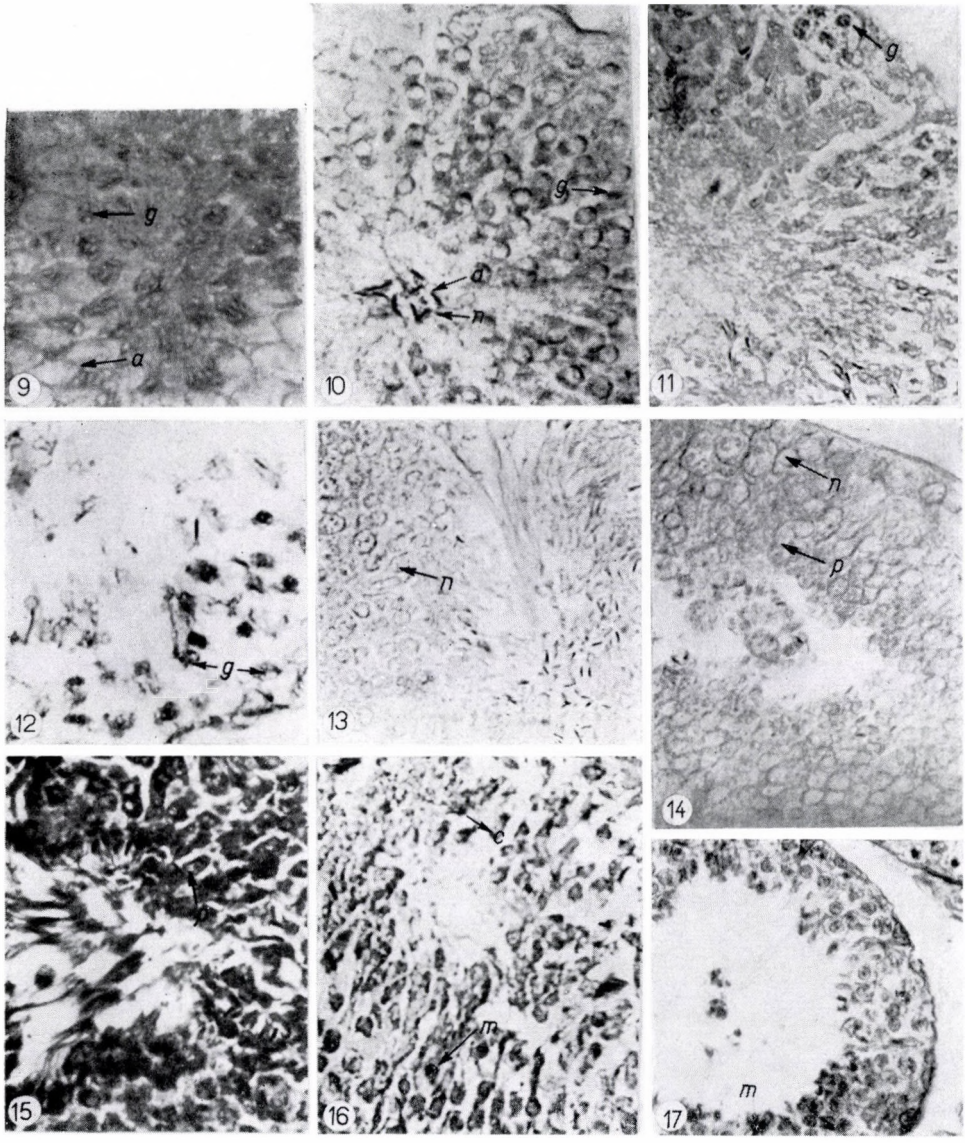

Fig. 9. F. pennanti. Early and late spermatids showing PAS positive regions. Note the reaction in the Golgi bodies (g). The reaction in the acrosome (a) is considerably weaked. Formol calcium fixed, × 1000

Fig. 10. F. palmarum. PAS positive reaction in the Golgi bodies (g) and the sperm nucleus (n); the acrosome (a) has lost its staining. Bouin's fixed, × 1000

Fig. 11. F. pennanti. Glycogen positive sites stained by Best's carmine. Note reaction in Golgi bodies (g). Carnoy fixed, × 1000

Fig. 12. F. palmarum. Glycogen positive Golgi bodies (g) indicated by Best's carmine. Bouin's fixed, × 1000

Fig. 13. F. pennanti. Alcian blue reaction in various nuclei (n). Formol calcium fixed, × 1000

Fig. 14. F. pennanti. Strongly reactive postnuclear granules and postnuclear cap (p), as

stained in contrast to the unstained "externum". The "internum" had distinct PAS positive granules arranged towards the periphery (proacrosomic granules) which later fused to form a single reactive granule, the proacrosome. The Golgi body then moved towards the nucleus, the proacrosome gradually increased in size and detached itself from the Golgi body to be deposited on the anterior proximity of the nucleus as the future acrosome. During these changes, PAS staining became weaker until in the final sperm the acrosome or the head cap contained little PAS positive material (Figs. 9 and 10). After deposition of the acrosome, the Golgi body sloughed off and was ultimately absorbed by the cytoplasm. A weak positivity was also noticed in the postnuclear cap.

To judge the reliability of the above reaction, sections were stained directly with Schiff's reagent without previous oxidation in periodic acid. The PAS reaction was negative, indicating the absence of free aldehydes. Next, glycol and other related groups were blocked by acetylation (LILLIE, 1954); no reaction was observed in any of the cell inclusions in any stage of sperm formation. The control sections, after treatment with dry pyridine to dissolve lipids, gave the same results as obtained by direct PAS method. This indicates that the PAS reaction is dependent on the availability of glycol and other related groups. Pretreatment of sections with diastase (PEARSE, 1960) gave negative results suggesting that the reaction may be due to glycogen, whereas digestion of galactogen by pectinase (GRAINGER and SHILLITOE, 1952) had little influence on the PAS reaction. According to these, the PAS reaction is due to glycol and related groups, especially glycogen. Although not thoroughly reliable, Best's carmine method (PEARSE, 1960) also gave a positive result in the same PAS positive regions (Figs. 11 and 12); this was a further proof of the presence of glycogen.

Acid polysaccharides were characteristically lacking in the various cytoplasmic inclusions but the nuclei of the two species gave faint reactions (Fig. 13) as revealed by the alcian blue (MOWRY, 1956) and dialyzed iron (HALE, 1946) methods. When the acid groups were blocked by methylation (FISHER and LILLIE, 1954), negative results were obtained. Demethylation (SPICER and LILLIE, 1959) gave positive results in the nuclei of the spermatocytes indicating the presence of carboxyl groups. Further, by using dialyzed iron combined with PAS (PEARSE, 1960), the nuclei showed a weak blue colour due to acid mucopolysaccharides (Figs. 14 and 15). In *F. pennanti*, purplish red staining was also noticed in the postnuclear region (Fig. 14).

revealed by combined dialyzed iron and PAS. The various nuclei (n) have also stained. Helley fixed, × 1000

Fig. 15 *F. palmarum.* Combined dialyzed iron and PAS reaction showing positive staining in the postnuclear region (p). Bouin's fixed, × 1000

Fig. 16 *F. palmarum.* Millon's reaction revealing tyrosine positive granules in the mitochondria (m) and the centriole (c). Bouin's fixed, × 1000

Fig. 17 *F. pennanti.* Sites of basic proteins shown by bromophenol blue. Note reaction in mitochondria (m). Carnoy fixed, × 1000

Acid groups were further recognized by metachromatic staining using azure A (KRAMER and WINDRUM, 1955) and toluidine blue (PEARSE, 1960). A weak positive orthochromasia in the nuclei was observed. Acidic methylation at 16°C (FISHER and LILLIE, 1954) resulted in the disappearance of azurophilia while saponification restored toluidine metachromasia, supporting the presence of carboxyl groups in the nuclei of the two species.

Proteins were detected by Millon's reaction as modified by BAKER (1956), bromophenol blue (MAZIA et al., 1953) and the performic acid-alcian blue (PEARSE, 1960) reactions. Millon's reaction distinguishes tyrosine, the only known amino acid with a hydroxyphenyl group. In *F. pennanti*, the reaction was very weak and hence no proper assessment of the presence of tyrosine in the cell inclusions was possible. In *F. palmarum*, however, the mitochondria were positive (Fig. 16) and in the final sperm, tyrosine was accumulated in the "middle piece" (Fig. 16). The centriole of the sperm also appeared to be tyrosine positive.

The protein rich mitochondria and the nuclei of the spermatocytes stained intensively with bromophenol blue (Figs. 17 and 18). The Golgi bodies and their derivatives were negative throughout spermatogenesis. In the spermatids, the intensity of the reaction increased in the mitochondrial regions while in the nuclei staining was observed in some granules scattered in the nucleoplasm in both species. The centriole, postnuclear cap and the flagella were also positive (Figs. 17 and 18). Specificity did not vary in various stages of spermatid differentiation and the results were similar in both species.

The reactions's reliability was judged by glocking free amino groups by deamination (BURSTONE, 1959). The reaction was completely inhibited, indicating the presence of free amino groups containing tyrosine, as evidenced also by Millon's reaction.

Further evaluation of proteins was carried out by ninhydrin-Schiff method which identifies protein bound amino groups and carboxyl groups. The mitochondria of the spermatocyte and the spermatid reacted strongly; while the reaction was weak in the nuclei and the postnuclear granules (Fig. 19 and 20). The postnuclear cap of *F. pennanti* stained more intensely (Fig. 19) than that of *F. palmarum* (Fig. 20).

These results were confirmed by acetylation and deamination; these procedures completely inhibited the reaction. Similarly, no positive staining was observed when the aldehyde groups had been blocked by hydroxylamine (BURSTONE, 1959). Methylation of carboxyl groups decreased the intensity of the reaction. It may, therefore, be concluded that the reaction is due to amino groups only. Thus the bromophenol blue and ninhydrin-Schiff reactions pointed towards the same general conclusion.

Performic acid-alcian blue (PEARSE, 1960) is a highly specific reaction for cystine. The reaction was very weak in the earlier stages but in very late

spermatids, accumulation of cystine was observed in the "middle piece" and on the nuclear membrane (Figs. 21 and 22). This means that cystine is present as a protein constituent in the above inclusions. Since no blocking methods for this reaction were carried out, it is possible to comment on the specific distribution and exact nature in which cystine occurs.

Nucleic acids were determined by Feulgen's reaction performed according to PEARSE (1960) and the methyl green-pyronine G method (JORDEN and BAKER, 1955). Feulgen positive granules were confined to the various nuclei,

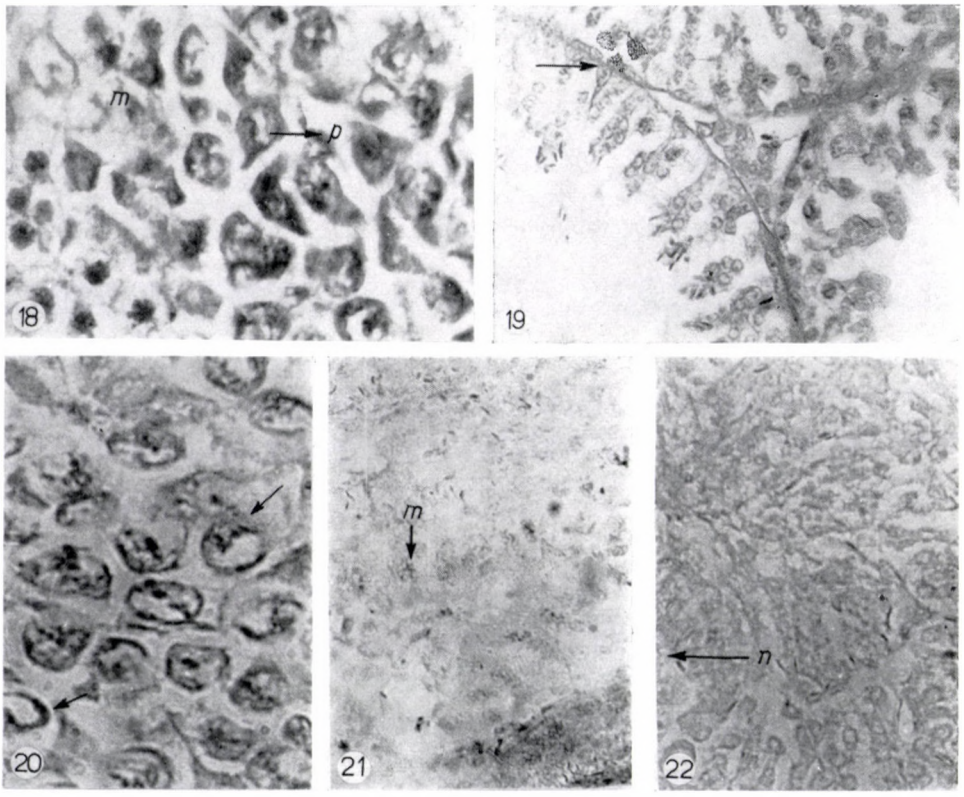

Fig. 18. F. palmarum. Bromophenol blue reaction in mitochondria (m) and postnuclear cap (p). Bouin's fixed, × 1500

Fig. 19. F. pennanti. Ninhydrin-Schiff reaction showing protein-bound amino groups. Carnoy fixed, × 800

Fig. 20. F. palmarum. Sites of protein-bound amino groups as revealed by ninhydrin-Schiff. Bouin's fixed, × 1500

Fig. 20. palmarum. Sites of protein-bound amino groups as revealed by ninhydrin-Schiff. Bouin's fixed, × 1500

Fig. 21. F. pennanti. Late spermatids containing cystine after performic acid-alcian blue staining. The reaction is particularly obvious in the mitochondria (m). Formol calcium fixed, × 1500

Fig. 22. F. palmarum. Performic acid-alcian blue staining showing positive reaction in the nuclei (n). Bouin's fixed, × 1000

distinguishing DNA from the rest of the chromatin material. Methyl green-pyronine G identifies both DNA and RNA. It stained the nucleus green (DNA) and the mitochondria red (RNA). The reaction persisted in all the stages of spermatogenesis. By digesting RNA by human saliva (BRADBURY, 1956), a positive reaction was obtained in the nucleus, due to DNA. Similarly, when DNA was extracted by 5 per cent trichloroacetic acid (TCA) (ALFERT and GESCHWIND, 1953), RNA was found to be present in the mitochondria. After TCA extraction, Feulgen's reaction was negative. Thus it is evident that DNA is present in the nucleus while RNA is located in the mitochondria and both these acids persist without any change during spermatogenesis is both the species.

Discussion

Qualitative analysis of the complex cell populations in the two squirrel species has been achieved by various cytochemical techniques. Basically, in the species under investigation the chemical composition of the various cell inclusions in different stages of spermatogenesis is similar, but there are certain differences in the intensity of the reactions. This is primarily, as far as the present investigations are concerned, due to the fact that all observations in *F. palmarum* have been made in fixed paraffin-embedded tissue, which might have been influencing the staining and thereby causing some differents from fresh frozen or fixed tissue of *F. pennanti*.

There are different views regarding the structure of the Golgi bodies in mammals during the various stages of spermatogenesis (MATHUR, 1965). The Golgi bodies in squirrels show the granular nature in the earlier stages, as observed by NATH et al (1957). This author did not mention anything further about the changing phases of the Golgi bodies in the squirrel, because he thought presumably that the granular nature was retained throughout spermatogenesis. In contrast, the present results clearly indicate that from the spermatid onwards the Golgi bodies aggregate and appear like a single body.

The chemical organization of the Golgiacrosome complex is indicative of a lipoid-polysaccharide constitution. In the Golgi body, lipids are concentrated in the "externum". Acid lipids along with choline containing lipids are present in the initial stages but disappear in later phases. The choline contents are very low wherever present. The proacrosomic granules are Sudan negative but show an acid character with Nile blue which persists in the proacrosome. In the final acrosome, the lipids are associated with the outer membrane, while the central core is negative. None of the earlier authors have reported the presence of lipids in the acrosome and hence it is not possible to correlate with them the present findings.

The positive PAS reaction in the Golgiacrosome system is due to the presence of glycogen in the internal cores of the Golgi body and the acrosome.

The lipoidal regions of these inclusions do not show any PAS positive substance. This suggests that the lipids and the simple polysaccharides occupy different zones and at no stage conjugate with each other to form a glycolipid complex in these inclusions which gives a positive reaction with both Sudan black and PAS. This is in accordance with our earlier observations on *Meriones* (MATHUR, 1965). Further, the possibility of the positivity of the PAS reaction due to other reactive substances like glycoproteins or mucoproteins is also ruled out as at no stage are proteins associated with either the Golgi bodies or the acrosome. LEBLOND and CLERMONT (1952), CLERMONT and LEBLOND (1955), LEUCHTENBERGER and SCHRADER (1950), and CLERMONT, GLEGG and LEBLOND (1955), have all reported PAS reactive material in mammalian acrosome. Further, LEBLOND AND CLERMONT (1952) and CLERMONT and LEBLOND (1955) state that the developing acrosome during spermateliosis becomes gradually less reactive, while OAKBERG (1956) states to the contrary that PAS reactivity in the developing acrosome of the mouse does not show any appreciable decrease in reaction. In the rodents studied by us an uniform PAS staining is maintained in *Meriones* but the acrosomes of the two squirrel species show a gradual decline. It appears that the variations in PAS reactivity may be due primarily to the labile nature of this reaction and its dependence on the fixative used, and the varying amounts of PAS positive material which is left over in the final sperm.

Essential components of the nuclei are DNA, neutral lipids, acid mucopolysaccharides (mainly carboxyl groups) and proteins. DNA is characteristic, as Feulgen positive granules and the intensity remains unchanged throughout spermatogenesis. Sudan positive material is mostly located on the nuclear membrane along with small concentrations of cystine. The lipoproteins are associated with the chromatin sharply distinguishable from Feulgen positive granules. The acid mucopolysaccharides and the proteins presumably form a carbohydrateprotein complex. The nucleus also gives a PAS positive test. If the nucleus is orthochromatic, the result of the PAS reaction is doubtful. According to PEARSE (1960), the PAS reaction may not be specific in the presence of carboxyl groups. Since the PAS reaction is not very distinct in the nuclei of the two species, this reaction might be due to Sudan positive or bromophenol positive materials and is not accounted for by the presence of any other type of polysaccharide. In that case, the positive PAS reaction in the inclusion should not be interpreted in the same way as in the Golgi bodies and the acrosome. It will thus be seen that the two organic complexes, lipid-protein and carbohydrate-protein, constitute and govern the activites of the nucleus, DNA being a separate entity.

The postnuclear region, consisting of postnuclear granules in the early spermatid and the postnuclear cap in the late spermatid, contains acidic lipids, mucoproteins and proteins. Acid lipids and protein are present till the formation of the final sperm, but mucoproteins are inhibited in later stages. Accord-

ing to GATENBY and WIGODER (1929), the postnuclear cap in the guinea pig spermatid originates from argentophile granules, the postnuclear granules which are present in the cytoplasm of the early spermatid and which are coalescing on the posterior aspect of the nucleus in the late spermatid. As observed in the present studies, the postnuclear granules never appear in the cytoplasm. The chemical constitution of the "granules" and the "cap" suggests a nuclear origin rather than a cytoplasmic one. This is evidenced further by the fact that the lipoprotein structure of the postnuclear cap is homologous to the lipoprotein nature of the nuclear membrane. The lipoprotein granules appear to be parts of the nuclear membrane and by the coalescence of these granules the posterior surface of the nuclear membrane becomes thickened into a girdle-shaped structure, the postnuclear cap. In *Meriones*, on the basic of the same phenomenon we have concluded to the nuclear origin of the postnuclear cap.

The mitochondria, the centriole and the flagellum are rich in protein and are lacking carbohydrates. The mitochondria besides being protein positive, also contain acid lipids, RNA and cystine positive materials. The centriole contains traces of choline with low amounts of acid lipids. In the flagellum, the acid lipid contents are very low. The centriole does not undergo any division into proximal and distal centrioles as in *Meriones* (MATHUR, 1965), but remains a single body from which the flagellum develops. It seems that the centriole and the flagellum have a lipoid-protein constitution in the initial phases but the lipid contents are soon lost or inhibited so that these organelles in the final sperm contain only the proteins. What happens further in the centriole is difficult to establish by light microscopy, because of the smallness of that structure.

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ZYTOCHEMISCHE ERFAHRUNGEN WÄHREND DER SPERMATOGENESE VON 2 INDISCHEN EICHHÖRNCHEN (FUNAMBULUS PENNANTI UND FUNAMBULUS PALMARUM)

R. S. MATHUR

Die Spermatogenese der 2 Eichhörnchenarten F. pennanti und F. palmarum vollzieht sich grundsätzlich ähnlich. Das Nebenkernchenfädchen ist aus einer Lipoid-Polysaccharid-Substanz aufgebaut. Das »Externum« enthält sauere Lipiden, das »Internum« dagegen einfache Polysaccharide, vornehmlich Glykogen. Eiweiß kann im Fädchen nicht nachgewiesen werden. Das Nebenkernchenfädchen sezerniert Nilblau-positive Proakrosomkörnchen, aus denen Proakrosom bzw. Akrosom (Endstadium) entsteht. Die saueren Lipiden verbleiben im Proakrosom unverändert und bilden die äußere Membran des Akrosoms, während das Innere desselben aus einer PAS-positiven Substanz aufgebaut wird.

Der Kern ist reich an DNA und enthält auch einen Lipoid-Polysaccharide-Eiweiß-Komplex. In der postnukleären Membran sind lipoproteinpositive, postnukleäre Körnchen zu beobachten, die im Laufe der Entwicklung die postnukleäre Hülle der Spermatiden bilden. Die postnukleären Körnchen und die postnukleäre Hülle sind nukleären Ursprungs.

Die granulierten Mitochondrien enthalten sauere Lipiden, RNA und Eiweiß. Im Fädchen erscheinen kleine Mengen von Zystin. Das Zentriolum teilt sich während der Spermatogenese nicht und bildet in der Spermatidenphase ein Flagellum. Zentriolum und Flagellum sind eiweißhaltig. Die in den früheren Phasen anwesenden Lipiden sind in den reifen Samenfäden nicht vorhanden.

ЦИТОХИМИЧЕСКИЕ НАБЛЮДЕНИЯ В СВЯЗИ СО СПЕРМАТОГЕНЕЗОМ ДВУХ ВИДОВ ИНДИЙСКИХ БЕЛОК (FUNAMBULUS PENNANTI И FUNAMBULUS PALMARUM)

Р. С. МАТУР

Сперматогенез двух видов белок **F. pennanti** и **F. palmarum** происходит в общем сходно. Волокно побочного ядрышка состоит из липоидно-полисахаридного вещества. «Наружная» часть содержит кислые липиды, а «внутренняя» часть — простые полисахариды преимущественно гликоген. Белок не может быть выявлен в ниточке. Волокно побочного ядрышка отделяет проакросомные зернышки, дающие положительную реакцию с нилблауом, из которых возникает проакросом или акросом (конечная стадия). Кислые липиды сохраняются в неизменном виде в проакросоме и образуют наружную перепонку акросома, в то время как внутренняя его часть состоит из ПАСК-положительного вещества.

Ядро богато ДМК и содержит также липоиднополисахаридно-белковый комплекс. В постнуклеарной перепонке наблюдаются липопротеин-положительные постнуклеарные ядрышки, образующие в ходе дальнейшего развития постнуклеарную оболочку сперматидов. Постнуклеарные ядрышки и постнуклеарная оболочка имеют ядерное происхождение.

Зернистые митахондрии содержат кислые липиды РНК и белок. В волокне появляются небольшое количество цистина. Центриолум во время сперматогенеза не делится и образует в сперматидной фазе жгутик. Центриолум и жгутик содержат белок. Имевшиеся в прежних фазах липиды в зрелых сперматоидах не содержатся.

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EFFECT OF PROLONGED THYROXIN AND METHYLTHIOURACIL TREATMENT ON THE EPIPHYSEAL CARTILAGE OF YOUNG RATS

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Seventy-two white male rats, 30 days old at the outset of the experiment have been treated with 10 μg/100 g body weight of thyroxin or 0.05 g/100 g body weight of methylthiouracil daily for 145 days to study the behaviour of the distal epiphyseal cartilage of the left third metacarpal bone.

Thyroxin enhanced maturation and destruction of cartilage, and the moderately increased rate of bone formation kept pace with this process. The appearance of chondroclasts was noted on the epiphyseal cartilage facing the diaphysis.

Methylthiouracil caused a reduction of chondrification and cartilage absorption; the rate of ossification was likewise decreased but still more rapid than cartilage breakdown. The cells of the epiphyseal cartilage were smaller, the zone of maturation was narrow, the matrix was poor in cells; the epiphyseal cartilage had not disappeared after 145 days treatment.

Thyroid hypofunction was recognized as responsible for the dwarfism of cretins as far back as the second half of the past century. The first experiments in this respect were performed by GUDERNATSCH [7], who administered thyroid tissue to tadpoles and found that they underwent metamorphosis earlier than their untreated mates. Subsequent investigations concerning the growth-promoting action of the thyroid in animals of higher order yielded varying and often contradictory results. SILBERBERG and SILBERBERG [19], reviewing the pertinent literature published till 1943, concluded that thyroxin was influencing linear growth by affecting endochondral ossification. Variations in and occasional contradictions of the literary data were attributed by these authors to species, sex and age differences, further to those in the dosage of the hormone and in the duration of the experiments. Of recent, HULTH and NYLANDER [9, 10] studied the behaviour of the epiphyseal cartilage in both hyperthyroidism and hypothyroidism.

The present experiments were designed to study in hyper- and hypothyroidism an epiphyseal cartilage which closes early under physiological conditions. The epiphysis of the third metacarpal bone, recommended by BECKS et al. [4], which was selected for study, closes at 120 to 140 days; accordingly, the treatment lasted from weaning to the age of 175 days.

Material and method

Seventy-two white male rats of our own breed were used. Date regarding them are assembled in Table 1. Body length was measured from the tip of the nose to the root of the tail. The age of the animals varied from 28 to 32 days at the outset of the experiments, and the maximum difference in their body weight amounted to 2 g.

The animals were divided into 3 groups, and each group into 4 sub-groups. One sub-group, each, of every group was killed at 40, 70, 91 and 145 days, respectively. (Data of Table 1 indicate mean results obtained in synchronously sacrificed sub-groups.)

Animals of Group 1 received subcutaneously 10 μg/100 g body weight of thyroxin (Hoffman—La Roche A. G., Basel) daily, dissolved in 0.1 ml of physiological saline.

Members of Group 2, the controls, received a subcutaneous dose of 0.1 ml physiological saline daily.

Members of Group 3 were treated orally with methylthiouracil daily, mixed of 0.05 g/100 g body weight to the food. The diet was identical in all groups, and all animals were kept under identical conditions.

The thyroid and the third left metacarpal bone of the sacrificed animals were used for microscopical study. The glands were fixed in 10 per cent formalin, the bones were decalcinated and fixed in Susa fluid. The sections were embedded in paraffin, stained with haematoxylin-eosin and azan.

Results

All test animals weighed less and were shorter than the controls after the treatment. The difference was especially pronounced in the group treated with methylthiouracil. Mean body temperature was higher in the thyroxin group and lower in the methylthiouracil group than in the controls (Table 1).

Table I

	Thyroxin			Control			Methylthiouracil		
Duration	Body weight g	Body length cm	Temperature °C	Body weight g	Body length cm	Temperature °C	Body weight g	Body length cm	Temperature °C
Initial value	35.63	11.37	38.00	36.45	11.50	38.20	36.33	11.56	38.20
40 days	74.00	14.68	39.00	78.00	14.60	38.20	76.83	14.30	37.53
70 Days	102.40	15.94	39.00	107.22	15.83	37.74	102.90	15.77	37.08
91 Days	131.25	16.82	38,20	144.00	17.14	37.45	124.00	16.00	37.08
145 Days	175.00	17.66	38.00	195.00	18.50	37.55	170.00	17.05	36.85

The fur of the animals in Group 1 was smooth, their movements vivacious, and they were more irritable than the controls. The thyroid gland was small in this group. The microscopic picture revealed large follicles engorged with colloid, further flattened follicular epithelium (Fig. 1).

Animals treated with methylthiouracil had a dishevelled fur and moved sluggishly. The thyroid was markedly enlarged. The microscopic picture showed empty follicles lined by high cylindrical epithelial cells. The picture resembled that of glandular hyperplasia (Fig. 2).

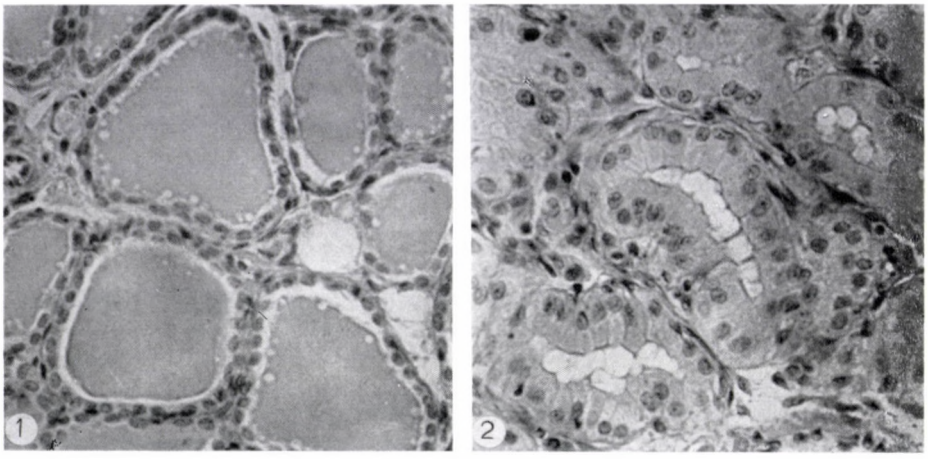

Fig. 1. Thyroid of 175-day old rat after 145 days thyroxin treatment. Haematoxylin-eosin × 375

Fig. 2. Thyroid of 175-day old rat after 145 days methylthiouracil treatment. Haematoxylin-eosin, × 375

Changes observed in the distal epiphyseal cartilage of the third metacarpal bone are described in the following, the nomenclature being borrowed from HAM [8].

Forty days treatment. In the thyroxin group, the zone of maturation in the epiphyseal cartilage was broader, the cells showed a fairly regular arrangement and were closely packed, osteoblastic activity was lively (Fig. 3).

In the control group epiphyseal cartilage and osteoblastic activity were normal (Fig. 4).

In the methylthiouracil group the epiphyseal cartilage was narrower, the cells were smaller and less regularly arranged, and the zone of maturation was narrower than in the other two sub-groups (Fig. 5).

In the thyroxine group the chondroclasts were fairly active, polynuclear cartilage-absorbing giant cells appeared on the epiphyseal surface facing the diaphysis, a phenomenon that became more pronounced after 70 and 91 days treatment.

Seventy days treatment. Changes at this stage were more pronounced.

In the thyroxin group the epiphyseal cartilage was much thinner, the zone of maturation broader and osteoblastic activity more intensive than in the control group. Chondroclastic activity was likewise intensive.

In the control group the epiphyseal cartilage was normal.

In the methylthiouracil group the proportion between chondrocytes and matrix had changed as far as there were less cartilage cells, and there was more

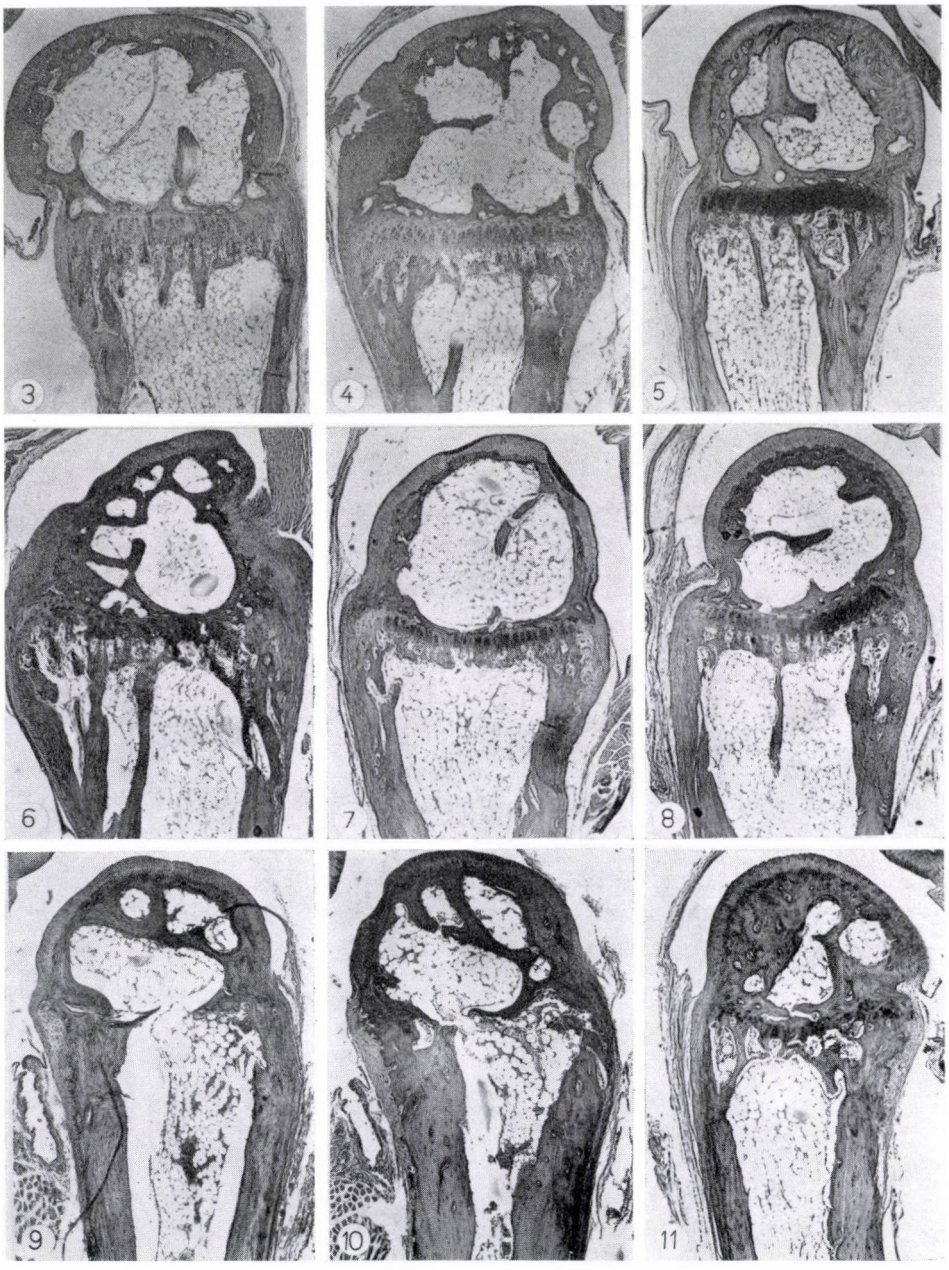

Fig. 3. Distal epiphysis of left third metacarpal after 40 days thyroxin treatment. Haematoxylin-eosin, × 20

Fig. 4. Distal epiphysis of left third metacarpal of 70 days old control animal. (Control of 40-day treatment) Haematoxylin-eosin, × 20

Fig. 5. Distal epiphysis of left third metacarpal after 40 days methylthiouracil treatment. Haematoxylin-eosin, × 20

matrix. The cells were arranged irregularly, the zone of maturation was narrow, and most of the cells therein were degenerated. There was abundant cartilage in the elementary bone trabecules; osteoblastic activity was less intense (Fig. 15).

Ninetyone days treatment. Changes were most conspicuous at this stage.

In the thyroxin group the epiphyseal cartilage consisted of 3 to 4 rows of cells only. Most of the cells were rounded and resembled those of the maturation zone. Absorption of cartilage extended at certain points to the bony end plate. Chondroclastic activity was most intensive. The extent of both bone formation and bone absorption was in excess of that in the control group (Figs. 6, 12).

In the control group the epiphyseal cartilage was perfectly normal (Figs. 7, 13). (Epiphyseal closure at the third metacarpal takes place at the age of 120 to 140 days in rats of the employed strain.)

In the methylthiouracil group the epiphyseal cartilage was thicker than in the other two sub-groups; the cell count diminished along with an increase of the matrix. The zone of maturation was narrow, the bone trabecules were thicker and contained more residual cartilage than in the other two sub-groups; in some cases chondrocytes surrounded by osteoid appeared in them. Osteoblastic activity was somewhat impeded, and chondroclasis considerably retarded (Figs. 8, 14).

Hundred and fortyfive days treatment. In the thyroxin group epiphyseal cartilage had disappeared, and the bony end plate above it was disappearing (Fig. 9). A great number of polynuclear giant cells was observed; their size varied between 20 and 60 μ, and the number of nuclei from 2 to 7 per section. The nuclei had a compact chromatin structure and contained 1 to 3 nucleoles. The cytoplasm was eosinophilic and included basophilic granules. The cells in question were lodged in their own lacunae next to the epiphyseal cartilage, and in their neighbourhood there were almost invariably other cells of mesenchymal origin (Figs. 7, 18, 19).

In the control group the epiphyseal cartilage had disappeared; the end plate, though perforated, was still present (Fig. 10).

Fig. 6. Distal epiphysis of left third metacarpal after 91 days thyroxin treatment. Haematoxylin-eosin, × 20

Fig. 7. Distal epiphysis of left third metacarpal of 121 days old control animal. (Control of 91 days treatment.) Haematoxylin-eosin, × 20

Fig. 8. Distal epiphysis of left third metacarpal after 91 days methylthiouracil treatment. Haematoxylin-eosin, × 20

Fig. 9. Distal epiphysis of left third metacarpal after a 145 days thyroxin treatment. Haematoxylin-eosin, × 20

Fig. 10. Distal epiphysis of left third metacarpal of 175-day old control animal. (Control of 145-day treatment.) Haematoxylin-eosin, × 20

Fig. 11. Distal epiphysis of left third metacarpal after 145 days methylthiouracil treatment. Haematoxylin-eosin, × 20

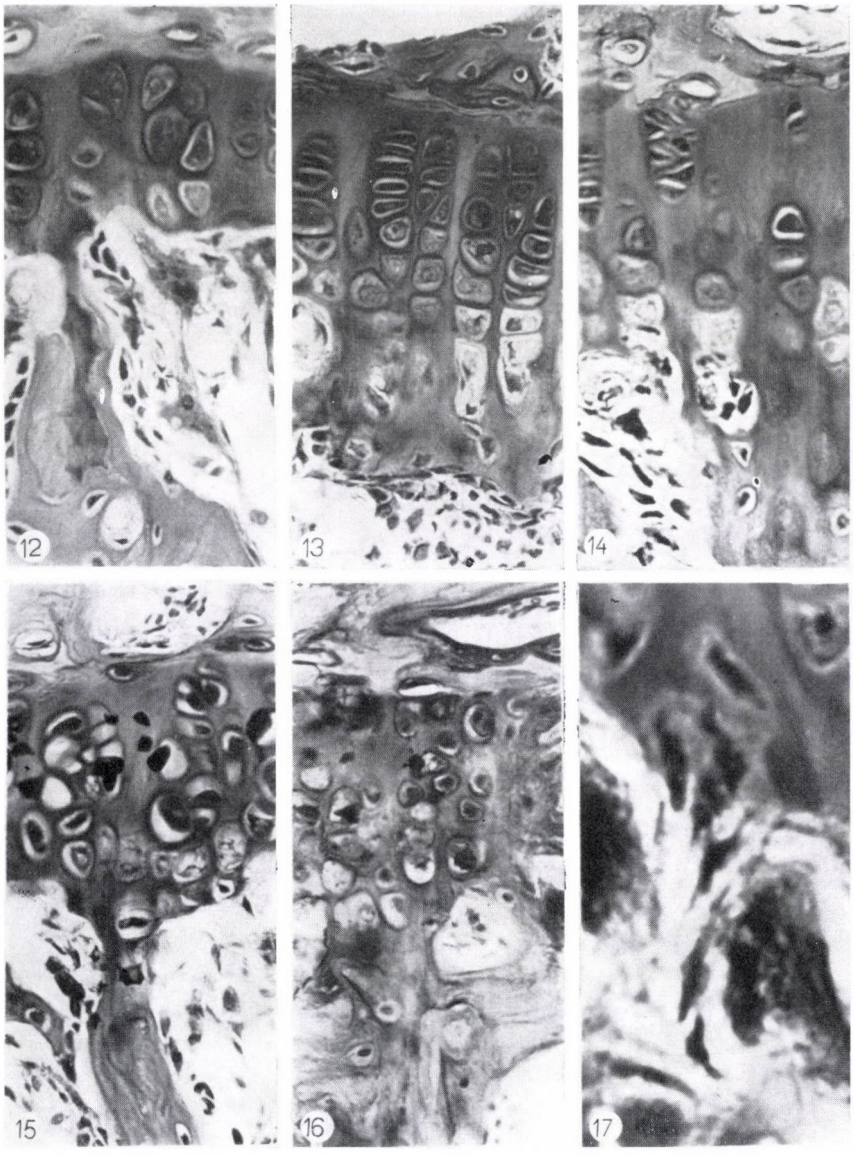

Fig. 12. Thyroxin treatment for 91 days. The epiphyseal cartilage is tenuous, its cells resemble those seen in the maturation zone of the controls. Note chondroclast next to epiphyseal cartilage. Haematoxylin-eosin; 375 × magnification of detail from Fig. 6

Fig. 13. Control of 91 day treatment. Haematoxylin-eosin; 375 × magnification of detail from Fig. 7

Fig. 14. Epiphyseal cartilage after 91 days methylthiouracil treatment. The matrix is relatively poor in cells, the zone of maturation narrow. Haematoxylin-eosin; 375 × magnification of detail from Fig. 8

Fig. 15. Epiphyseal cartilage after 70 days methylthiouracil treatment. The cells are irregularly arranged, there are numerous atrophied, degenerated cells; the zone of maturation is narrow. Haematoxylin-eosin, × 375

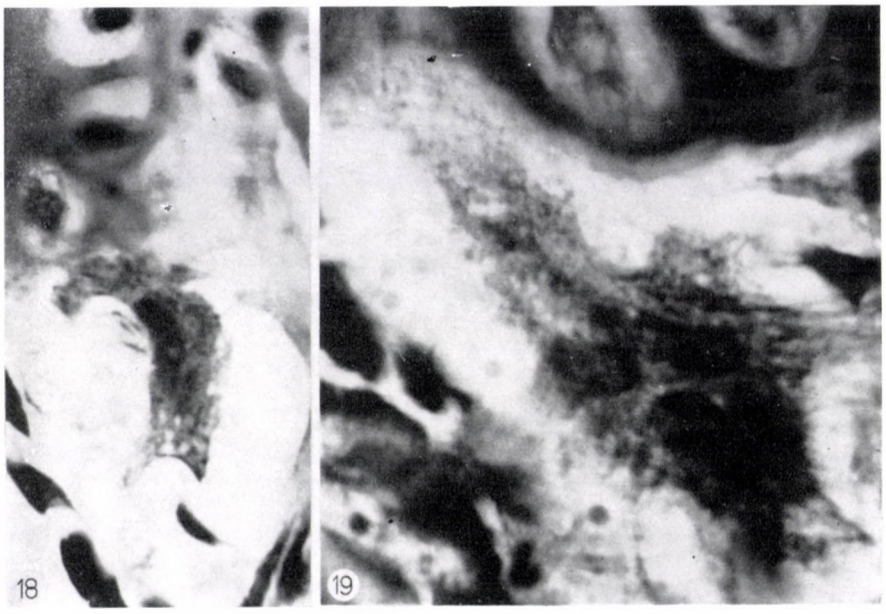

Fig. 18. Chondroclast. Haematoxylin-eosin, × 1100
Fi . 19. Chondroclast. Haematoxylin-eosin, 1600 × magnification of detail from Fig. 6

In the methylthiouracil group the epiphyseal cartilage was broad; the matrix was relatively poor in cells; the cells were small, irregularly arranged, degenerating; the zone of maturation was narrow. The thick elementary bone trabecules contained much cartilage and sometimes chondrocytes surrounded by osteoid. Osteoblastic activity was retarded (Figs. 11, 16).

Let us now sum up the changes that had taken place in the course of the experiment.

Thyroxin. The rate at which the cartilage was maturing and absorbed was accelerated; the epiphyseal cartilage grew thinner more rapidly, than in the control group. Osteoblastic activity was moderately increased, and chondroclasts appeared on the diaphyseal surface of the epiphyseal cartilage.

Controls. Epiphyseal closure occurred between the 120th and 145th day of life.

Methylthiouracil. The epiphyseal cartilage still existed at the end of the experimental period. Maturation and absorption of the cartilage were inhibited,

Fig. 16. After 145 days methylthiouracil treatment epiphyseal cartilage is poor in cells, and contains small irregularly arranged cells; the zone of maturation is narrow. Haematoxylin-eosin; 375 × magnification of detail from Fig. 11
Fig. 17. Chondroclasts on the surface of the epiphyseal cartilage next to the diaphysis, with mesenchymal elements among them. Haematoxylin-eosin, × 1100

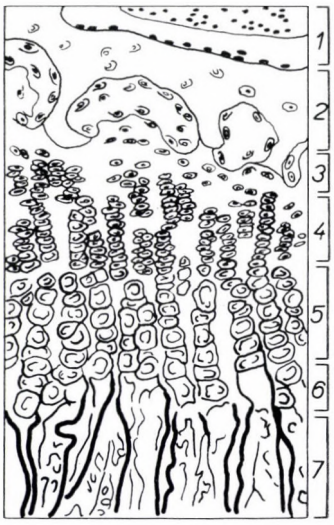

Fig. 20. Zonal division of the epiphyseal cartilage after HAM
1 Marrow of epiphysis
2 Bone of epiphysis
3 Zone of resting cartilage
4 Zone of young proliferating cartilage
5 Zone of maturing cartilage
6 Zone of calcifying cartilage
7 Developing trabecules of metaphysis

osteoblastic activity was impaired. The epiphyseal cartilage exhibited signs of grave damage after 70 days treatment; these signs were less serious on the 91st day, but once more very grave at 145 days.

Discussion

Since the monograph of SILBERBERG and SILBERBERG [19] numerous authors have concerned themselves with endochondral ossification in hypothyroidism and hyperthyroidism [1, 2, 3, 9, 10, 18, 20]. KROMPECHER's work [12] contains a classical description of normal ossification.

While there is no diversity of opinion regarding the effect of hypothyroidism on endochondral ossification, the effect of hyperthyroidism constitutes a subject of controversies. Experimental hypothyroidism is usually induced by thyroidectomy, further by the administration of radioiodine or of thiouracil derivatives, while hyperthyroidism by the administration of thyroid or thyroxin.

The amount of thyroxin synthesized by the rat is estimated by DONHOFFER [5] at 5 μg per day; two-to fourfold this amount was administered in the present experiments, and of methylthiouracil the same amounts as those used by KROMPECHER [16].

Medium doses of thyroxin promote, large doses delay and small doses fail to influence linear growth [19]. Although the linear growth of our test animals hardly differed from that of the controls, the epiphyseal cartilage responded to treatment vigorously. Its effect was evident after 40 days, and became increasingly pronounced until the end of the experiment. Epiphyseal closure started early, and the bony end plate of the epiphysis disappeared after 145 days treatment, at the age of 175 days. Early epiphyseal closure as a consequence of thyroxin treatment is due to the increased metabolism in the cartilage which promotes maturation and breakdown (relationship between the zones of proliferation and maturation, chondroclasts). Osteoblastic activity is enhanced and keeps pace with the absorption of cartilage, and simultaneously a slight increase of bone decomposition was observed. These findings are in harmony with the observations of FORST et al. [6], whereas increased osteoclastic and chondroclastic activity in thyroxin-treated animals was only assumed by HULTH and NYLANDER [9], whose experiment was too short actually to observe this. That the cells show a regular arrangement in the zone of maturation is regarded by these authors as a sign of intensified growth, whereas it is with respect to chondroclasis that we consider it significant, since one of the forms of chondroclasis is correlated with cellular activity in the zone of maturation [13]. NOBACK et al. [17] found that thyroxin treatment enhanced the development of ossification centres in the newborn rat.

Absorption of bone and cartilage is reduced in hypothyroidism. The clavicle and scapula of cretins frequently contain cartilage even after the 5th decade [22]. Having inhibited thyroid function by means of ^{131}I, SILBERBERG and SILBERBERG [20] found the epiphyseal cartilage to persist in the mouse experiments. These phenomena, confirmed by our observations, are in our opinion brought bouta by the following mechanism. Hypothyroidism facilitates the persistence of the epiphyseal cartilage by delaying its maturation (cells are few and small, the zone of maturation is narrow) and by slowing down the rate of absorption (the maturation zone is narrow, chondroclastic activity practically nil, the elementary bone trabecules contain more cartilage, sometimes chondrocytes surrounded by osteoid). Although the effects of hypothyroidism were observable after 40 days already, they became fully manifest after 70 days in the present experiments. At 91 days, the epiphyseal cartilage conveyed the impression as if it had structurally adapted itself to the changed conditions. Alterations were most striking after 145 days of treatment. The cells of the epiphyseal cartilage were smallest and their arrangement was most irregular at that time; a great number of degenerated cells were observed. These findings are in good harmony with the observations of other authors [9, 10, 17, 20].

Osteoblastic activity was reduced by methylthiouracil, and the changes became graver with the progress of treatment. This notwithstanding, the diminution of cartilage absorption caused a homogeneous bony end plate to

form on the distal surface of the epiphyseal cartilage by the end of the experiment, a phenomenon due to that bone formation was more rapid than cartilage breakdown.

A physiological equilibrium between chondrification and cartilage destruction as also between bone formation and bone destruction is a precondition of normal ossification [14, 15]. Its upset leads to pathologic bone formation. The alterations observed in the present experiment were mainly due to an upset of the balance between cartilage formation and breakdown as also to a less pronounced disturbance of the balance between bone formation and breakdown.

In hyperthyroidism, the histological effect of thyroxin-increased renal excretion of calcium and phosphorus with a normal blood calcium level [11] and secondary basophilic hyperpituitarism have to be considered [22]. In connexion with primary hypothyroidism induced by methylthiouracil, the problem of the peripheral action of thiouracil derivatives (inhibition of oxidases) arises.

As regards chondroclasis, KROMPECHER [13] distinguishes three ways of cartilage absorption: (1) the distal cells of the epiphyseal cartilage (i. e. those of HAM's maturation zone) reabsorb the matrix in their vicinity; (2) there appear polynuclear giant cells, chondroclasts, of mesenchymal origin which absorb the matrix of the cartilage only; (3) osteoclast-like giant cells are involved in the removal of elementary bone trabecules which contain both cartilaginous and osteoid tissue. The giant cells observed in the present experiments presumably belonged to the second and to the third category. The assumption that it is along the vessels, that they gain assess to the distal surface of the epiphyseal cartilage, is substantiated by the presence in their neighbourhood of other mesenchymal elements. The first form of cartilage absorption as described by KROMPECHER, [13] and others [21] was likewise observed in the course of our experiments: we found that the epiphyseal cartilage persisted for a long time if the zone of maturation was narrow or absent. Having observed an increased number of chondroclasts in thyroxin-treated animals, it seems justified to assume that they appear only under the hormonal and enzymatic conditions of hyperthyroidism, a problem that cannot be settled before elucidating the role of other endocrine glands.

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ÜBER DIE WIRKUNG DER PROLONGIERTEN THYROXIN- UND METHYLTHIOURACILBEHANDLUNG AUF DIE EPIPHYSENFUGE VON JUNGEN RATTEN

G. LÉVAI und I. ZS. NAGY

Im Laufe einer 145tägigen Thyroxin- [10 μg/100 g Körpergewicht/Tage] und Methylthiouracil- [0,05 g/100 g Körpergewicht/Tag] Behandlung wurde das Verhalten der Epiphysenfuge bei 72, 30 Tage alten Albinorattenmännchen untersucht. Das Testobjekt der Untersuchung war die distale Epiphysenfuge des linken III. Metakarpus.

Bei der mit Thyroxin behandelten Gruppe waren gesteigerte Knorpelreife- und -zerstörung zu beobachten; parallel mit dieser Erscheinung verlief ein mäßig gesteigerter Knochenbildungsprozeß. An der diaphysären Oberfläche der Knorpelscheibe der Epiphyse erschienen Chondroklast-Riesenzellen.

Bei den mit Methylthiouracil behandelten Tieren waren verminderte Knorpelreife- und -zerfall vorzufinden, die ebenfalls verlangsamte Knochenbildung übertraf jedoch das Maß des Knorpelabbaus. Die Zellen der Epiphysenfuge waren kleiner, die Reifzone schmal, die Grundsubstanz zellarm, außerdem ließ sich die Epiphysenknorpelscheibe selbst nach 145tägiger Behandlung deutlich erkennen.

ДЕЙСТВИЕ ДЛИТЕЛЬНОГО ПРИМЕНЕНИЯ ТИРОКСИНА И МЕТИЛТИОУРАЦИЛА НА ЭПИФИЗАРНЫЙ ХРЯЩ КРЫС В СТАДИИ РОСТА

Г. ЛЕВАИ, и. Ж. НАДЬ

Авторы исследовали на протяжении 145 дней изменения эпифизарного хряща крыс-самцов под влиянием дачи тироксина и метилтиоурацила. Наблюдения проводились на 72 крысах, которым к началу опыта было 30 дней. Наблюдения проводились над дистальным эпифизарным хрящом левой третьей запястной кости. Животные получали 10 мкг/100 г веса тела тироксина и 0,05 г/100 г веса тела метилтиоурацила в день.

У животных, получавших тироксин, наблюдалось усиленное созревание и усиленная гибель хряща. Умеренно усиленное образование костей не оставало от темпов гибели хряща. На диафизарной поверхности эпифизарного хрящевого диска появлялись хондрокластические гигантские клетки.

У животных, получавших базетирин наряду с замедлением созревания и гибели хрящей, наблюдалось замедленное образование кости, степень которого превышала степень разрушения хряща. Клетки эпифизарного хряща меньшие, зона созревания узкая, основное вещество более бедное клетками, чем в норме и еще после 145-дневной затравки наблюдается эпифизарный хрящевой диск.

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THE ROLE OF MITOCHONDRIA IN THE EARLY MORPHOGENESIS OF CHICK EMBRYOS

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If the blastoderm of young chick embryos is treated with Janus green B, mitochondrial dehydrogenase areas, typical of the given ontogenetic stage, are formed, and the dye is reduced, a phenomenon indicative of dehydrogenase activity. The difference between the rate at which the dye is taken up in the early phase of development and that at which it is reduced justifies the assumption that morphogenesis has three stages; the morphological change is always preceded by two phases of submicroscopic or molecular transformation. Janus green B at high concentrations has a teratogenic effect. The resulting monsters exhibit a phase specificity corresponding to the detected presumptive areas. Janus green B is linked to a specific lipoprotein of the mitochondrium and affects the energy-consuming biological processes. Uncoupling of the oxidative phosphorylation gives rise to a pathological shunt which is responsible for the teratogenic effect.

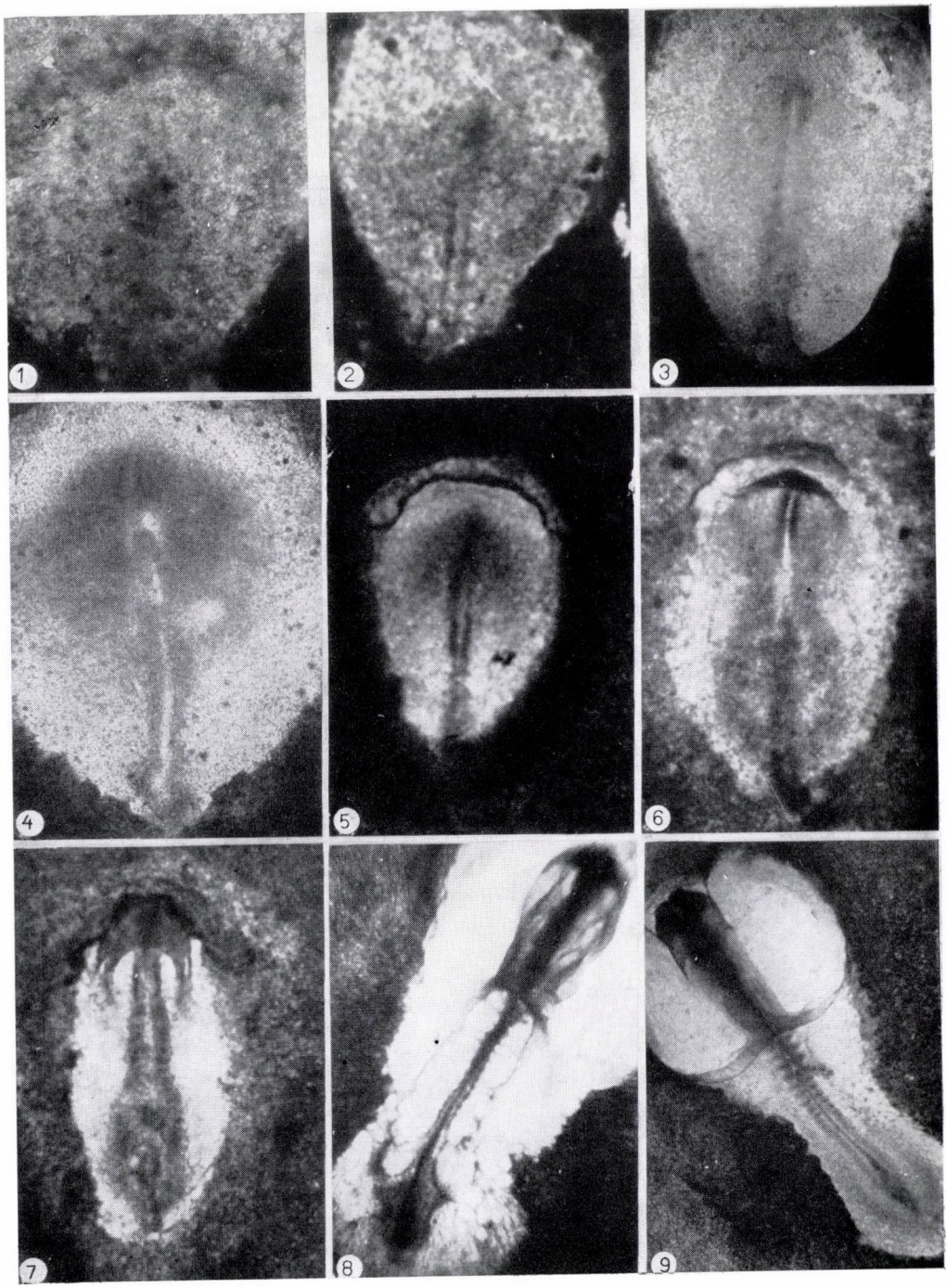
Janus green B stains the mitochondria electively, presumably by being linked to a specific substance therein. When the stain affects energy-consuming biochemical processes, it probably disturbs energetic processes by virtue of its specific binding point. Starting from this assumption we have shown the carcinogenic action of Janus green B [1], the effect of the dye as an interphase poison [2, 3], its teratogenic [4] and leukaemogenic [5] action, as well as its so-called biological oxygen effect [6, 7]. We succeeded in isolating and chemically analysing the lipoprotein-enzyme-dye complex which arises as a result of the elective staining with Janus green B and which plays a central role in biological oxidation.

Binding of the dye and its reduction to leukosafranine requires the presence of mitochondria and is indicative of biological oxidation. Reports concerning the embryological aspects of this phenomenon are scarce [12 through 18].

Material and method

The eggs of Leghorn hens were incubated at 38°C from zero to 72 hrs at constant vapour content. After isolation of the embryonic disk under physiological conditions, the embryo was freed from the vitelline membrane and incubated for 60 to 90 min. under aerobic and partially anaerobic conditions in microchambers with abruptly changeable gas phase. Prior to incubation the epiblast and hypoblast were treated with 6.25×10^{-3} to 6.25×10^{-4} M Janus green B in great excess. Binding and reduction of the dye were recorded by means of coloured photographs and moving pictures. Control examinations were performed in vivo by means of the indophenol test, with and without sodium azide inhibiton, the formazan

Table 1



reaction, with and without malonic acid inhibition. Fixed preparations were also made to register the early phases. The fixing fluid contained 1800 ml of saturated picric acid solution, 700 ml of neutral formalin, 66 ml of glacial acetic acid and 1300 ml of 5 per cent trichloroacetic acid. The sections were embedded in methyl benzoate — celloidin paraffin, and stained with haematoxylin — acid fuchsin — "tuchechtgelb" and iron haematoxylin. The dye in the applied concentrations were not teratogenic under anaerobic conditions and allowed protracted observation.

Results

Although the entire surface of the embryonic disk had been covered with Janus green B, separately placed and differently shaped areas intensive bluish green in colour appeared on the blastoderm after 12 to 15 min. Location and size of these areas were invariably identical in identically developed embryos. While the whole blastoderm was exposed to the dye, the latter was bound and reduced under partially anaerobic conditions to its leuko form through a phase of cherry-red diethylsafranine at certain "typical" points only. It was possible to follow the three-stage reduction of the dye *in situ*.

After Janus green B treatment of the epiblast and hypoblast, the mitochondrial dehydrogenase areas showed the following distribution in the course of morphogenesis. At the stage of the early primitive streak, diffuse mitochondria were found over the entire substance of the streak; they were more closely packed at the caudal base (Table 4, Figs. 1—4). After 10 to 12 hours the mitochondria were grouped along the axis of the primitive streak: a phenomenon which proves that the primitive streak corresponds to the blastopore.

Table 1

Fig. 1. Chick embryo No. 3529. Conical early primitive streak with wide base, after 13 1/2 hrs incubation

Fig. 2. Chick embryo No. 3539 after 16 1/2 hrs, at the middle stage of primitive streak; maximal enzymatic activity in the lateral mesoderm

Fig. 3. Chick embryo No. 3538 after 15 hrs, at the stage of long primitive streak. Absence of mesodermal enzymatic activity cranio-lateral from Hensen's node

Fig. 4. Chick embryo No. 3845 after 20 hrs; early head process. Enzymatic activity in Hensen's node and the surrounding foliage-like preaxial mesoderm, as well as in the upper third of the primitive streak, in the fields of the lateral mesoderm next to the primitive streak

Fig. 5. Chick embryo No. 3563 after 21 1/2 hrs. Stage of late head process. Marked enzymatic activity in the head process, in Hensen's node, on both sides of the primitive streak and in the preaxial and paraaxial mesoderm. Incipient regression of primitive streak

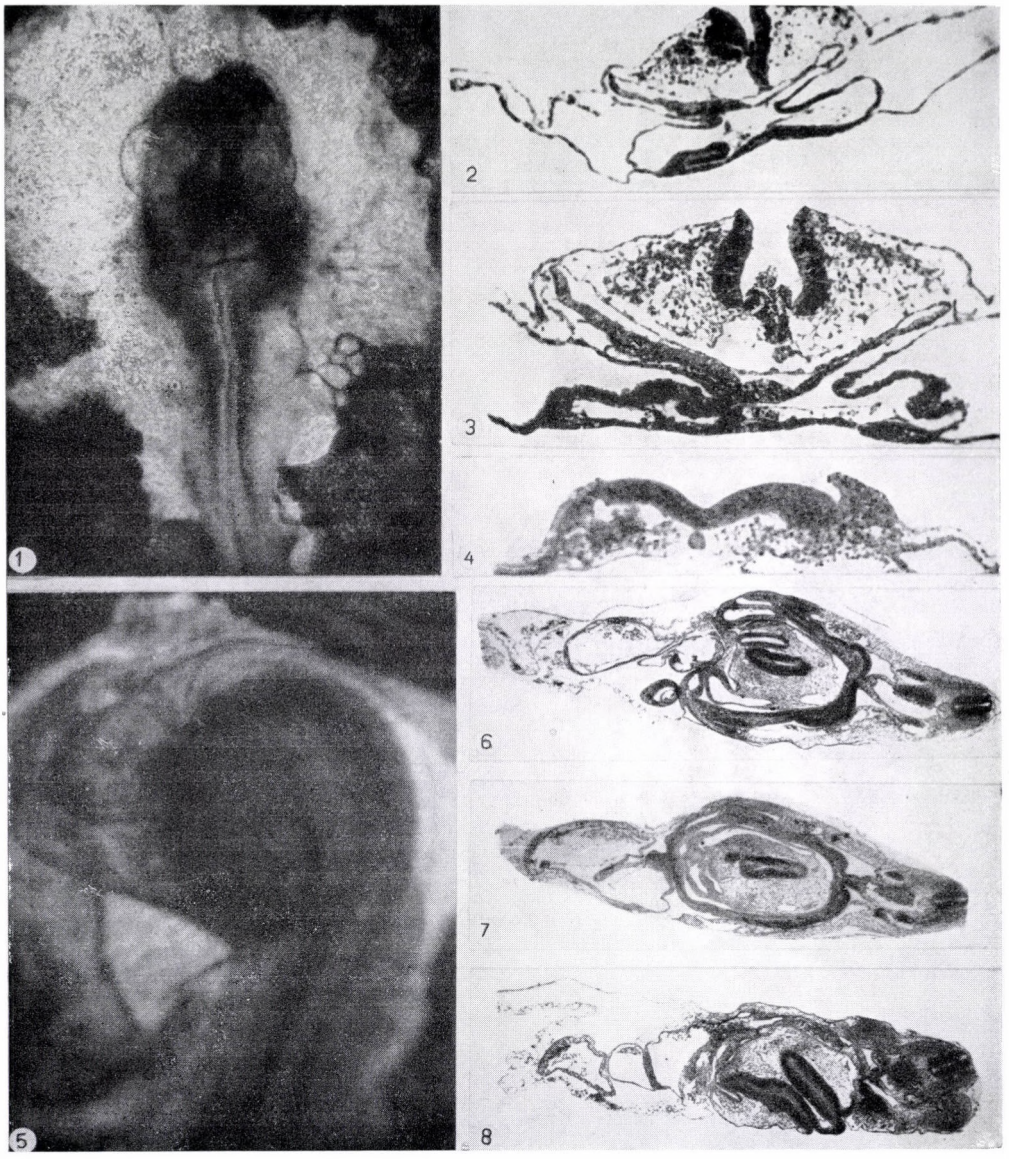
Fig. 6. Chick embryo No. 3445, after 22 1/2 hrs., at the stage of early head fold. Intensive enzymatic activity at the anterior opening of the foregut, in Hensen's node and the caudal part of the primitive streak

Fig. 7. Chick embryo No. 3535 at 27 hrs — four pairs of somites. Intensive enzymatic activity in the cephalic segment, in the somites, in the unsegmented mesoderm, and especially in Hensen's node

Fig. 8. Chick embryo No. 3486. Treatment of the internal lamina of the membrana serosa with 0.05 ml of 6.25×10^{-4} M Janus green B at zero hour of incubation. Situation at 48 hrs at the 18 somite stage, with marked hydropic degeneration in the somites and also paraaxially

Fig. 9. Chick embryo No. 3349. Fenestration at zero hour of incubation; treatment of embryonic disk with 0.05 ml of 6.25×10^{-4} M Janus green B; closure. At 35 hrs, the three brain vesicles are perceptible and the heart shows a slight displacement to the right. As a result of hydropic degeneration, there is subectodermal vesiculation cranially to the edge of the entrance of the foregut in the symmetrical part corresponding to the expanded area of the subcephalic pouch

Table 2



As from the 14th—16th hour, the staining extended into the deeper axial and later to the lateral mesodermal layer; movement of the tissue from the paraaxial areas of the epiblast through the linear axis of the primitive streak could be followed to the deeper layers. Dye uptake and reduction could be observed in a centrifugal direction but in a deeper layer (Table 1, Fig. 2; Table 4, Figs. 3, 4). Hensen's node displayed a more pronounced mitochondrial dehydrogenase activity from the 12th hour; this persisted until the formation of the primitive streak. It was especially intensive before the formation of the head process, and persisted through the entire course of regression (Table 1, Figs 1—7; Table 4, Fig. 8). It follows that regression is not a passive mechanical phenomenon concomitant to the elongation of the head process and to the formation of the notochord, but is actively involved in the progress of morphogenesis. This is further indicated by the fact that cranio-caudally advancing symmetrical areas could be observed in the epiblast during the whole period of regression.

Staining of the head process became marked only when it induced the development of the medullary plates above it (Table 1, Figs. 5, 6; Table 4, Figs. 3—4).

It is a decisive and invariable rule that the entrance and the marginal parts of the foregut stain intensively and selectively when the medullary folds emerge from the embryonic plane; this intensive staining persists after the mitochondrial dehydrogenase activity has extended to the ependyma of the brain ventricles at the incipient three-vesicle stage (Table 1, Fig. 7; Table 4, Figs. 5, 6).

The same phenomenon can be seen on both sides of the prosencephalon prior to the protrusion of the brain vesicles. Intensely staining areas are to be found on the ectoderm itself, at the entrance of the foregut and on both sides laterocranially to it (Table 5, Figs. 5, 6).

The applied staining has decided the problem whether the terminal bud and the tail bud should be regarded as separate centres of organization. The

Table 2

Figs 1 to 4. Chick embryo No. 3508. Internal lamina of serous membrane treated with 0.05 ml of 6.25×10^{-4} M Janus green B at zero hour of incubation. After 51^{h} 45' of incubation, a craniorachischisic monster (corresponding to an embryo after 35 to 38 hrs of incubation) was found. Patency of the neural groove as well as the excessive proliferation of the neural crest (Fig. 3) and the medullary plate (Fig. 1) are well distinguishable in the cross sections. Fig. 2 shows the cross section of the distended capillary which invades the neural groove. Triazide fixation, iron haematoxylin staining. Microtar photograph

Fig. 5. Chick embryo No. 3395. 6.25×10^{-4} M Janus green B at zero hour; the egg was opened after 67 hrs of incubation, and a living omphalocephalic embryo was found. It had irregular pulsating tubes arranged stepwise in the mesencephalic area

Fig. 6. Cross section of Fig. 5. Note the unilocular, endothelially lined myoepithelial pocket beside a portion of the dysplastic brain

Fig. 7. Biventricular cardiac tube from Fig. 5

Fig. 8. Detail from Fig. 5, Triventricular cardiac tubes in the area of rachischisic hindbrain. Figs. 6—8: triazide fixation, iron haematoxylin staining. Microtar photographs

facts that the remnant of the primitive streak retained its intensive enzymatic activity through the whole course of regression, the movement of the paraaxially arranged symmetrical areas, and that the remnant of the primitive streak was still able to convert the indifferent tissue elements of the blastoderm's caudal portion, argue in favour of the supposition that one is dealing with separate organizatory centres. Therefore, the theory that the tail bud serves only for a rearrangement of tissues cannot be accepted.

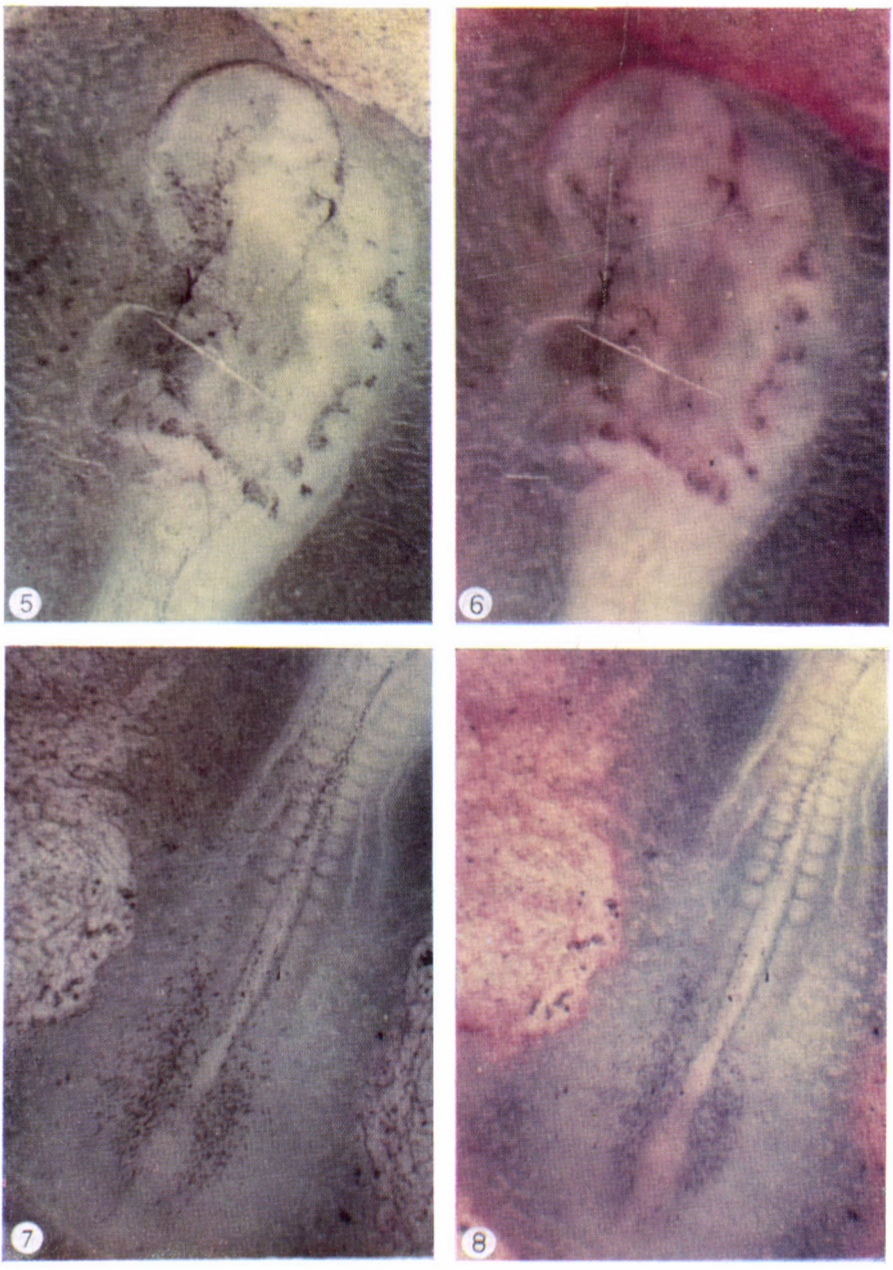
Higher concentrations of Janus green B in the air and oxygen phase have been applied in the present investigations in order to observe its teratogenic effect. The freaks shown in Table 2 originate partly from the omphalocephalic group of monsters. The phase-specificity of teratogenesis evidently depends on the position of the mitochondrial dehydrogenase areas since the developmental disturbance of these presumptive areas, caused by concentrated Janus green B, dominates the subsequent morphological picture.

Discussion

Mitochondrial distribution in the early stage of morphogenesis has been observed in normal control chick embryos in the course of the present investigations. The dehydrogenase is strongly bound by the mitochondria. It is clear from our experiments as also from those of LAZAROV and COOPERSTEIN [12] and SHOWACRE and DUBUY [18] that reduction of Janus green B is indicative of dehydrogenase activity. The difference between the rates at which the dye is taken up and reduced (Table 4, Figs. 5—8; Table 3, Fig. 3) allows the conclusion that morphogenesis has three stages. In the first, the enzyme is synthesized (the dye is bound but not reduced); this is followed by enzymatic action (reduction of the dye) which precedes the phase of morphologic changes. Morphological transformation is, thus, always preceded by a two-stage submicroscopic or molecular process. ATP is synthesized in the mitochondria which are, therefore, decisive factors in the metabolism of energy-consuming processes of growth; their distribution admits, thus, of certain conclusions to the controlling role played by the mitochondria in the course of embryogenesis. This metabolic role of the mitochondria is obvious if we consider the great difference in the quantity of ATP produced from the same amount of glucose in the course of glycolysis and in that of oxidative phosphorylation.

It has been noted in the foregoing that Janus green B is linked to a particular lipoprotein of the mitochondrium, a property which facilitates the study of the metabolic role of mitochondria. GREEN et al. [8—11] have shown that it is possible to split the mitochondrium into further enzymatically active particles. They claim that all substances (alcohols, detergents, etc.) which act upon the lipides of a macromolecular system are capable of detaching active particles from the mitochondrium. They demonstrated the presence of three

Table 5



Figs. 5—6. 45 1/2 hours chick embryo No. 5799 at the onset of the reaction
Figs. 7—8. Ditto after 30' of reduction

Table 5

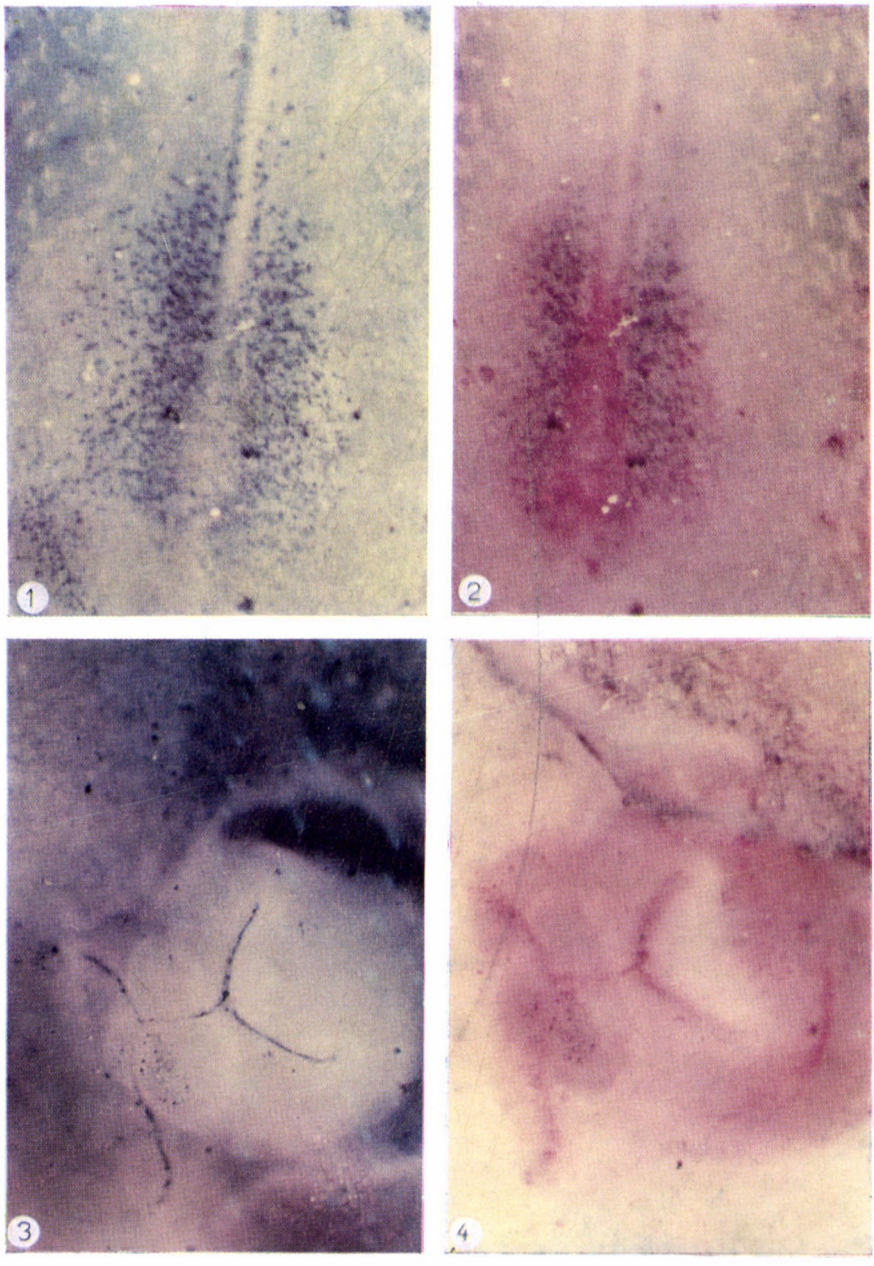


Fig. 1. 48 hours chick embryo (No. 5800) at the onset of the reaction
Fig. 2. Ditto after 35' of reduction
Figs 3—4. Chick embryo No. 5802. The internal lamina of the serous membrane was treated with 0.05 ml of 6.25×10^{-4} M Janus green B at the beginning of incubation. Result after 29 hrs: pygopagus in the early phase of development. Activity in Hensen's node. Maximum activity of dye in the primitive streak after 15 min. of partially anaerobic phase. Cherry-red reduction after 35 min. of partially anaerobic treatment. No dye reduction in the backward bent extra-embryonic parts

Table 4

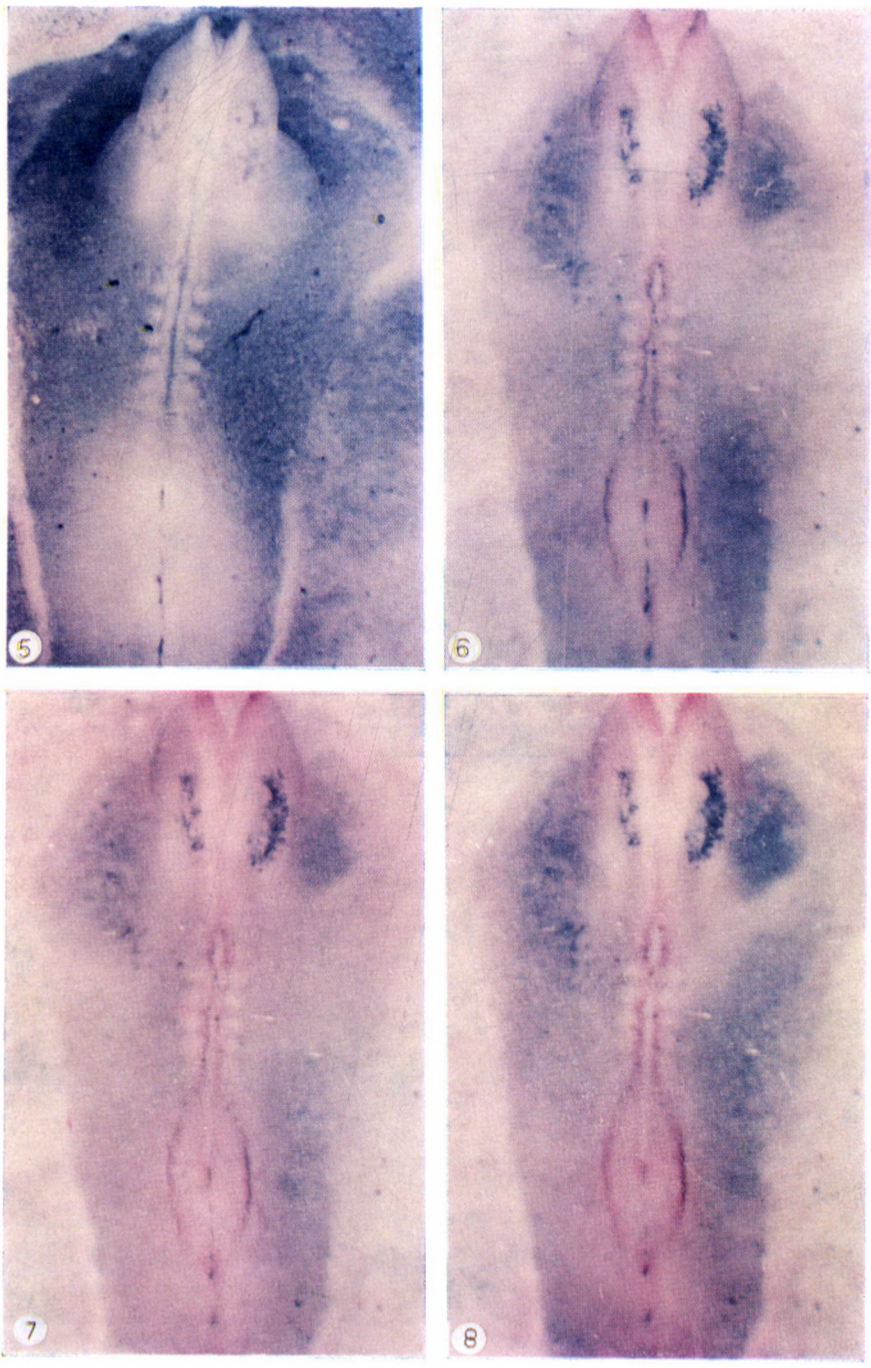


Fig. 5—8. 30 hours chick embryo (No. 5803): reduction from the beginning of the reaction till the 30th min. at 53' (not represented here), complete reduction of the dye, except in the blue areas

Tables 3 — 5

The figures of these tables show early living chick embryos deprived of vitelline membrane, treated with 3.125×10^{-4} M Janus green B. After an air-phase incubation of 15 min., the embryos were placed under partially anaerobic conditions. Reduction of the dye and rate of dehydrogenase activity are recorded on photographs

Table 3

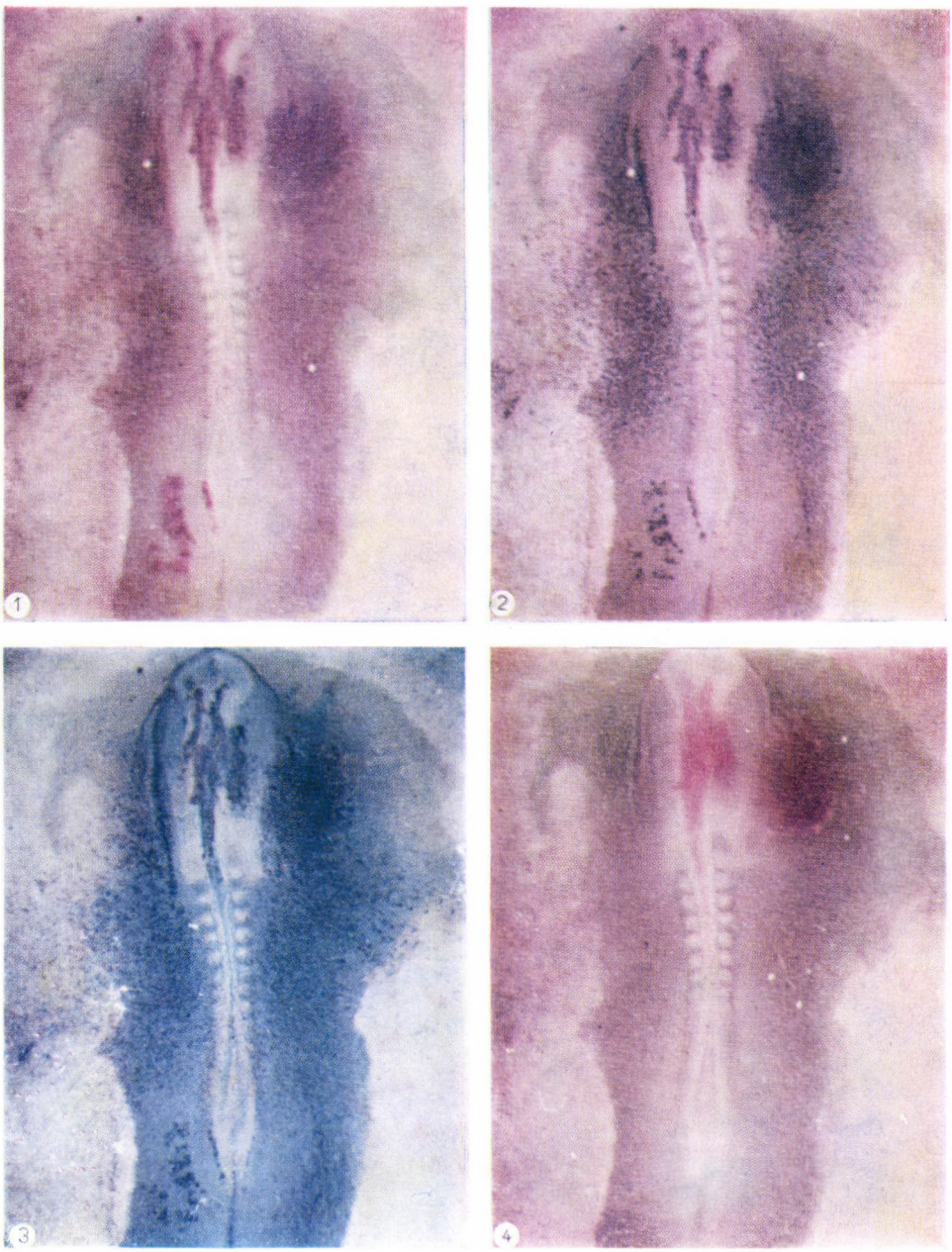


Fig. 1. 33 hours chick embryo at the onset of the reaction; *Fig. 2.* Ditto after 4' of reduction *Fig. 3.* Ditto after 10' of reduction; *Fig. 4.* Ditto after 30' of reduction

Table 4

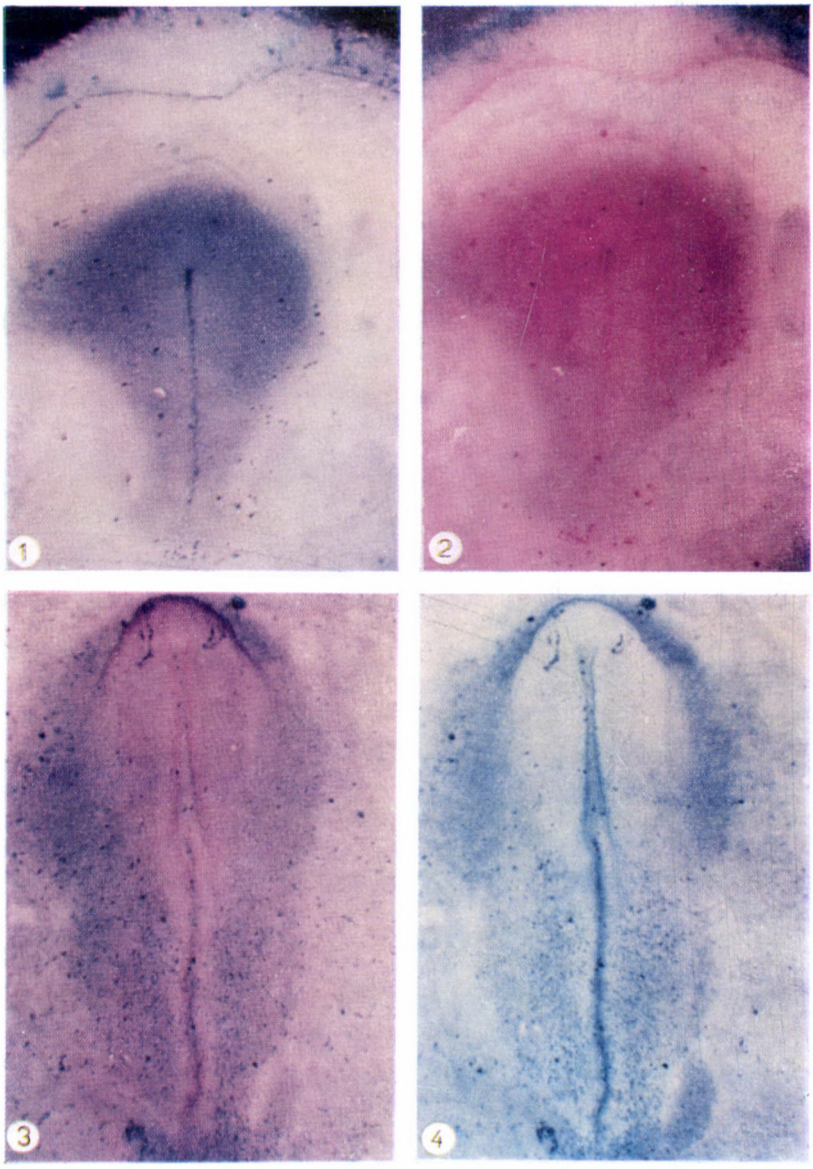


Fig. 1. 16 hours chick embryo (No. 5891) after 10' of reduction
Fig. 2. Ditto after 35' of reduction
Fig. 3. 23 hours chick embryo (No. 5809) after 7' of reduction
Fig. 4. Ditto after 11' of reduction

specific lipoproteins in the mitochondrium each of which binds a respiratory enzyme so that the lipoproteins may be regarded as functional bridges for electron transport. What happens if to this system of lipoproteins Janus green B is linked, a substance which has a special affinity for this system and is, therefore, the uncoupling substance of oxidative phosphorylation? A pathologic shunt is formed so that the energy, gaining access to another part of the macromolecular system, releases a pathologic process the teratogenic results of which (in cases of adequately high doses) are well illustrated by the accompanying pictures.

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DIE ROLLE DER MITOCHONDRIEN IN DER FRÜHEN MORPHOGENESE DER HÜHNEREMBRYONEN

S. BRAUN

Wenn das Blastoderm von frühen Hühnerembryonen mit Janusgrün B (JgB) behandelt wird, bilden sich Mitochondrien-Dehydrogenase Felder, die typisch sind für das jeweilige ontogenetische Stadium. Der Farbstoff wird reduziert als Zeichen der Dehydrogenase-Aktivität. Der Unterschied zwischen der Rate bei welcher der Farbstoff in der frühen Entwicklungsphase aufgenommen wird und der, bei welcher er reduziert wird, rechtfertigt die Annahme, dass die Morphogenese ein dreistufiger Vorgang ist. Den morphologischen Veränderungen gehen immer zwei Phasen von submikroskopischen oder molekularen Transformationen voraus. Bei hohen Konzentrationen wirkt JgB teratogen. Die infolgedessen entstandenen Missbildungen sind phasenspezifisch und entsprechen den jeweiligen detektierten präsumptiven Feldern. JgB ist an ein spezifisches Lipoprotein der Mitochondrien gebunden und wirkt auf die Energie verbrauchenden biologischen Prozesse ein. Die Entkopplung der oxydativen Phosphorylierung verursacht einen pathologischen Shunt, welcher für die teratogene Wirkung verantwortlich ist.

ЗНАЧЕНИЕ МИТОХОНДРИЕВ В РАННЕМ МОРФОГЕНЕЗЕ КУРИНОГО ЭМБРИОНА

Ш. БРАУН

В ранней стадии морфогенеза под влиянием различных концентраций Янус грын-Б введенного на бластодерму куриного эмбриона возникают поля митохондриевой дегидрогеназы, соответствующие данной стадии. Краска — как признак активности дегидро-

геназы — расщепляется. В отдельных стадиях раннего развития из разности между скоростью связывания краски и скоростью ее расщепления можно делать выводы о трехступенчатом процессе морфогенеза. Согласно этому морфологически видимому изменению во всех случаях предшествует двуступенчатое субмикроскопическое или молекулярное изменение. В случае большей концентрации вышеуказанного агента возникают хорошо определяемые живые уроды. Здесь пресумпхондрий связывается к специфическому липопротеину и оказывает влияние на нуждающиеся в энергии биологические процессы. Разрушением окислительной фосфорилации возникает патологический шунт, ответственный за тератогенное действие.

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PHENOL REACTION OF COLLAGEN FIBRES AND ACID MUCOPOLYSACCHARIDE CONTENT OF CONNECTIVE TISSUE

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The phenol reaction of the collagen fibres in various kinds of connective tissue has been studied and it has been found that the phenol reaction permits conclusions as to the close correlation between the collagen protein and acid mucopolysaccharides, on the one hand, and the quantity of acid mucoid substance, on the other.

A close correlation is assumed to exist between the acid mucopolysaccharide (MPS) components of the connective tissue and the collagen fibres. JACKSON [2] has shown the important role of the MPS of the ground substance in the stabilisation of the collagen fibres.

Under natural conditions the collagen fibres show positive birefringence alongside their longitudinal axis [8]. The direction and size of that birefringence is influenced by numerous factors, such as EBNER's [1] phenol reaction, among others. The essence of that sensitive and specific reaction is that the phenol molecules are oriented at rectangles to the micellary structure of collagen fibres. The intensity of the reaction in the function of phenol concentration has been studied by JOBST [3], who, by using a xylol-phenol solution, has found that the positive birefringence of the collagen fibres turns negative at a 2.4 per cent phenol concentration. The reaction is influenced by the variable microstructural properties of collagen. It has been shown by ROMHÁNYI [6, 7] that the acid compenents of collagen fibres play a significant role in the quality of the phenol reaction. After sulphuration the collagen fibres give no phenol reaction, or they become isotropic. This would mean that the SO_4 groups inhibit the orientation of the phenol molecules to the sulphurated collagen fibres, thus the phenol reaction may be influenced by the acid MPS of the connective tissue ground substance. The behaviour of the phenol reaction may accordingly be used for drawing conclusions as to the variability of the acid MPS content of collagen fibres, or as to their structural components. We have therefore studied the behaviour of the phenol reaction given by collagen structures of different acid MPS concentrations.

Materials and methods

Albino rats, weighing about 120 g, were used. The collagen fibres and acid MPS concentrations of various kinds of connective tissue (skin, bone, articular and epiphyseal cartilage, cornea, sclera and aorta) were studied. The tissues were fixed in 10 per cent neutral formalin, embedded in paraffin and cut into sections about 5 μ thick. Bones were decalcinated in 3 per cent sodium ethylenediamino tetraacetate. Collagen fibres precipitated in vitro by NÉMETH—CSÓKA's method [5] were used in the following way. The collagen fibres obtained from tail tandon were treated for 24 hours with 0.4 per cent acetic acid at 4°C, then the collagen solution obtained after centrifugation and filtration was precipitated in 5 per cent NaCl and 1 per cent chondroitin sulphate A. The precipitate was fixed in alcohol, embedded in paraffin and cut up into sections. After deparaffination the following topochemical reactions were carried out.

Phenol reaction. The sections used for this purpose were covered with Canada balsam containing 50 per cent phenol, while other sections were covered with Canada balsam containing phenol at increasing concentrations, as recommended by ROMHÁNYI. Some results were characterized by the phenol index [5], the quotient of the birefringence measured in Canada balsam containing 50 per cent phenol and that measured in pure Canada balsam; the quotient is independent of the fibre diameter.

Precipitation metachromatic staining [6]. The sections were stained with 0.02 per cent pH 4 toluidine blue and stabilized with 3 per cent potassium ferricyanide. The sections were examined in monochromatic red light and the metachromatic index of the structure studied was determined according to ROMHÁNYI.

Results

The sections were covered with Canada balsam containing increasing concentrations of phenol and the birefringence of the collagen fibres in the different kinds of connective tissue was studied in the function of phenol concentration. The phenol reaction was different in the different kinds of connective tissue. Whereas collagen structures marked in the ground substance of cartilage, and first of all in that of epiphyseal cartilage, showed inversion of birefringence at high phenol concentrations in connective tissues less rich in acid MPS the fibres turned negative at lower phenol concentrations. This was most marked in epiphyseal cartilage, in which the birefringence of collagen structures was still slightly positive at a phenol concentration of 7.5 per cent, when in the zone of calcification and the bone trabecules they showed negative birefringence, while the collagen fibres of the media turned merely isotropic. Of the collagen fibres those precipitated in 5 per cent NaCl turned isotropic at a phenol concentration of 5 per cent, while those precipitated in chondroitin sulphate A showed optical inversion at phenol concentrations higher than 6 per cent. At the corneal-scleral junction the collagen fibres of the sclera showed negative birefringence at a 6 per cent phenol concentration whereas those of the cornea turned merely isotropic at that concentration (Fig. 1).

To demonstrate that it is the acid side radicals that act as inhibitors in the phenol reaction, some sections were treated with testicular hyaluronidase (Permease, Gilag), and others were methylated [4]. After such treatment the birefringence of the collagen fibres of the above mentioned kinds of connective tissue turned negative at the same point and at lower phenol concentration. The latter could be observed mainly in connective tissues otherwise rich in acid

MPS, whereas the collagen fibres of connective tissues possessing originally hardly any acid MPS ground substance gave a practically unaltered reaction (Fig. 2). The effect of hyaluronidase and methylation was controlled by staining with pH 4 toluidine blue.

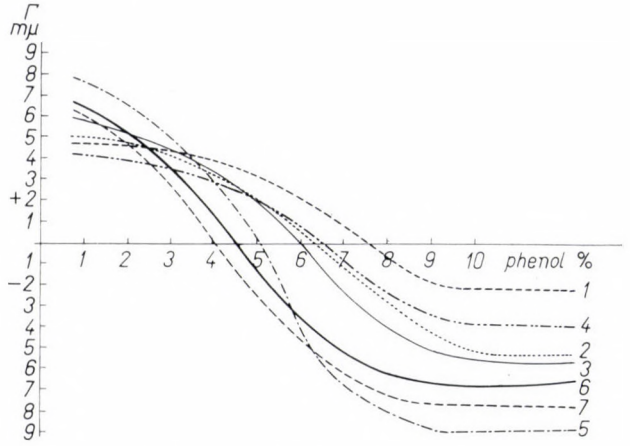

Fig. 1. Phenol reaction of collagen fibres differing in acid MPS content
1. Epiphyseal cartilage — 2. Collagen fibres reconstr. in ChS A. — 3. Cornea — 4. Aorta media — 5. Collagen fibres reconstr. in NaCl — 6. Aorta adventicia — 7. Skin

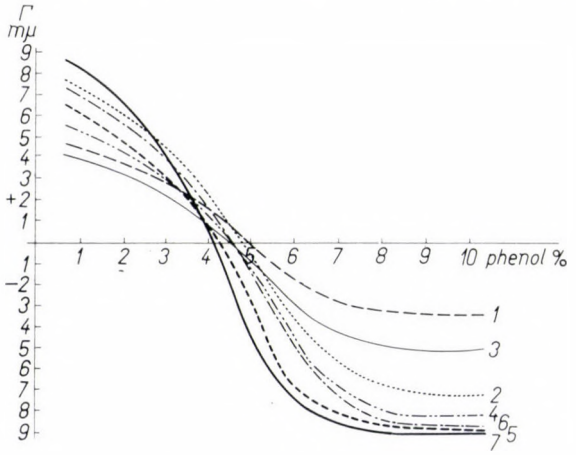

Fig. 2. Phenol reaction in different types of collagen fibres following methylation and following testicular hyaluronidase treatment
1. Epiphyseal cartilage — 2. Collagen fibres reconstr. in ChS A. — 3. Cornea — 4. Aorta media — 5. Collagen fibres reconstr. in NaCl — 6. Aorta adventicia — 7. Skin

In the next step the phenol value and the metachromatic index of the various kinds of connective tissue collagen structures were compared. It was found that the kinds of connective tissue showing a high metachromatic index

had a low collagen fibre phenol index, and vice versa. Following hyaluronidase treatment or methylation the phenol index increased. The metachromatic index varied between 4 and 5 in the epiphyseal and articular cartilage, as well as in the sclera, with collagen fibres precipitated in chondroitin sulphate A, and those of the aortic media, while the collagen fibres of these kinds of connective tissue showed a phenol index lower than 1. As compared to these tissues, the connective tissues poor in acid MPS had a much lower metachromatic index, and a phenol index higher than 1. Following hyaluronidase treatment and methylation the phenol index increased to above 1 in the collagen fibres of the tissues high in acid MPS.

Discussion

It has been assumed that the behaviour of the phenol reaction of collagen fibres would be influenced by the acid, first of all by the sulphate, groups of the connective tissue ground substance, allowing conclusions as to the correlation between the collagen fibres and the acid MPS components of the connective tissue.

It has been pointed out by ROMHÁNYI that after sulphuration the collagen fibres give no phenol reaction, since the acid groups inhibit the oriented association of phenol molecules to the collagen fibres, and, also, that for example in the embryonic cartilage, high in acid mucoid, the collagen structures show possess a slight phenol effect [7].

The important role played by the acid mucopolysaccharides of the ground substance in the stabilization, maturation and physico-chemical behaviour of the collagen fibres has been extensively investigated, first of all by JACKSON, and a close correlation has been assumed to exist between the collagen structure and the acid components of its ground substance, although the exact nature of this correlation has not been clarified.

We have shown that the collagen fibres of connective tissue possessing a ground substance high in acid MPS yielded a weak phenol reaction. A similar observation was made by NÉMETH—CSÓKA; in his experiments the collagen fibres precipitated in chondroitin sulphate showed lower phenol values than the fibres precipitated in other agents. VISZLÓY has found that in patients suffering from osteogenesis imperfecta, a condition in which the acid components of the ground substance are increased, the collagen fibres turned negative at higher phenol concentrations only [9].

According to our experiments the phenol reaction of the collagen fibres is influenced by the acid MPS content of the ground substance. On this basis it has been surmised that in connective tissues containing a large quantity of acid MPS the acid groups inhibit sterically the oriented association to the

collagen fibres, of the likewise acid phenol molecules. In view of this a close correlation has been assumed to exist between the collagen fibres and the acid MPS components of the ground substance. In addition, the present investigations allow conclusions as to the quantity of acid MPS firmly bound to the collagen fibres in the various kinds connective tissue.

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CÜBER DIE WIRKUNG DES SAUEREN MUKOPOLYSACCHARIDGEHALTS AUF DIE PHENOLREAKTION DER KOLLAGENFASERN

T. NEUMARK

Anläßlich der Untersuchung der Phenolreaktion der Kollagenfasern der verschiedenen Gewebearten wurde festgestellt, daß aus der Intensität der entstandenen Phenolreaktion teils auf den zwischen dem Kollageneiweiß und den saueren Mukopolysacchariden bestehenden engen Zusammenhang, teils auf die Menge der saueren Mukoidsubstanzen gefolgert werden kann.

ФЕНОВАЯ РЕАКЦИЯ КОЛЛАГЕНОВЫХ ВОЛОКОН В ЗАВИСИМОСТИ ОТ СОДЕРЖАНИЯ КИСЛЫХ МУКОПОЛИСАХАРИДОВ В СОЕДИТЕЛЬНОЙ ТКАНИ

Т. НЕЙМАРК

Автор исследовал феноловую реакцию коллагеновых волокон различных видов соединительной ткани и нашел, что по феноловой реакции можно делать выводы относительно тесной связи белков коллагена с кислыми мукополисахаридами и относительно количества кислого мукоидного вещества.

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HISTOCHEMICAL EXAMINATION OF VAGINAL EPITHELIUM AND ENDOMETRIUM WITH SPECIAL REFERENCE TO MUCOPOLYSACCHARIDES

F. Tóth and R. Gimes

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Glycogen, acid and neutral mucopolysaccharides have been studied in the cells of the vaginal epithelium and endometrium. Alkaline and acid phosphatase, non-specific esterase, succinic-lactic-dehydrogenase and diphosphopyridine-nucleotide reactions have been performed. It was found that the superficial cells of the vaginal epithelium as also the cells in layers 1, 2 and 3 contained, in addition to glycogen, large amounts of neutral and acid mucopolysaccharides. Vaginal epithelial cells contained the largest amount of glycogen in the periovulatory period; it was associated with a high level of oestrogen. Glycogen in the endometrium showed a peak concentration in the secretory phase; it was associated with a high level of progesterone. Acid mucopolysaccharides were present in all phases of the menstrual cycle. They were comparatively abundant in the intermediary layer of the vaginal epithelium and the endometrium during the periovulatory period. Neutral mucopolysaccharides accumulated in the endometrium in the second half of the secretory phase and filled even the peritubular lymph spaces. Metabolic changes observed in the different phases of the menstrual cycle are ascribed to circulatory changes in the vaginal epithelium and the endometrium. It is suggested that, among others, local factors of circulation may be responsible for the identical changes produced by two different hormones (progesterone and oestrogen) in the glycogen and mucopolysaccharide contents of the vaginal epithelium and endometrium.

Stockard and Papanicolaou [54] in 1917 were the first to describe cyclic changes in the vaginal epithelium of guinea pigs. Ten years later, Dierkes [15] published his observations on human material; he found a correlation between the menstrual cycle and the changes occurring in the vaginal epithelium, and demonstrated that, like the endometrium, the vaginal epithelium gave a typical response to sex hormones. Of especial value was the observation that vaginal smears allowed conclusions as to the quantity and composition of the sex hormones. Since then, a great number of authors has discussed the morphology, enzymology and histochemistry of the vaginal epithelium, and it would seem that nothing has been left unelucidated in this field after the detailed investigations and painstaking researches of Botella-Llusia, De Brux, Graham, Haam, Koss, Murray, Pundel, Rosa, Runge, Shorr, Stoll, Wied, Zinser and numerous others. Yet, it appears that the mechanism through which the response of the vaginal epithelium to hormonal influences is mediated has not been cleared up. Hormones exert no direct effect on epithelial cells. Moreover, the origin and composition of the substance termed "horn", contained in the cytoplasm of superficial cells, are likewise unknown,

so that a number of histological and morphological problems have still to be solved. This study had the aim to approach the solution of these problems.

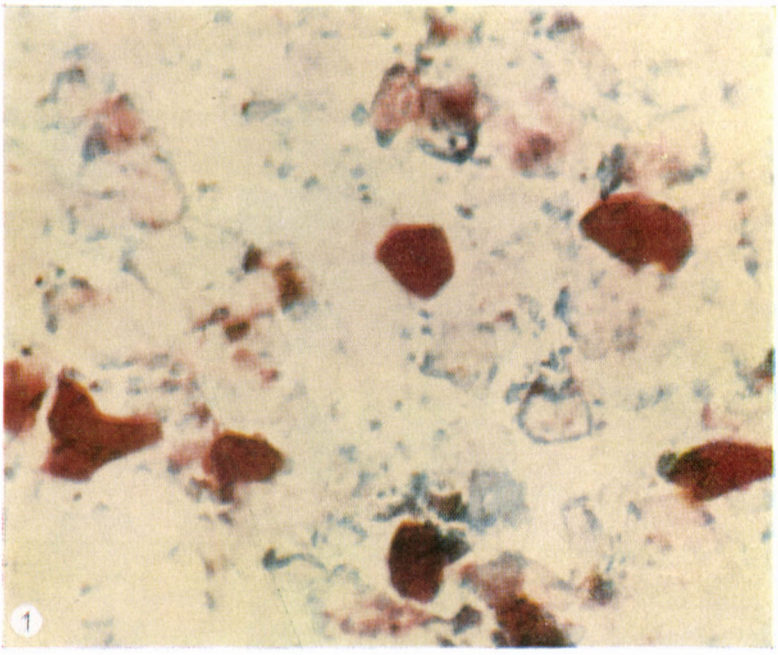
Material and method

Fifty-five healthy young female subjects, in different phases of the menstrual cycle, constituted the material of this study. Endometrial biopsy specimens were collected from 18 of them. Fifteen of the females were pregnant; two of them were in the first 3 weeks of pregnancy which was revealed by examining the curettings. Serial examination of vaginal smears was performed in the other 13 cases; the smears were obtained every other day from the boundary between the middle and the upper third of the vagina. Vaginal smears, intended for enzymatic examinations, were immediately placed in incubating solutions, while material intended for the determination of glycogen and polysaccharides in 96 per cent alcohol and Shabad's fixative which latter contains $Zn(NO_3)_2$ for the inhibition of bacterial digestion and phloridzin (L. Light and Co., Ltd.) in a mixture of 10 per cent formalin and 96 per cent alcohol for the inhibition of glycolysis. A 10 per cent solution of neutral formalin served as fixative in most cases. For staining, Schiff's periodic acid (PAS); Ritter-Oleson's dye; Best's carmine; 0.25 per cent toluidine blue; methylgreen-pyronine; Feulgen's reaction; Papanicolaou's stain; gallocyanin; Fettrot (fat-red); Nile blue; haematoxylin-eosin; Van Gieson's dye and Azan's dye were applied. Digestion was performed with ribonuclease, diastase, saliva, hyaluronidase, pepsin, trypsin and combined pepsin-trypsin were applied. For the purposes of PAS reaction, oxidation with 0.23 per cent sodium periodate during 5 minutes and staining with Schiff's reagent during 60 min. was applied. Ritter-Oleson A, B and C indicate the results of acetylation; material *A* was dyed without preliminary treatment; material *B* was stained after acetylation; material *C* was first acetylated, then saponified and finally stained. Hyaluronidase digestion was performed with 150 U/ml, in physiological saline, at 37°C for 16 hrs. A 1 per cent solution of diastase (L. Light and Co., Ltd.) in 0.01 M phosphate-buffered 0.8 per cent solution of NaCl was employed for digestion which lasted 2 hrs at 37°C. Pepsin digestion was made at 37°C by means of a 2 mg/ml solution in 0.02 N hydrochloric acid for 2 hrs. Digestion with trypsin, at a concentration of 1 mg/ml in pH 8 phosphate buffer was performed at 37°C for 2 hrs. In the combined procedure, digestion with pepsin for 6 hrs. was followed by trypsinization for 12 hrs. Alkaline and acid phosphatase as well as non-specific esterase reactions were carried out by means of the azo-kupplung method with naphthol-As-phosphate as substrate. The procedures of FUHRMANN [20] and ROSA [52] were employed for the succinic dehydrogenase (SDH), lactic dehydrogenase (LDH) and diphosphopyridinenucleotide-diaphorase (DPN) assay. As to the numbering of the layers of the vaginal epithelium, No. 1 means the superficial and No. 2 the next layer; the intermediate layer is divided into an upper (3a) and a deeper zone (3b); the parabasal layer bears the number 4, and the basal one is marked 5.

Results

Glycogen and mucopolysaccharides (MPS) in the vaginal epithelium

Cells from layers 1, 2 and 3 gave an intensive PAS reaction. The histochemical results for PAS-positive substances are assembled in Table 1. The fact that the brilliant red colour showed a slight paling after digestion with diastase, saliva, pepsin, trypsin and combined pepsin-trypsin (Fig. 1), permits of the conclusion that the cells of the three first layers contain glycogen as well as a large amount of MPS. An insignificant amount of finely granulated PAS-positive material was demonstrated in the cells of layers 4 and 5; most



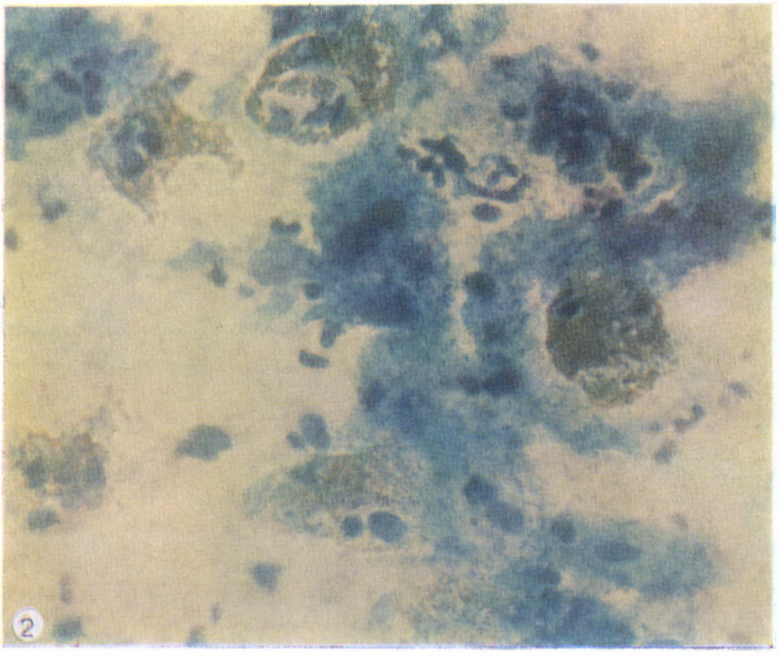


Fig. 1. Neutral mucopolysaccharides in superficial cells of vaginal epithelium. Ritter-Oleson staining after diastase digestion, × 160

Fig. 2. Fine metachromatic particles in a superficial epithelial cell. Toluidine blue, × 320

of this material disappeared after digestion with diastase. Staining with Ritter-Oleson's dye yielded the most intensive red colour in the cytoplasm of superficial cells with pycnotic nuclei. All shades of red, from purple to pink, were observed in the cells of the three upper layers. Other cells of these layers stained blue which would, with Hale's combined staining, indicate the presence of acid MPS. Treatment with toluidine blue actually revealed metachromatic granulations in the majority of these cells (Fig. 2). Granules of acid MPS often covered almost the entire nucleus. Their amount was largest in the cells of layers 1 and 2. These observations are in contradiction to those made in specimens of the vaginal wall. Here the PAS reaction was practically negative

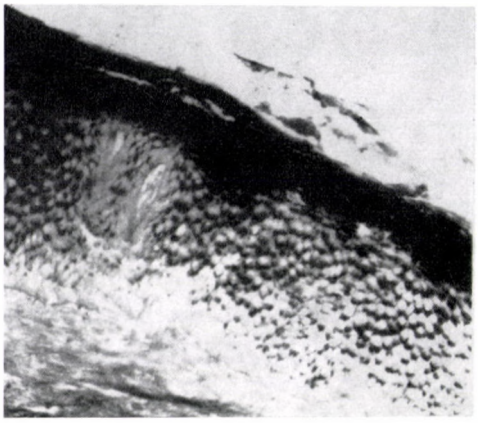


Fig. 3. Accumulation of mucopolysaccharides in layers 1 and 2 of biopsy specimen. Ritter—Oleson staining after diastase digestion, × 80

in layers 3b and 4, weak in layer 2, and practically unchanged in layer No. 1 (Fig. 3). The intercellular ground substance strongly adsorbed colloidal iron, gave a marked blue reaction with Ritter-Oleson's stain and a metachromatic one with toluidine blue which became much weaker after hyaluronidase digestion. The amount of the substance was largest in layer 3 and considerably less in layers 1 and 2.

The glycogen and MPS content of the vaginal epithelial cells showed no significant change under the effect of different hormones; it was comparatively highest in the 3rd and 4th months of pregnancy and considerably diminished in cases of colpitis. The excised material contained a large amount of glycogen in the 2nd and 3a layers at the preovulatory stage of the proliferative phase, while the amount of MPS increased toward the end of the secretory phase. The quantity of glycogen reached a peak in the 3rd and 4th months of gestation.

Ribonucleic acid

It was in the cells of layers 4 and 5 that the largest amount of pyroninophilic substances was found in both the biopsy and the exfoliative material (Fig. 4). Pyroninophilia practically disappeared after ribonuclease digestion. The amount of RNA rapidly diminished in the cells of the superficial layers. The nucleoles of the parabasal and basal cells contained pyroninophilic matter the bright red colour of which contrasted with the green staining of the desoxyribonucleic acid. The nuclei were surrounded by a narrow bright zone, and it was around this zone that the vividly red RNA was seen. Its amount, with a maximum in the 3rd and 4th months of pregnancy, did not seem to depend on hormonal influences.

Desoxyribonucleic acid

Like RNA, most of the DNA was located in the parabasal and basal layers. It formed a fine network in the nuclei, but gradually condensed into coarse lumps in the cells of the upper layers, to form structureless spherules in the superficial layer. Such condensation of DNA is the most characteristic oestrogenic effect which fails to appear in the menopause and in other cases of oestrogen deficiency (e.g. in Turner's syndrome).

Lipoids

A slight amount of larger or smaller granules reacting with "Fett-rot" was observed in the cells of the superficial epithelial layers. Both number and size of the granules diminished toward the deeper layers. It was, on the other hand, in the cells of layers 4 and 5 as also in the intercellular fibres of layer 3 that the largest amount of lipoid was recorded in the excised material. No correlation seemed to exist between the lipoid content of the cells and the action of hormones.

Enzyme reactions

These reactions were invariably weaker in the vaginal smears than in the biopsy material.

Alkaline phosphatase was negative in the vaginal smears and comparatively most intensive in the basal zone of the excised specimens. It became more pronounced in the proliferative phase, and reached a peak during ovulation. The endothelial cells of the vessel walls, too, gave a marked reaction.

Acid phosphatase activity in the desquamated cells seemed to be limited to the cytoplasm of the superficial cells with pycnotic nuclei, whereas a moderate activity was observed in the basal and parabasal cells and a stronger

reaction in layers 1 and 3 of the biopsy material. Activity became more pronounced during ovulation and toward the end of the secretory phase.

Non-specific esterase reaction was negative in the vaginal smears, and moderate in the superficial and basal layers of the excised material at the outset and termination of the menstrual cycle. Activity appeared also in layer 3a in midcycle.

Dehydrogenase, SDH, LDH and DPN. Activity in the biopsy material

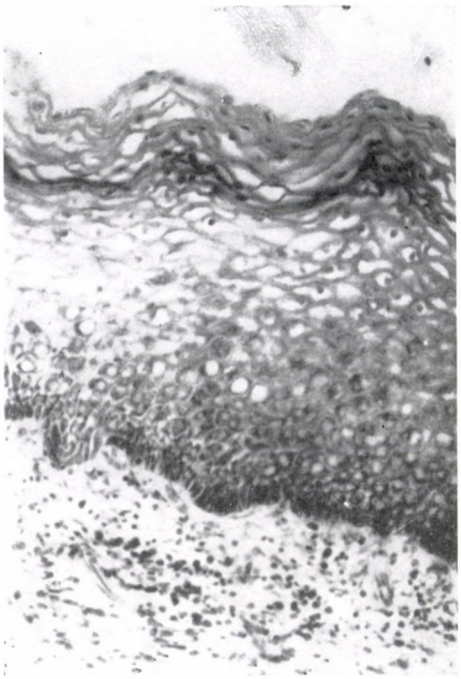


Fig. 4. Accumulation of pyroninophilic matter in the cells of layers 4 and 5. Methylgreen-pyronine, × 80

was strongest in the superficial and basal layers at the outset and termination of the cycle. Administration of oestrogen increased the intensity of reactions.

Endometrial glycogen and MPS

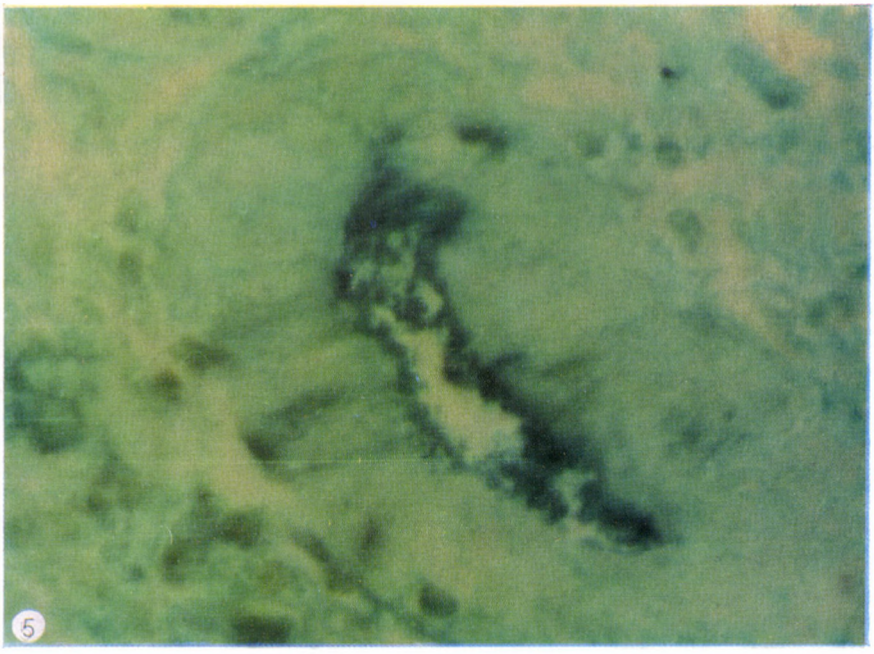
The amount of glycogen gradually increased in the epithelial cells of the endometrial glands as from the middle phase of the cycle. After forming fine granules in the basal portion of the epithelial cells, it filled the entire cytoplasm in a diffuse form. The greatest number of granules appeared in the second half of the secretory phase; they were situated subnuclearly and accumulated

in the basal part of the tubules of the endometrial glands. A moderate amount of acid MPS around the glands was found between the 8th and 10th day of the proliferative phase: the amount increased after ovulation. Club-shaped structures connected the apex of the epithelial cells with the matter contained in the lumen of the glands (Fig. 5). The various phases of secretion formation and transfer could well be observed under the microscope. Neutral MPS appeared in the second half of the secretory phase. Glycogen, acid and neutral MPS were observed side by side in the cytoplasm of the epithelial cells and the glandular lumina. The neutral MPS completely filled the peritubular lymph spaces in the second half of the secretory phase, a phenomenon particularly well observable in Ritter-Oleson-stained sections after digestion with diastase. The PAS-positive substance stained bright red and sharply outlined the distended peritubular lymph capillaries (Fig. 6). Only scattered fine powder-like particles of glycogen were seen in the ground substance. It was around the time of ovulation, between the 12th and 18th day the cycle, that the amount of acid MPS reached a peak. The major part of the MPS disappeared after treatment with hyaluronidase in the postovulatory period, a proof that hyaluronic acid was their main component. Hyaluronidase removed but a minor part of these MPS in the later stages of the menstrual cycle. It was in this phase that neutral MPS began to accumulate. The endometrium of pregnantes contained considerable amounts of glycogen, acid and neutral MPS. The amount of glycogen was many times more than its maximum in the secretory phase. The cytoplasm of decidual cells was almost completely filled with granules and lumps of glycogen (Fig. 7). The quantity of acid MPS did not diminish in the ground substance; neutral MPS began to accumulate in the placental villi from the 4th month of gestation. The amounts of RNA and DNA reached their respective maxima in the proliferative phase.

Changes in enzymatic reactions

It was only in the epithelial cells of the glands and the endometrial cylindrical epithelium that the examined reactions were strong enough for evaluation, while in the stroma occasional weak reactions were only observed.

Alkaline phosphatase in the endometrium was most pronounced in midcycle and during pregnancy, acid phosphatase in the second half of the cycle. In contrast, non-specific esterase was not pronounced in any phase. SDH activity was most intensive from the middle of the proliferative until the middle of the secretory phase. LDH and DPN activity increased rapidly from the time of ovulation and remained marked until the end of the secretory phase. LDH and DPN activity increased rapidly from the time of ovulation and remained marked until the end of the secretory phase. Dehydrogenase and acid phosphatase activities were strongest in the decidual cells during pregnancy.



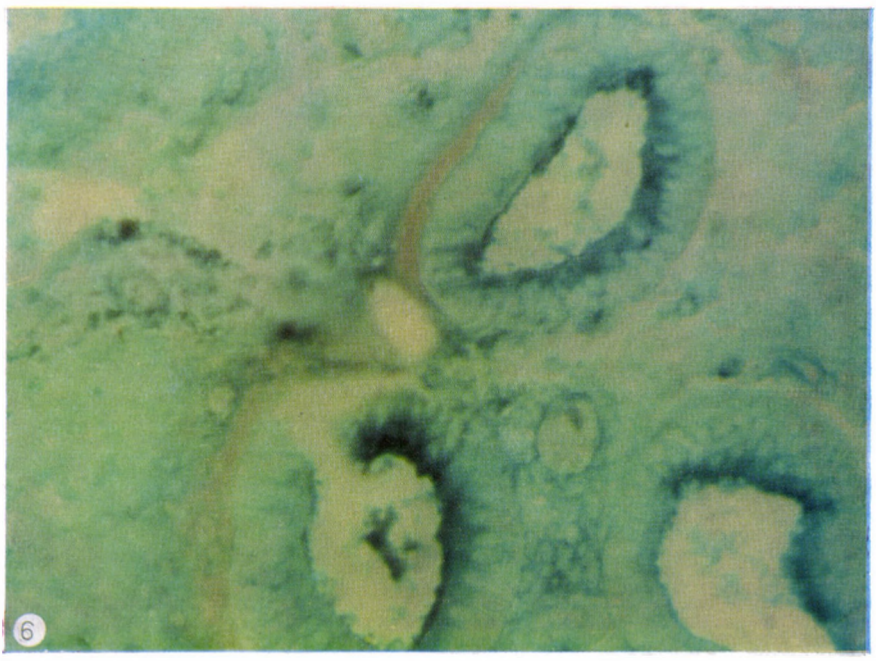


Fig. 5. Lumen of endometrial gland and apical portion of epithelial cells engorged with mucopolysaccharides. Ritter-Oleson staining after diastase digestion, × 400

Fig. 6. Red-staining PAS-positive matter fills and sharply outlines dilated peritubular lymph spaces. Ritter-Oleson staining after diastase digestion, × 160

Discussion

The present investigations had two aims, *viz.* [1] to study the various processes of metabolism by means of up-to-date methods and describe the properties of and the changes in the substances and enzymes involved; [2] to compare the results obtained and to present a brief summary after their comparative evaluation. The material covers wide range, and detailed treatment has therefore been reserved to certain new points. Comparative examination and evaluation of the glycogen and MPS contents of the vaginal epithelium and endometrium are unavoidable since, otherwise, different reactions to identical hormones cannot be interpreted. We have omitted to expatiate

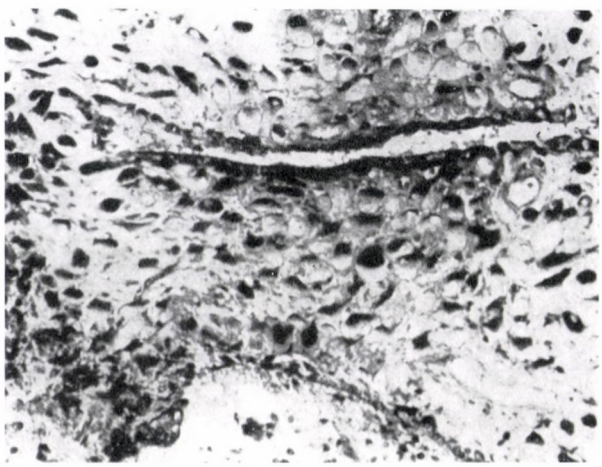


Fig. 7. Cytoplasm of decidual cells almost completely filled with glycogen granules. PAS, × 160

upon known results [5, 8, 9, 10, 13, 17, 19, 21, 22, 27, 35, 38, 42, 43, 46, 50, 51, 52].

Data as to the glycogen and MPS-contents of the vaginal epithelium and endometrium and the changes produced by different hormones are contradictory [2, 3, 4, 5, 7, 11, 12, 14, 16, 18, 23, 24, 28, 29, 30, 31, 37, 39, 40, 44, 45, 53, 55, 56, 57]. This may be due to differences in fixation and staining.

We performed a number of histochemical analyses for the identification of PAS-positive substances, and it can be seen from Table I that epithelial cells in the vaginal smears contained much glycogen and a considerable amount of glycoprotein, acid and neutral mucopolysaccharides. As well-known, it is the free OH radical of the 1 : 2-glycol group which renders the PAS-reaction positive; a positive reaction may, however, be obtained with the NH_2 radicals of the amino-alcohols as well. Acetic anhydride dissolved in pyridine acety-

Table

The applied histochemical methods for identification of PAS-positive substances in the exfoliated vaginal epithelium cells

	Applied methods	Cornified squamous cells	Precornified squamous cells	Intermediate cells
1	PAS reaction without oxidation	—	—	—
2	PAS reaction	++++	++++	+++
3	PAS reaction after diastase digestion	+++	+++	+++
4	PAS reaction after saliva digestion	+++	+++	+++
5	PAS reaction after pepsin extraction	+++	+++	+++
6	PAS reaction after trypsin extraction	+++	+++	+++
7	PAS reaction after comb. pepsin—trypsin extraction	+++	+++	+++
8	PAS reaction after phenylhydrasine reaction	—	—	—
9	Best's carmine stain	++++	++++	+++
10	Best's carmine stain after diastase digestion	+++	+++	+++
11	Ritter-Oleson "A" (untreated section)	++++	++++	+++
12	Ritter-Oleson "B" (section after acetylation)	—	—	—
13	Ritter-Oleson "C" (section after acetylation and deacetylation)	++++	++++	+++
14	Ritter-Oleson's stain after diastase digestion	PAS +++ Hale +++	PAS +++ Hale +++	PAS +++ Hale +++
15	Ritter-Oleson's stain after saliva digestion	PAS +++ Hale +++	PAS +++ Hale +++	PAS +++ Hale +++
16	Ritter-Oleson's stain after pepsin extraction	PAS +++ Hale +++	PAS +++ Hale +++	PAS +++ Hale +++
17	Ritter-Oleson's stain after trypsin extraction	PAS +++ Hale +++	PAS +++ Hale +++	PAS +++ Hale +++
18	Ritter-Oleson's stain after comb. pepsintrypsin extraction	PAS +++ Hale +++	PAS +++ Hale +++	PAS +++ Hale +++
19	R. Oleson's stain after hyaluronidase extraction	PAS +++ Hale +++	PAS +++ Hale +++	PAS +++ Hale +++

	Applied methods	Cornified squamous cells	Precornified squamous cells	Intermediate cells
20	Toluidin blue stain	+++	++	++
21	Toluidin blue stain after hyaluronidase extraction	+	+	+
22	Toluidin blue stain after pepsin extraction	+++	+++	+++
23	Toluidin blue stain after trypsin extraction	+++	+++	+++
24	Toluidin blue stain after comb. pepsin-trypsin extraction	+++	+++	+++
25	Ninhydrin-Schiff reaction	+	+	—
26	Millon reaction	+	+	+
27	Fettrot stain	+	+	+
28	Nile blue stain	+	+	+

lates the OH radical of the 1 : 2-glycol group and the amino derivatives, thereby preventing periodic acid oxidation. Negativity of subsequent PAS reactions indicated the presence of the said radicals. If acetylation is followed by saponification with diluted alkali, the original condition (i.e. free OH radical) will be restored in the presence of the 1 : 2-glycol group, but will not be restored in that of amino derivatives. The reacting radical is of carbohydrate origin if the PAS reaction, rendered negative by acetylation, becomes once more positive after saponification. This applies especially to material fixed in absolute alcohol where lipoids are eluted.

The above results justify our supposition that it is not only the direct hormonal action which is responsible for changes in the glycogen and MPS-contents of the cells and the ground substance, but local circulation and oxygenation may be involved as well. Acid MPS — embedded in and chemically bound to proteins — constitute the most important component of the interstitial ground substance. The connective tissue has both static and dynamic functions; it plays an independent active role in metabolism. Far from being a mere storehouse of fluids, the extracellular compartment actively promotes the flow of intercellular fluid transporting O_2, CO_2 and the metabolic products. This flow is mainly determined by circulatory conditions, colloid and hydrostatic pressure, capillary permeability and the pH. Permeability depends largely on the condition of the basement membrane composed of acid MPS. Its polymerization, water contents and gel-sol condition determine the size

and permeability of the capillary mural pores. Hyaluronidase and oestrogen, for instance, increase permeability. Besides acid also neutral MPS are contained in the connective tissue. Every cell of the organism contains more or less mucoprotein. The fact that neutral MPS mostly occur in tissues with a poor blood supply (cartilage, vitreous, cardiac valves) has led to the conclusion that bradytrophia and anaerobic glycolysis are responsible for their formation. It has been found [1, 33] that the first effect of moderate hypoxia on bradytrophic tissues is the production of acid MPS, a phenomenon of adaptation. With increasing hypoxia, metabolism returns from aerobic to anaerobic glycolysis; a further decrease of the oxygen supply induces processes of degeneration and gives rise to the accumulation of neutral MPS, a phenomenon that has been verified histologically in cases of pulmonary tuberculose, mouse cancer and crural ulcer. Mucopolysaccharides so produced are removed *via* the distended lymph vessels, and the level of MPS rises in the serum at the same time. LETTERER [36] ascribes an active role to acid mucopolysaccharides in both anabolic and catabolic processes.

For the correct interpretation of the present results, we have to touch upon the effect of oestrogens on angiogenesis. MARKEE [41], studying the behaviour of endometrial tissue transplanted to the anterior chamber of female monkeys, has shown that angioarchitectonic endometrial processes occurring in the course of the menstrual cycle are due to changes in the oestrogen level. Oestrogens promote angiogenesis, while their 50 per cent diminution induces vasoconstriction, congestion, hypoxia and, finally, the menstrual cycle are due to changes in the oestrogen level. Oestrogens promote angiogenesis, while their 50 per cent diminution induces vasoconstriction, congestion, hypoxia and, finally, the menstrual discharge of blood. Endometrial blood flow gradually increases during the phase of proliferation, reaches a maximum about the time of ovulation, remains unchanged in the secretory phase, diminishes rapidly before menstruation, whereafter a state of prestasis ensues. According to DE BRUX and BORY [12], oestrogens promote mitosis in the ripening of cells, i.e. their development into superficial cells.

Our investigations have shown that the amount of glycogen in the vaginal epithelium is largest at the time of acute hyperaemia, under the favourable circulatory conditions in the periovulatory period and in the secretory phase. Endometrial glycogen reaches its highest level in the secretory phase and in pregnancy. Acid MPS are present during the entire cycle; their amount (especially in the ground substance) is largest about the time of ovulation. Neutral MPS reach a peak in the second half of the secretory phase; even the peritubular lymph spaces of the endometrium are engorged with them. In the vaginal epithelium they mostly accumulate in the superficial cells, in the layer farthest from the circulation. The superficial cells of the vagina also contain acid MPS. Desquamated epithelial cells lodged in the vagina encounter

completely changed conditions of metabolism. Besides glycogen also acid and neutral MPS are polymerized in their cytoplasm. These are the dominant substances in the cells of layer 3a as well, whereas the cells of layer 3 (especially those of layer 3b) contain hardly anything but glycogen, a phenomenon showing that detached cells in the vagina, deprived of circulation, are compelled to follow the anaerobic way of metabolism.

The enzyme reactions examined in this study play a significant role in various metabolic processes. Acid and alkaline reactions, for instance, are — on account of phosphorus transport — important factors in carbohydrate and protein metabolism, whereas it is by the transfer of hydrogen that dehydrogenase reactions constitute essential factors in the same metabolic processes. Like enzymes in general, these too are both positive and negative catalysers: they promote now polymerization, now depolymerization. Reduced blood circulation, a diminution of oxygenation, seems to shift the equilibrium toward polymerization. It thus becomes clear why vaginal glycogen is most abundant during the proliferative phase and endometrial glycogen during the secretory phase, i.e. both at the time of peak circulation. The examined enzyme reactions were most pronunced during the proliferative phase in the vaginal epithelium, and during the secretory phase in the endometrium. Acid phosphatase was positive only in those epithelial cells of the vagina which had been detached from the superficial layer; all the other reactions were negative, a phenomenon emphasizing the importance of acid phosphatase in the metabolism of epithelial cells shed into the vagina. Alkaline phosphatase activity was strongest around the time of ovulation both in the vaginal biopsy material and the endometrium. Acid phosphatase activity in the vaginal epithelium seemed to become more intensive at the time of ovulation and at the termination of the secretory phase. It would follow that oestrogen promotes alkaline, progresterone acid, phosphatase activity. It is, however, more probable that the activity of the two enzymes is intensified under the combined effect of both hormones. This assumption is confirmed by their increased activity in the vaginal epithelium at the time of ovulation, and in the endometrium during gestation. The interrelations of hormones and enzymes are still more or less *terra incognita*.

Neutral MPS were most abundant during the second half of the secretory phase partly in those epithelial cells of the vaginal smears which had originated from the superficial layer, and partly in the endometrium. If it is assumed that these changes, as well as the products of metabolism depend on the degree of blood supply, the question arises as to the cause of these circulatory changes, further, whether all observed phenomena are exclusively governed by them. The findings of MARKEE [41] who demonstrated the effect of oestrogen on the endometrial vessels are convincing. PRILL [47], by administering 5 mg of oestradiolpropionate, increased endometrial blood flow by 200 per cent within 30 min. HAGERMANN and VILLEE [25, 26] demonstrated in endometrial

homogenate oestrogen-conditioned $DPN^+ + TPNH \rightarrow DPNH^+ + TPN^+$ systems which may be important factors of protein synthesis. In addition to the endometrium, DPN—TPN transdehydrogenase was observed in the placenta, mamma and hypophysis, but nowhere else. This would explain the mechanism through which oestrogens produce their specific effect on the target organs. Oestrogens may participate in metabolic processes in the form of co-enzymes, and it is perhaps by being attached to inactive protein molecules (which may occur in the said organs only) that they turn into active enzymes. Rona [49] holds that both oestrogen and progesterone might be involved in MPS metabolism.

Krompecher [34] claims that the blood level of MPS is governed by the thyroid. As far as can be judged, the sex hormones exert, conjointly with the nervous apparatus, a specific effect upon the vascular conditions of the target organs, the synthesis of glycogen and MPS, as also on various enzyme systems. Circulatory changes induced by oestrogens may give rise to new conditions of metabolism in which enzyme systems serving the synthesis of glycogen and MPS, among others, show a behaviour completely different from what they display under the effect of oestrogens *in vitro*.

It is not our intention to explain all metabolic changes by the alterations of circulatory conditions. All we feel safe to claim is that oestrogen and progesterone play an important role in the synthesis of glycogen and MPS contained in the epithelial cells of the vagina and the endometrium.

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ÜBER DIE HISTOCHEMISCHE UNTERSUCHUNG DES SCHEIDENEPITHELS UND DES ENDOMETRIUMS MIT BESONDERER RÜCKSICHT AUF DIE MUKOPOLYSACCHARIDEN

F. TÓTH und R. GIMES

Mit Hilfe verschiedener Verfahren (alkalische und sauere Phosphatase-, aspezifische Esterase-, Sukzino-lakto-dehydrogenase- und Diphosphopyridin-nukleotidadiaphorase-Reaktion) wurden der Glykogen- bzw. der sauere und neutrale MPS-Gehalt der Scheidenepithelzellen und des Endometriums untersucht. Es wurde festgestellt, daß die oberflächlichen Scheidenepithelzellen und die in die Scheide desquamierten Zellen der ersten, zweiten und dritten Schicht außer Glykogen auch neutrale und sauere Mukopolysaccharide enthalten. Anläßlich der Untersuchung von, aus Biopsiematerial verfertigten Präparaten ergab sich, daß der Glykogengehalt der Scheidenepithelzellen — nebst hohem Östrogenspiegel — in der Periovulationsphase am höchsten liegt; im Endometrium war der Glykogengehalt dagegen — nebst hohem Progesteronspiegel — in der Sekretionsphase am höchsten. Die saueren Mukopolysaccharide waren in sämtlichen Zyklusphasen nachzuweisen; in der Grundsubstanz des Endometriums und der intermediären Schicht des Scheidenepithels war der Mukopolysaccharidgehalt in der Periovulationsphase am bedeutendsten. Im Endometrium vollzieht sich das Anhäufen der neutralen Mukopolysaccharide — die im Laufe dieser Periode selbst die peritubulären Lymphspalten ausfüllen — in der zweiten Hälfte der Sekretionsphase. Die vorgefundenen Stoffwechselveränderungen wurden mit den verschiedenen Perioden des Menstruationszyklus und den Blutkreislaufsverhältnissen des Endometriums bzw. des Scheidenepithels verglichen. Es wird angenommen, daß die auf Wirkung der 2 verschiedenen Hormone zustandekommenden Glykogen- und MPS-Veränderungen auch die lokalen Kreislaufsverhältnisse beeinflussen.

ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ВЛАГАЛИЧНОГО ЭПИТЕЛИЯ И ЭНДОМЕТРИЯ С ОСОБЫМ УЧЕТОМ МУКОПОЛИСАХАРИДОВ

Ф. ТОТ, и Р. ГИМЕШ

Авторы исследовали в эпителиальных клетках влагалища и в эндометрии содержание гликогена, кислых и нейтральных мукополисахаридов. Они проводили реакции определения щелочной и кислой фосфатаз, неспецифической эстеразы, сукцино-лактодегидрогеназы и дифосфоридин-нуклеотиддиафосфоразы. Они установили, что поверхностные эпителиальные клетки влагалища и клетки 1-го, 2-го и 3-го слоев, десквамированных во влагалище, содержат наряду с гликогеном большое количество нейтральных и кислых мукополисахаридов. В эпителиальных клетках влагалища, исследованных на биоптическом материале, больше всего гликогена обнаруживалось в периовуляционном периоде при высоком содержании эстрогенов в крови. В эндометрии же больше всего гликогена удалось выявить в секреторной фазе, при высоком содержании прогестерона в крови. Кислые мукополисахариды обнаруживаются во всех стадиях цикла. В основном веществе промежуточного слоя эндометрия и влагалищного эпителия они в наибольшем количестве обнаруживаются в периовуляционном периоде. Количество нейтральных мукополисахаридов в значительной степени увеличивается в эндометрии во второй половине секреторной фазы и они полностью заполняют также и перитубулярные лимфатические щели. Обнаруженные авторами метаболические изменения они сравнивали с различными этапами менструального цикла и с условиями кровообращения в эндометрии и во влагалищном эпителии. По мнению авторов в изменениях содержания гликогена и мукополисахаридов в эндометрии и во влагалищном эпителии роль могут играть также и местные факторы кровообращения.

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ENTWICKLUNG UND KERATINISATION DER MENSCHLICHEN EPIDERMIS MIT BESONDERER BERÜCKSICHTIGUNG DER BARRIERE

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Die Morphologie der epidermalen Barriere wurde mit histochemischen Verfahren, an der Sohlenhaut menschlicher Foeten, an der Sohlen-, Rücken-, Brust- und Bauchhaut gesunder Personen und Psoriasis- und Ekzemkranken mit der Ritter-Oleson-, Gram-, Kongorot-, Sudan III- und Sudanschwarzfärbung, der Schiffschen Poressigsäure-Reaktion, der Chevremont-Frédéricschen und Barnett-Seligmanschen Reaktion zum Nachweis von SH-Gruppen untersucht.

1. Die sog. Übergangszone läßt sich in allen Körperregionen mit Kongorot gut färben. Außerdem ist sowohl mit Lipoidfärbung als auch mit modifizierter, sorgfältig differenzierter Gramfärbung eine kompakte Schicht im unteren Teil der Keratinschicht zu beobachten. Diese Zone ist PAS-positiv. Von den histologischen Enzymreaktionen ist nur die unspezifische Esteraseaktivität unmittelbar unter der Übergangszone nachweisbar. Die Sulfhydrilreaktionen sind in der unteren Partie der Keratinschicht am ausgeprägtesten. Die Übergangszone ist auch Baker-positiv, als Beweis des für diese Schichte charakteristischen Lipoid-Keratin Komplexes (Sakáll — Mali).

2. Unsere Befunde an Menschenembryonen stimmten weitgehend mit Matoltsys an Hühnerembryonen gewonnenen Resultaten überein. In der Haut menschlicher Embryonen kann man erst am Ende des 5. Monats eine supragranuläre homogene Schicht im Stratum corneum beobachten.

3. Auf Grund der Beobachtungen kann angenommen werden, daß die epidermale Barriere auch morphologisch nachweisbar und aller Wahrscheinlichkeit nach als eine mehr oder weniger kompakte Schicht eines ausgeprägten Keratinlipoidkomplexes in der unteren Partie des Stratum corneum lokalisiert ist, und diese breiter ist als die Szakállsche-Schicht.

4. Da in der Übergangszone Enzymaktivität (Esterase und Phosphatse) nachzuweisen war, wird in Übereinstimmung mit Sakáll und Mali angenommen, daß die Barriere eine biologisch aktive Schicht darstellt.

Ein Teil unserer Untersuchungen wurde in der Morphologischen Abteilung des Instituts für Experimentelle Medizin der Ungarischen Akademie der Wissenschaften durchgeführt; für diese Hilfe sei auch an dieser Stelle gedankt

Eine der wichtigsten Funktionen des Hautorgans besteht darin, daß es den Organismus gegen physikalische und chemische Schäden schützt, d. h. die darauf einwirkenden Strahlen filtert, das mit der Haut in Kontakt kommende Wasser und die darin gelösten Substanzen gewissermaßen zurückhält und verhindert, daß diese in den Kreislauf gelangen. Dieser funktionelle Faktor gab den Forschern Anlaß dazu, das für diese Funktion verantwortliche morphologische Element und die Lokalisation der Barriere, welche das Eindringen von Wasser und Chemikalien in den Organismus verhindert oder vermindert, festzulegen.

Rein setzte bereits 1924 voraus, daß in der Haut eine negativ geladene Schicht das Eindringen von Anionen verhindert und daß sich diese Schicht

an der Grenze zwischen verhornter und unverhornter Epidermis befindet. Später ergänzte ROTHMAN REINs Auffassung durch die Annahme, daß es sich um zwei Schichten handele, welche an der äußeren Oberfläche stark sauer, innen schwach basisch reagieren. BLANK (1953) experimentierte mit Diffusionskammern und fand ein Erlöschen der Barrierfunktion nach der Ablösung der 8. Leukoplastschicht.

SZAKÁLL (1952) konnte mit Hilfe der Wolffschen Cellophanpflastertechnik die Barriere als selbständige Membran isolieren. Seitdem wird die Membran allgemein (z. B. MALKINSON und ROTHMAN [1]) in die sog. *Übergangszone lokalisiert.* Histologisch bildet diese Übergangszone die kompakte Keratinschicht über dem Stratum lucidum. STEIGLEDER [3, 4] wies auf den Widerspruch hin, daß das Stratum lucidum bzw. Stratum compactum nur in der Handfläche und der Sohle morphologisch nachweisbar ist. In der Haut der meisten Körperregionen sind unmittelbar über der Keratohyalinschicht Keratinlamellen in meistens disjunktiertem Zustand zu beobachten, welche die Aufgabe einer Schutzschicht nicht erfüllen können. Neben dieser anatomischen Barriere dürften andere Bestandteile der Epidermis die Barrierefunktion ausüben. Trotz dieses morphologischen Wiederspruchs erfüllt aber die Hautoberfläche einer jeden Körperregion mehr oder minder eine Barrierefunktion.

STEIGLEDER beobachtete mit Mikroradiogrammen eine, über der Keratohyalinschicht gelagerte, schmale, für weiche Rtg-Strahlen gar nicht oder nur in einem geringem Grade durchlässige Schicht. Er fand sie auch in solchen Körperregionen, in denen ein Stratum lucidum nicht nachweisbar ist. Laut MARZULLI und TREGEAR's Permeabilitäts-Untersuchungen mit radioaktivem Tri-n-propylphosphat müßten wir annehmen, daß außer der SZAKÁLLschen Schicht auch andere Epidermisschichten eine Barrierfunktion ausüben.

Ein Faktor der Barrierefunktion ist die Keratinisation der Epidermis. Intrauterin ist dieser Vorgang noch unvollkommen, wie das sowohl morphologische als auch histochemische Befunde beweisen. MATOLTSY untersuchte den Ablauf der Keratinisation in der Haut von 6—20 tägigen Hühnerembryonen, an mit Hämatoxylin-Eosin gefärbten Paraffinschnitten und auch mit der BARNETT-SELIGMANschen Sulfhydryl-Reaktion. Nach diesen Untersuchungen zeigte sich die Keratinisation d. h. die an SH-Radikalen reiche Keratinschicht, erst in der Mitte des embryonalen Lebens. Stabilisiertes, endgültiges Keratin war erst 2 Tage vor der Geburt nachweisbar. Die chemischen Untersuchungen von DEME und GARAZSI [11] an Rattenembryonen ergaben annähernd gleiche Resultate. Unsere Untersuchungen an menschlichen Embryonen — welche wir später ausführlich erörtern werden — gaben ähnliche Ergebnisse, wie die von MATOLTSY [8]. In diesen Experimenten wendeten wir die Gramfärbung an, da nach SZODORAYS (1931) früheren Untersuchungen, die die Hypothese UNNAS, daß die Keratinisation in den unteren Zellreihen anfängt, zu bestätigen scheinen, eine aufwärtsgraduierte Grampositivität der

Tonofibrillen nachweisbar ist (Abb. 1), die als beginnende Keratinisation angesehen werden kann. Nach den meisten Autoren (CARRUTHERS, DERKSEN, SELBY, RUDALL, STOUGHTON, FLESCH und VAN SCOTT) fängt die Keratinisation in der Basalschicht an. Die Tonofibrillen und interzellulären Brücken sind Vorstadien des fibrösen Keratins.

Neben der Keratinisation spielt bei der Ausbildung der Barrierefunktion noch ein, die Permeabilität vermindernder Faktor, der Lipoidgehalt der oberen Zellschichten der Epidermis eine Rolle. SZAKÁLL [13] schreibt auf Grund seiner Funktionsuntersuchungen: »Die Autoren nehmen an, daß das Rohkeratin eine Matrix bildet, in der die Lipoide durch einen unbekannten Mechanismus

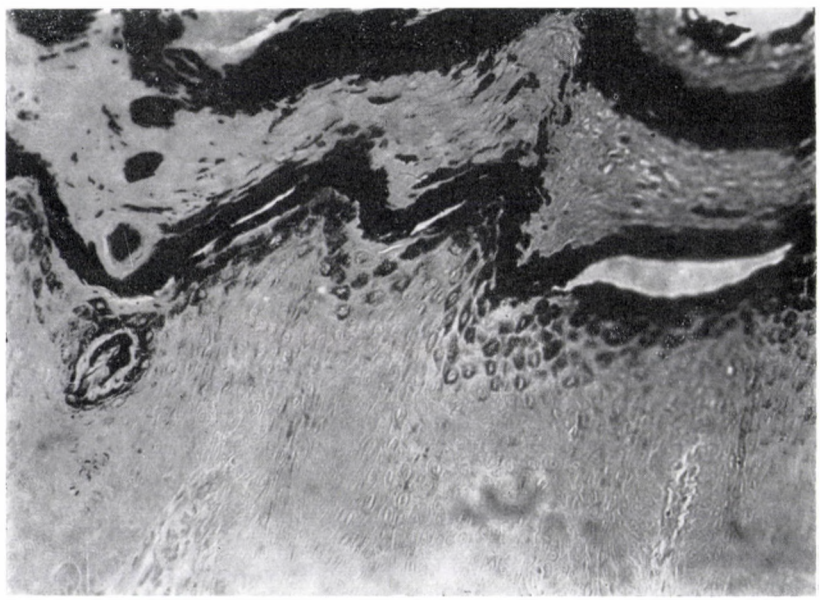


Abb. 1. Keratohyalinkörner und Übergangszone in menschlicher Sohlenhaut. Gramfärbung

fest eingebettet sind und daß diese ihrerseits den Wasserdurchtritt bestimmen«. Bei der Untersuchung der Permeabilität scheinen die histologischen Untersuchungen über den Lipoidgehalt sehr wichtig zu sein, da die chemischen Untersuchungen keinen sicheren Aufschluß über die genaue Lokalisation der Lipoidschicht geben. MALI [10] führte (1956) ausgedehnte Funktionsuntersuchungen in dieser Richtung durch. Er stellte fest, daß an der Barrierezone die Fette (Cholesterin) gemeinsam mit dem kompakten Keratin die Diffusion der Was-sermoleküle durch diese Membran verhindern. Die sogenannte MALI-Schicht liegt offensichtlich unter der SZAKÁLLschen Schicht.

Wir führten bei einigen Foeten die Übergangszone anzeigenden histochemischen Untersuchungen durch.

Methoden

An der Sohlenhaut frisch verstorbener 2—9 monatiger Foeten wurden teils in eingebetteten, teils in Gefrierschnitten folgende Färbungen und Reaktionen durchgeführt: Hämatoxylin-Eosin, Cresylviolett, Ritter-Oleson (Hyaluronidase und Verdauungskontrolle), Gram-, Kongorot-, Sudan III-, und Sudanschwarzfärbung, die Lilliesche, die Peressigsäure-Schiff-Reaktion, die Chevremont-Frédéricsche, Barnett-Seligmansche und die Bakersche Reaktion schließlich den SH-Nachweis.

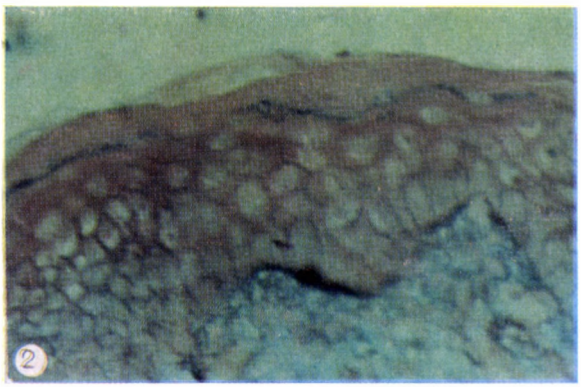
Diese Reaktionen führten wir auch an normaler Sohlen-, Rücken-, Brust-, Bauch- und Schenkelhaut aus, und auch an Papeln von Psoriasis und Ekzem. Mit dem Kryostat wurden dünne Schnitte für Succinodehydrogenase, alkalische und säurephosphatase Azofarbstoff nach VADÁSZ und nicht-spezifische Esterase (nach David-Orstein) verfertigt.

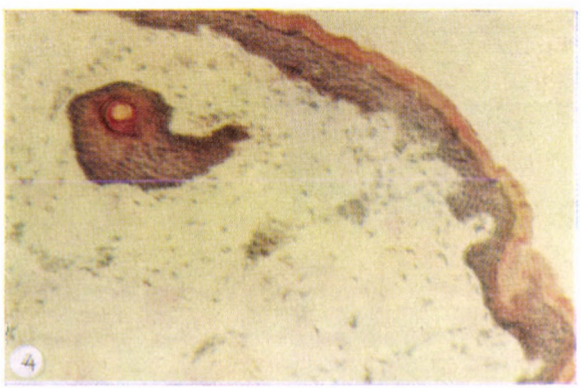
Ergebnisse

1. In der untersuchten Sohlenhaut fielen die vor dem 4. Monat für die Barriere charakteristischen histochemischen Reaktionen negativ aus und ebenso fehlte die Keratinschicht. Tonofibrillen waren zuerst im 3—4. Monat wahrnehmbar. Im 4. Monat entsteht in der Haut der Foeten an der Oberfläche der Epidermis eine Gram-positive Zone. In der Haut der 4—5 monatigen Foeten war im Stratum granulosum eine Keratohyalinkörnelung nachweisbar. Das Keratohyalin zeigt Hale- (Abb. 2.) und Gram-Positivität, die Keratinschicht ist mit Kongorot, Gram und PAS färbbar. Bei älteren Foeten waren Gram- und Kongo-Positivität verstärkt und in der unteren Partie der Keratinschicht lokalisiert. Lipoidfärbungen waren vom 5. Monat an positiv. Unsere Ergebnisse in Hinblick auf SH stimmten mit denen von MATOLTSY überein, der in Hühnerembryonen die SH-Gruppen etwa in der Mitte des embryonalen Lebens vorfand und erst später höhere Konzentrationen nachweisen konnte. In den so behandelten embryonalen Hautschnitten kann man im allgemeinen im 5—6. Monat eine homogene suprabasale Keratinschicht beobachten, die ein Vorstadium der morphologischen Barriere sein dürfte. Diese morphologische Schicht kann man besonders in den mit Kongrot und nach Gram behandelten Schnitten gut beobachten (Abb. 3).

2. In normalen Hautgebieten erwachsener Personen sind die Keratohyalinkörner mit der RITTER—OLESONschen Färbung Hale-positiv, die Keratinschicht ergibt in ihrer ganzen Breite eine positive PAS-Reaktion. Ein ähnliches Bild zeigen die Haut der Sohle, Psoriasis- und Ekzem-Haut, mit der Abweichung, daß bei Parakeratose das Stratum granulosum meistens fehlt.

Mit modifizierter Gram-Färbung kann man bei sorgfältiger Differenzierung, besonders in akanthotischen Gebieten die aufwärts zunehmende Gram-Positivität der Tonofibrillen gut beobachten, welche mit den Keratohyalinkörnern eine stark positive Schicht der Übergangszone bildet. Eine schwache Färbung ist hie und da auch im Stratum disjunctum auffindbar. Die Positivität des Keratohyalins und der Übergangszone an der Sohle ist in der normalen Haut in der Mehrzahl der Fälle zu beobachten. In dem parakeratotischen Keratin





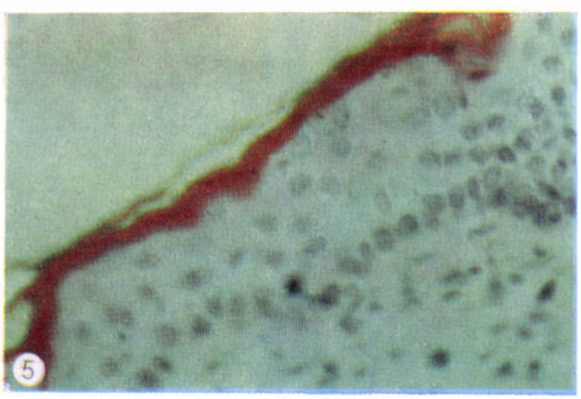


Abb. 2. Hale-positive Keratohyalinkörner in der Haut von 5 Monat alten Foeten
Abb. 4. Die sog. »Übergangszone« wird mit der Bakerschen Reaktion gut sichtbar
Abb. 5. Starke saure Phosphatase-Reaktion in der Hornschicht. VADÁSZsche Azofarbstoff-Methode

der Psoriasis sowie bei Ekzem ist dagegen die Reaktion schwach. Die Gram-Positivität der beschriebenen Strukturen ist nach FLESCHS [5-7] und unseren Untersuchungen wahrscheinlich durch die Anwesenheit sauerer Mukopolysaccharide bedingt. Nach eigenen Untersuchungen läßt sich die Übergangszone am besten mit der Kongorot-Färbung in der Haut der Sohle und auch anderer Körpergebiete darstellen. Bei Parakeratosis färbt sich dagegen die Keratinschicht in ihrer ganzen Breite kaum oder gar nicht. Es ist unbekannt, an welche Substanzen sich die Kongo-Positivität knüpft. Alte histologische Handbücher bezeichnen sie als eine elektive Eleidin-Färbung.

Mit den verwendeten Lipoidfärbungen — besonders mit der Bakerschen

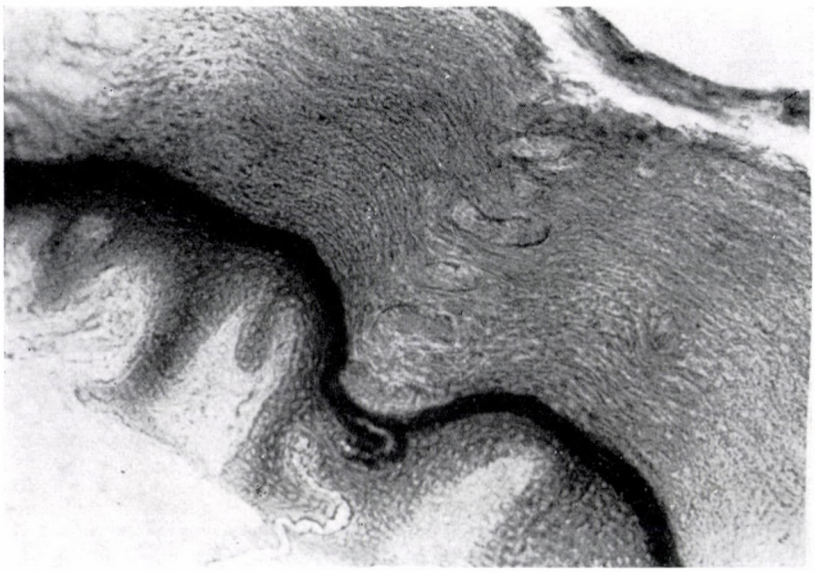


Abb. 3. Kongorotfärbung in der Haut von 5 Monat alten Foeten

Reaktion — ist die Übergangszone in der gesunden Epidermis (Abb. 4) und Sohlenhaut gut darstellbar, auch die Oberfläche der Keratinschicht färbt sich in der Regel positiv. Bei Psoriasis und Ekzem färbt sich die Keratinschicht meistens in ihrer ganzen Breite. Mit der Bakerschen Reaktion ist ein Teil der Keratohyalinkörner nachweisbar.

In der Barriere lassen sich SH-Gruppen in großer Menge und bei Parakeratose in der ganzen Keratinschicht nachweisen.

Von den untersuchten Enzymen ist die Aktivität der Succinodehydrogenase in der Basalschicht am stärksten; sie vermindert sich in Richtung der Hornschicht, und hört in der Übergangszone auf.

Der Nachweis der alkalischen Phosphatase-Aktivität mit der VADÁSZschen Methode gelingt nur im Kapillar-Endothel.

Die saure Phosphatase-Aktivität ist in der *sog. Übergangszone am stärksten* (Abb. 5). In der psoriatischen und ekzematischen Epidermis erstreckt sie sich über die ganze Hornschicht.

Die unspezifische Esterase — nach einigen Autoren teilweise Lipase— akkumuliert sich unter der Übergangszone. Die Hornschicht gibt nur bei Parakeratose eine positive Reaktion.

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DEVELOPMENT AND KERATINIZATION OF THE HUMAN EPIDERMIS WITH SPECIAL REFERENCE TO THE BARRIERS

L. SZODORAY und KLÁRA NAGY-VEZEKÉNYI

The morphology of the epidermal barriers has been studied by histochemical methods in the sole of human foetuses, the sole, back, chest and abdomen of healthy persons and patients suffering from psoriasis and eczema, applying Ritter—Oleson's, Gram's, Congo red, Sudan III and Sudan black dyes the periodic acid reaction of Schiff, the Chevremont—Frédéric and the Barnett—Seligman reactions for the demonstration of SH groups.

1. The so-called transitory zone stains well with Congo red in every skin area. Lipid staining and thoroughly differentiated Gram staining reveal a compact zone in the lower part of the keratin layer. This zone is PAS-positive. Of the enzymes only a non-specific esterase activity is demonstrable immediately under the transitory zone. The sulphhydryl reaction is the most marked in the lower parts of the keratin layer. The transitory zone is Baker-positive, proving the presence of the lipid-keratin complexes characteristic of these layers (Szakáll—Mali).

2. The results for human embryos were in close agreement with those obtained by Matoltsy for chick embryos. In the stratum corneum of human embryos it is late in the 5th month that a supragranular homogeneous layer appears.

3. It is concluded that the epidermal barrier is demonstrable morphologically, and apparently located as a more or less compact keratin-lipid layer in the lower part of the stratum corneum, and that it is broader than Szakáll's layer.

4. As in the transitory zone esterase and phosphatase activity could be demonstrated, in agreement with Szakáll and Mali it is believed that the barrier is a biologically active layer.

РАЗВИТИЕ И КЕРАТИНИЗАЦИЯ ЧЕЛОВЕЧЕСКОГО ЭПИДЕРМИСА С ОСОБЫМ УЧЕТОМ БАРЬЕРА

Л. СОДОРАИ и К. НАДЬ-ВЕЗЕКЕНЬИ

Авторы исследовали при помощи гистохимических методов морфологию эпидермального барьера на коже подошвы человеческих зародышей, на коже подошвы, спины, груди и живота здоровых лиц и больных с псориазом и с экземой. Исследования проводились при помощи окраски по Риттеру—Олесону, Граму, окраски с конгоротом, суданом III и судановым черным, реакции Шиффа, реакций Шевремон—Фредерика и Барнета—Зелигмана для выявления групп SH.

1. Так называемая переходная зона во всех областях тела хорошо окрашивается конгоротом. Кроме того как при помощи липоидной окраски, так и с видоизмененной, тщательно дифференцированной окраской по Граму наблюдается компактный слой в нижней части кератинового слоя. Эта зона ПАСК-положительная. Из гистологических энзимных реакций непосредственно под переходной зоной можно выявлять только активность неспецифической эстеразы. Сульфгидрильные реакции наиболее выраженные в нижней части кератинового слоя. Переходная зона дает также и положительную реакцию по Бекеру, что является доказательством характерного для этого слоя липоид-кератинового комплекса (Сакалл—Мали).

2. Наши данные, полученные на человеческих зародышах, в значительной мере совпадали с данными, полученными Матольчи на куриных зародышах. В коже человеческих зародышей только к концу 5-го месяца можно наблюдать супрагранулярный гомогенный слой в роговом слое.

3. На основании наблюдений можно предполагать, что эпидермальный барьер локализован и в морфологически выявляемой форме и по всей вероятности как более или менее компактный слой выраженного кератин-липоидного комплекса в нижней части рогового слоя и что этот барьер шире, чем слой Сакалла.

4. Так как в переходной зоне можно было выявить энзимную активность (эстеразы и фосфатазы) авторы предполагают в согласии с Сакаллом и Мали, что барьер представляет биологически активный слой.

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ÜBER GEWISSE EIGENTÜMLICHKEITEN DES PHYSIKALISCHEN ABBAUS DER MARKSCHEIDE BEI DER EXPERIMENTELLEN ALLERGISCHEN ENZEPHALOMYELITIS DER HUNDE

L. LÁZÁR, T. MAROS und IBOLYA KOVÁCS

(Eingegangen am 5. April, 1965)

Bei der experimentellen allergischen Enzephalomyelitis (EAEM) der Hunde wurden mit lichtmikroskopischen Methoden die einzelnen Typen des physikalischen Abbaus der Markscheide untersucht. Auf Grund der anfänglichen Zeichen der Demyelinisation konnten drei Typen unterschieden werden:

1. Die primäre Demyelinisation, die durch Bildung von Myelinkugeln entsteht, und bei welcher eine Antigen-Antikörper-Reaktion die Abwicklung der Myelinperiode von der Außenfläche der Markscheide verursacht.
2. Die sekundäre Demyelinisation, die mit Erosionen der Oberfläche der gequollenen Markscheide beginnt, und welche durch Einwirkung der aktivierten RES-Elemente in mit Mononukleären infiltrierten Gebieten hervorgerufen wird.
3. Die sekundäre Demyelinisation, die mit Zacken- und Stachelbildungen an der Oberfläche der gequollenen Markscheide beginnt, und welche mit der Schädigung und Mobilisation der Oligodendroglia-Elemente in Zusammenhang zu bringen ist.

Die genannten Typen kommen meistens vermischt, allein nur selten vor. Das mikroskopische Bild macht die WALLERsche Degeneration, die infolge der Neuronenschädigungen entsteht, noch verwickelter.

Es konnte kein grundlegender Unterschied zwischen der EAEM der Hunde und der menschlichen multiplen Sklerose (MS) gefunden werden.

Die eigentümlichsten Veränderungen, die bei Enzephalitiden und Enzephalopathien mit Demyelinisation zustande kommen, werden durch den Abbau der Markscheide und dessen Folgen verursacht. Obwohl sich mit der Desintegration der Markscheide eine fast undurchsichtliche Literatur beschäftigt, kann auch heute noch nicht behauptet werden, daß alle Probleme der Demyelinisationsvorgänge klargelegt wären. So steht es immer noch zur Diskussion, ob die Demyelinisation bei der multiplen Sklerose (MS), und auch bei der experimentellen allergischen Enzephalomyelitis (EAEM) vom primären oder sekundären Charakter ist ferner, ob bei der EAEM die zelluläre Infiltration des betroffenen Nervengewebes nach der Demyelinisation entsteht, oder — in umgekehrter Reihenfolge — ob die zelluläre Infiltration an Stellen der fokalen Demyelinisationen als sekundäre Folgeerscheinung zustande kommt, und schließlich, ob die primäre und sekundäre Demyelinisation in der physikalischen Phase des Markscheidenabbaus einen gemeinsamen morphologischen Charakter hat. Mit den vorliegenden Untersuchungen trachteten wir die obenerwähnten Fragen zu beantworten und gleichzeitig weitere Daten zur richtigen Interpretation jener morphologischen Bilder zu liefern, welche wir in EAEM-Modellversuchen an Hunden beobachtet haben (MAROS u. Mitarb.,

1964). Wir suchten auch nach Zusammenhängen zwischen den lichtmikroskopischen Charakteristika des Demyelinisationsprozesses, der Bedeutung der mitwirkenden zellulären Elemente und den Typen der immunologischen Reaktion bei der EAEM. Schließlich verglichen wir anhand der gewonnenen Ergebnisse die morphologischen Eigenschaften des EAEM mit der menschlichen MS.

Untersuchungsmaterial und Methodik

Die Untersuchungen wurden am Gehirn von 20 erwachsenen Hunden durchgeführt, denen nach vorangehender Pertussis-Vakzination fünftäglich eine heterologe Gehirnemulsion mit Freunds Adjuvant intrakutan in die Haut des Genickes und des Rückens verabreicht wurde. Die Tiere wurden nach Erscheinen der neurologischen Symptome in verschiedenen Zeitpunkten getötet, zur Zeit als es zu einem derartigen Verschlechterung des Allgemeinzustandes gekommen ist, daß eine spontane Verendung zu fürchten war. Die Angaben der Modellexperimente wurden bereits bekanntgegeben (MAROS, 1964).

Das Zentralnervensystem und gewisse Teile des peripheren Nervensystems wurden in 1 : 9 Formalinlösung, die eine Hemisphäre in Bromformol fixiert. Gefrierpräparate wurden nach LÁZÁRscher (1955) Markscheidenfärbung oder Silberimprägnation, Paraffinschnitte nach Nissl-, Masson- und Hämatoxylin-Eosin Färbung untersucht.

Ergebnisse

Es ist eine Eigentümlichkeit der EAEM, daß nach jeder Antigenverabreichung immer neuere Herde entstehen, bzw. daß in einem Teil der älteren perivaskulären Demyelinisationsherden neuere Exazerbationen zustande kommen (MAROS, LÁZÁR, KOVÁCS, 1964).

Bei jedem Tier sind die einzelnen Phasen der Demyelinisation von den anfänglichen exsudativen perivaskulären Erscheinungen bis zur Glianarben bzw. sklerotischen Herden gut zu verfolgen.

Die Frühveränderungen, d. h. die Erweiterung der perivaskulären Räume und Invasion derselben durch hämatogene Elemente, das perifokale Ödem und die hämatogene Infiltration der Nachbargebiete sind nur mit einer Quellung der Markscheide vergesellschaftet. Diese reversible Reaktion der Markscheide wird irreversibel und führt zur Demyelinisation, wenn das Nervengewebe dauernd oder wiederholt mit Blutplasma bzw. Extravasat infiltriert wird. Im letzteren Falle weist die Markscheide eine unebene Oberfläche auf, nachher kommt es zur Desorganisation ihres Neurokeratingerüstes, und endlich zerbröckelt sie sich zu Myelinkugeln.

In älteren, subakuten Herden und in deren Nachbarschaft verläuft der Markscheidenabbau nach der hyperergischen Exazerbation der histio-lymphopolyblastischen Infiltrationen in derselben Weise entlang.

Derselbe Typ des physikalischen Abbaus der Markscheide ist auch in den charakteristischen chronisch-gliotischen Herden wahrzunehmen, wenn es zu Exazerbationen mit exsudativen Erscheinungen kommt. Man sieht die

eigentümliche Abwicklung-Abreißung der verschieden großen Myelinkugeln von der Außenfläche der gequollenen Markscheide, bis schließlich an ihrer Stelle nur eine Reihe von Myelinkugeln zu beobachten ist (Abb. 1). Die diffuse akute Demyelinisation der weißen Substanz der Hemisphären wird meistens durch den genannten »Myelinkugel-Typ« des physikalischen Markscheidenabbaus

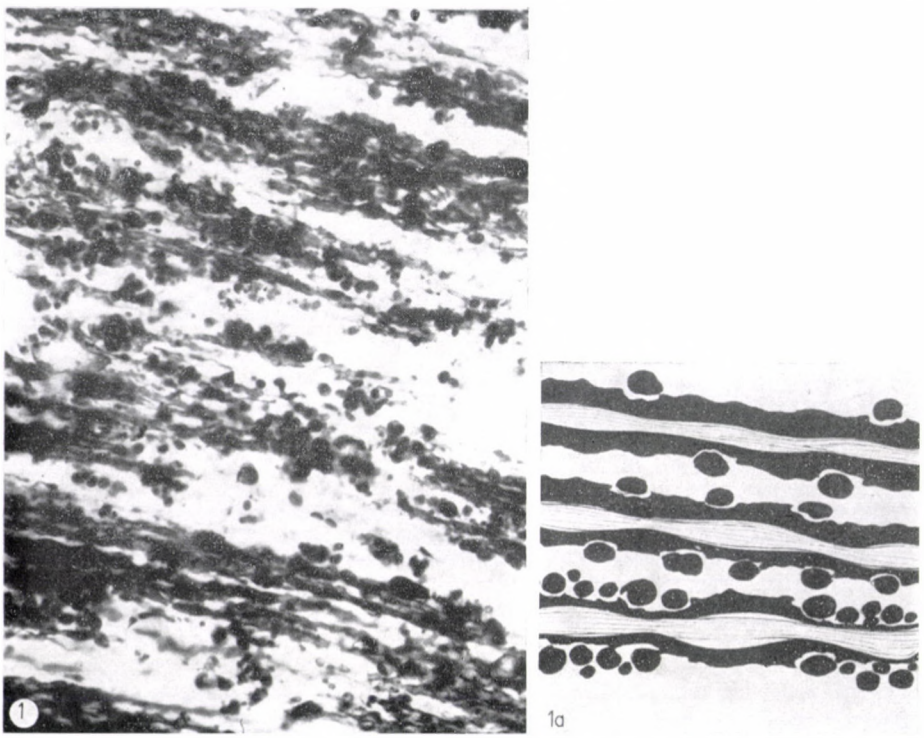


Abb. 1. N. opticus. Zerfall der Markscheiden zu Myelinkugeln. Markscheidenfärbung. Vergr. Ok.: 6×, Obj.: 24/0,42
Abb. 1a. Zerfall der Markscheide zu Myelinkugeln

charakterisiert (Abb. 2). Der Myelinkugelbildung folgend wird das ganze Gebiet von mit Gliazellen vermischten Granulationselementen überschwemmt, d. h. den physikalischen Abbau der Markscheide folgt die zelluläre Infiltration.

Eine Demyelinisation ist aber auch an solchen Stellen zu beobachten, wo das Nervengewebe in umschriebenen perivaskulären Herden, oder diffus mit lympho-polyblastischen Elementen überhäuft ist. An den genannten infiltrierten Stellen, wo der Prozeß von subakuten Charakter ist, kommen oft Erscheinungen vor, die als Erosionen den äußeren Schichten der Markscheide zu deuten sind; auch dieser Veränderungen folgt ein physikalischer Abbau (Abb. 3). Diese Demyelinisationsform ist in den langen Bahnen des Gehirn-

stammes und des Rückenmarkes häufiger, kommt aber auch in der weißen Substanz der Hemisphären vor.

Zwischen den (akuten, subakuten oder chronischen) Herden der weißen Substanz der Hemisphären findet man oft eine Vermehrung der Zeilen von Oligodendrogliareihen, mit Quellung des Zellkerns und Anreicherung des

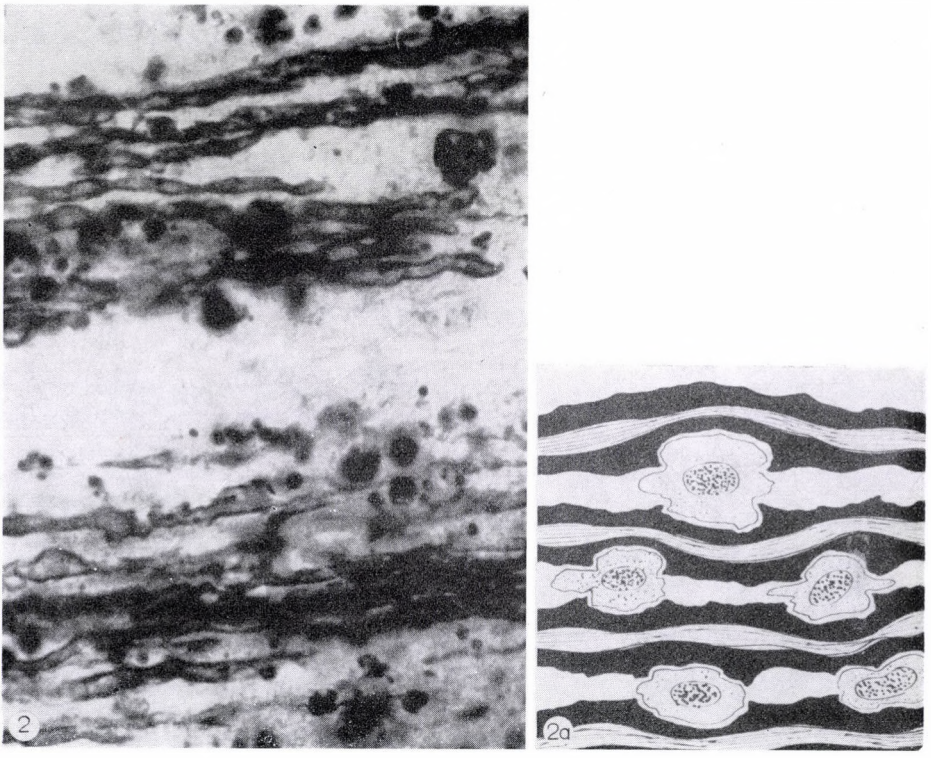


Abb. 2. N. opticus. Erosionen an der Oberfläche der gequollenen Markscheide. Markscheidenfärbung. Vergr.: Ok.: 6 ×, Obj.: 45/0,65
Abb. 2a. Schema des Markscheidenabbaus durch Erosionen

Zytoplasmas. Ensteht an diesen Stellen eine Demyelinisation, so wird sie durch "Myelinfortsätze", "Myelinstacheln" charakterisiert, welche sich von der Außenfläche der gequollenen Markscheide abschnüren (Abb. 4). Das Myelin wird von den Nervenfasern sozusagen abgerissen. Diese Form des physikalischen Abbaus der Markscheiden ist auch im Opticus-System eine häufige Erscheinung.

Meistens kommen die genannten Formen des Markscheidenabbaus vermischt vor, um so mehr, da sich die akuten exsudativen Herde meistens auf solche Herde ansetzen, welche einen subakuten oder chronischen Prozeß

spiegeln. Noch verwickelter wird das Bild dadurch, daß nach direkter Schädigung der Neuronen ein, die WALLERsche Degeneration begleitender Myelinabbau auch einsetzt.

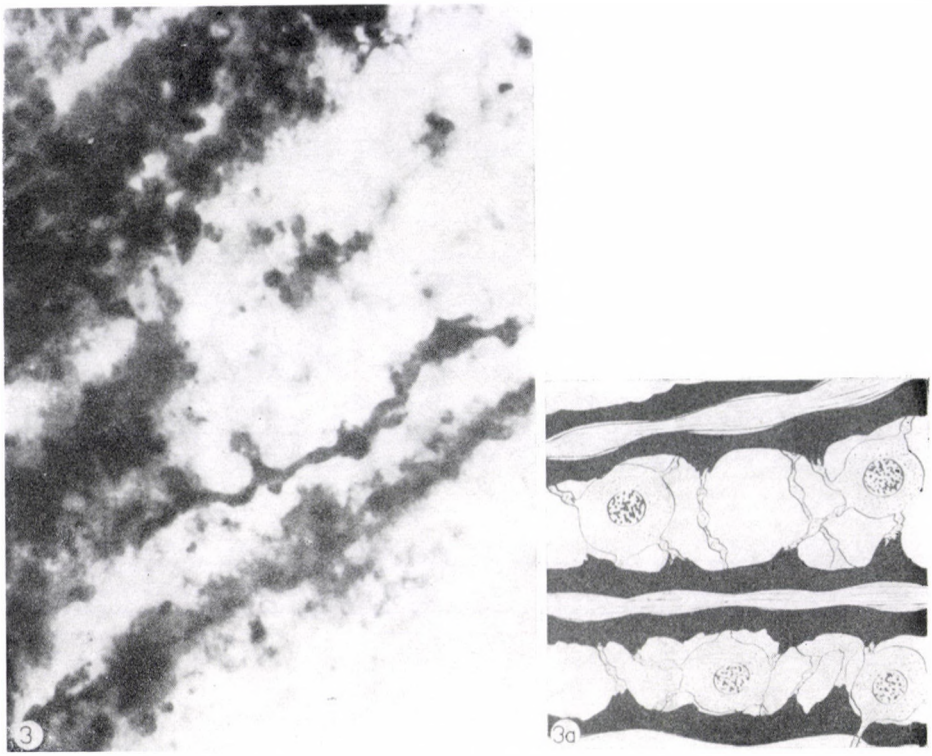


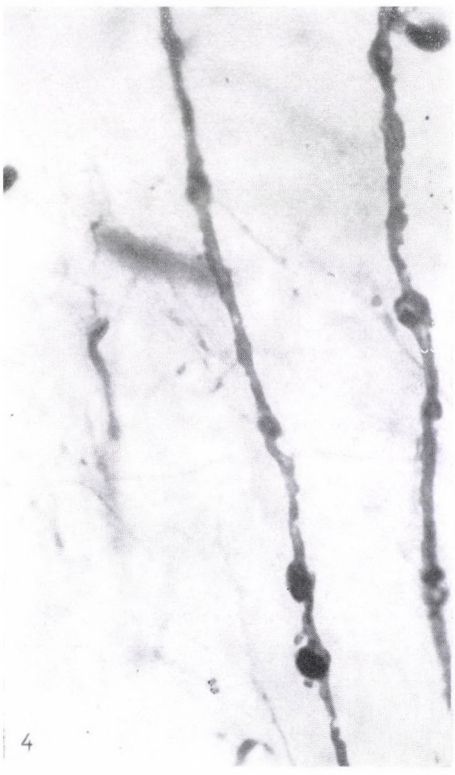
Abb. 3. N. opticus. An der gequollenen ungleichmäßig dicken Markscheide sind »Stacheln«, »Zacken« zu beobachten. Markscheidenfärbung. Vergr.: Ok.: 6 ×, Obj.: 45/0,65

Abb. 3a. Schema des beginnenden Markscheidenabbaus durch »Myelinstacheln« und »Myelinzacken«

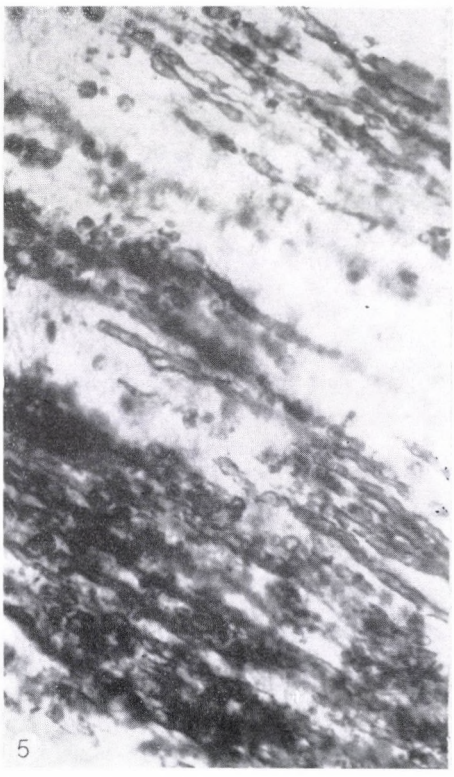
Besprechung

In der Umgebung des perivaskulären entzündlichen Hofes, in den diffus ödematösen Gebieten, und auch an Stellen der hyperergischen Exazerbationen wird es klar, daß in jenen Formen der Demyelinisation, wo dieser Prozeß der Aktivation der zellulären Elemente vorangeht, der markscheidenschädigende Faktor direkt auf das Myelin einwirkt. Die Funktionsstörung der Blut-Gehirn-Barriere (LEHOCZKY, 1962, HALASY-LEHOCZKY, 1962, SEITELBERGER, JELLINGER, 1963) ermöglicht die Einströmung des im Serum vorhandenen demyelinisierenden Faktors (APPEL, BORNSTEIN, 1964) in die perivaskulären Gewebe. Der Serumfaktor ist von Antikörper-Natur (NIEDICK, KUWERT, 1963), und

entsteht infolge der Einwirkung der Antigene auf die RES-Elemente (BÖHME, 1963, 1964). Als Antigen fungieren die mit der heterologen Gehirnemulsion eingeführten Substanzen des Myelins (LEVINE, WENK, 1963). Die Rolle der als Adjuvant injizierten Mykobakterien oder anderen Gramnegativen Mikroorganismen ist nicht ganz klar (SHAW, ALVORD, KIES, 1964). Daß die anti-



4



5

Abb. 4. Polus temporalis. An den Konturen der Fasern sind anfängliche Zeichen des Markscheidenabbaus durch Erosionen und Stacheln zu beobachten

Abb. 5. N. opticus in demselben Sehfeld. Anfängliche Zeichen des Myelinabbaus: Erosionen Stacheln und Myelinkugelchen die sich von der Markscheide wegreißen. Markscheidenfärbung Ok.: 6 ×, Obj.: 24/0,42

Myelinsubstanzen sich zur Markscheide binden, ist mit Immunofluoreszenzmethoden gut kontrollierbar (FIELD, RIDLEY, CASPARY, 1963). Die zur Demyelinisation führenden Antigen-Antikörper Verbindungen kommen vermutlich auch im lebenden Organismus in ähnlicher Weise zustande, wie in der Nervengewebekultur. Das Serum des Tieres mit EAEM, oder des Menschen mit MS enthält solche Substanzen, die in vitro Demyelinisation herbeiführen (SCHATZ, ADELSON, 1958, BORNSTEIN, 1963).

Das zur Schwannzellen-Kultur gegebene anti-Schwannzellen-Serum löst zuerst die Zellwand und deren Membransysteme, und danach auch das Zyto-

plasma auf. Inaktiviertes Serum hat nur nach Zugabe von Komplement eine ähnliche Wirkung (PETTE, PETTE, 1963). Dieser Antikörper ist scheinbar ein Lysolezithin, der die Markscheide, die doch von Schwannzellen abstammt, angreift (WEBSTER, 1957). Ähnlicherweise wirken auch die Lezithinase enthaltenden Stoffe, sowie einige Schlangengifte, Clostridium-Filtrat usw. (MORRISON, ZAMECNIK, 1950, WEIL, 1930, WEIL, HEILBRUNN, 1941). Auf Wirkung der myelindestruierenden toxischen Stoffe kommt es zur Desorganisation der dickeren dunklen Linien zwischen den Myelinperioden der Markscheide (BARTON, 1962, PETERSOHN, 1963). Danach zerfallen die aufgewickelten Myelinperioden zu kleineren oder größeren Myelinkugeln. Anhand der obigen Befunde ist der von uns beobachtete Markscheidenzerfall in Myelinkugeln die Folge einer Antigen-Antikörper-Wirkung. Im wesentlichen handelt es sich um eine Myelinolyse, welche zur axo-myelinischen Dissoziation (FOG, 1947, 1957) führt, und als primäre Demyelinisation zu betrachten ist.

Die andere, durch Erosionen charakterisierte Form des physikalischen Abbaus der Markscheide können wir als direkte Einwirkung der zelligen Elemente deuten. Dieser Typ entsteht in mit Zellen infiltrierten Zonen. Nach der Meinung von BUBIS und LUSE (1964), sowie WAKSMAN und ADAMS (1955) ist die Demyelinisation zum Erscheinen der mononukleären Zellen im Nervengewebe gebunden, und beginnt zuerst mit dem Kontakt zwischen Histiozyten und Myelin. Eine demyelinisierende Wirkung der RES-Elemente wurde auch von anderen Autoren beobachtet (BÖHME, 1963, WEINSTEIN, WOOLF und MEYNELL, 1963). JOHNSTON, GREEN und HEADINGTON (1963) haben neben Tuberkulose und Sarkoidose Leukoenzephalopathien mit Demyelinisation beobachtet. DRAGÄNESCO, GYERGYAY, PETRESCO und IONESCO (1963) berichteten über perivaskuläre Demyelinisation bei einer durch retikulohistiozytäre Granulome charakterisierten Enzephalitis. D'AGOSTINO, PEASE und KERNOHAN (1963) beobachteten einen Fall von multifokaler progressiver Leukoenzephalopathie in den Hemisphären und dem Gehirnstamm, die sich zur RES-System-Erkrankung anschloß.

Unserer Meinung nach ist der mit Erosionen einhergehende Abbau der Markscheide einer direkten Einwirkung der RES-Elemente zuzuschreiben und als solches für die subakute Demyelinisation charakteristisch.

Der dritte Demyelinisationstyp, der in der physikalischen Phase des Markscheidenabbaus zu beobachten ist, und welchen die an der Außenfläche der Markscheide entstehenden unregelmäßigen Stacheln oder Zacken charakterisieren, dürfte mit einer primären Schädigung und Mobilisation der Oligodendroglia-Elemente erklärt werden.

Mit dieser Ansicht schließen wir uns LUMSDENS (1957) Behauptungen an, wonach die dem Myelinabbau vorangehende Myelinquellung mit der hypoxischen oder andersartigen Schädigung der Oligodendroglia-Elemente in Zusammenhang steht. Diese Schädigung der Oligodendroglia-Elemente, die in den

osmotischen Verhältnissen des Myelins eine wichtige Rolle spielen, können bei der EAEM, auch ohne Teilnahme entzündlicher Komponente, zur irreversiblen Myelinveränderungen und Demyelinisation führen (ROIZIN, KOLB, 1957). Die Schädigung und Dedifferenzierung der Oligodendrogliazellen erklärt diese Form des Markscheidenabbaus, welche trotz der axo-myelinischen Dissoziation als sekundäre Demyelinisation zu deuten ist.

Schließlich kann also in der Demyelinisation bei Hunden mit EAEM eine primäre und auch eine sekundäre Demyelinisation eine Rolle spielen. Bei der akuten primären Demyelinisation ist die zellige Infiltration des Herdes sekundär, im Gebiet der subakuten zellulären Infiltration haben wir aber mit einer sekundären Demyelinisation zu tun, bei welcher der primäre Faktor die Aktivität der RES-Elemente und der Gliazellen ist.

Der Auffassung LUMSDENS (1957), wonach in allen allergischen Enzephalomyelitiden nur sekundäre Demyelinisation vorkommt, können wir also nicht völlig beistimmen.

In Einverständnis mit FOGS (1963) Behauptungen können wir zwischen den Demyelinisationsformen bei der EAEM der Hunde und der menschlichen MS keine grundlegenden Unterschiede finden, da in beiden Prozessen dieselbe Faktoren wirken und im wesentlichen beide auf Grund einer disseminierten zerebrospinalen Periphlebitis entstehen.

Die für die menschliche MS charakteristische herdförmige oder diffuse progressive Demyelinisation (HENNEAUX, 1959) unterscheidet sich nicht wesentlich von jenen Typen, die bei der EAEM des Hundes vorkommen.

Konklusionen

Bei der EAEM der Hunde sind drei Type des Markscheidenabbaus zu unterscheiden:

1. In den akuten allergischen Herden und an den Stellen der hyperergischen Exazerbation zerfällt die Markscheide in Myelinkugeln. Bei diesem Prozeß kommt eine charakteristische Erscheinung vor: von der Außenfläche der Markscheide reißen sich verschieden große Myelinkörnchen und Kugelchen ab, wahrscheinlich infolge einer Antigen-Antikörper-Reaktion, die sich im Niveau der Myelinperioden abspielt. Diese Form des Markscheidenabbaus ist als primäre Demyelinisation zu deuten, welche von einer Mobilisation der zellulären Elemente gefolgt wird.

2. Den subakuten, herdförmigen oder diffusen zelligen Infiltrationen entsprechend entsteht eine sekundäre Demyelinisation mit langsameren Ablauf. Für diesen Typ der Demyelinisation ist es charakteristisch, daß sie mit Erosionen der Oberfläche der Markscheide beginnt. Diese Erosionen entstehen infolge der myelinschädigenden Wirkung der zelligen Elemente, hauptsächlich der RES-Elemente.

3. Den Beginn der dritten Form der Demyelinisation charakterisieren die Myelin-Zacken oder Stacheln, die sich von der Außenfläche der Markscheide wegreißen. Diese ebenfalls sekundäre Demyelinisationsform kann man mit der primären Schädigung bzw. Mobilisation der Oligodendroglia-Elemente in Zusammenhang bringen.

Bei der EAEM der Hunde entstehen die genannten drei Demyelinisationstypen meistens gemischt mit Präponderanz der einen oder der anderen Form. Das histologische Bild kann noch verwickelter werden, wenn zu den erwähnten Formen gleichzeitig ein solcher Markscheidenabbau hinzukommt, der mit der WALLERschen Degeneration verbunden ist.

Die wesentlichen Unterschiede zwischen der EAEM der Hunde und der menschlichen MS sind nicht in den morphologischen Eigentümlichkeiten, sondern vielmehr in den Abweichungen des Krankheitsablaufes zu suchen.

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SOME CHARACTERISTICS OF THE PHYSICAL BREAKDOWN OF THE MYELIN SHEATH IN THE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS OF THE DOG

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In the experimental allergic encephalomyelitis of the dog authors have studied by light microscopic methods the various types of the physical breakdown of the myelin sheath. It has been found that on the basis of the initial changes of demyelinization three forms may be distinguished:

1. Primary demyelinization resulting from the formation of myelin spheres, in which the rolling off of the myelin periods from the surface of the myelin sheath is caused by the antigen-antibody reaction.

2. Secondary demyelinization beginning with a "pitting" of the surface of the swollen myelin sheath, produced by the activated RES elements in the areas infiltrated by mononuclear cells.

3. Secondary demyelinization beginning with the appearance of myelin indentations and spikes, which may be correlated with lesion to and mobilization of oligodendroglial elements.

These types usually occur mixed and are seldom found alone. The degeneration of Waller due to lesions to the neurone makes the microscopic pattern of demyelinization even more complicated.

Authors have found no fundamental differences between the demyelinization occurring in the dog's EAEM and that demonstrated in SM of man.

НЕКОТОРЫЕ ОСОБЕННОСТИ ФИЗИЧЕСКОГО РАЗРУШЕНИЯ МИЕЛИНОВОГО ВЛАГАЛИЩА ПРИ ЭКСПЕРИМЕНТАЛЬНОМ АЛЛЕРГИЧЕСКОМ ЭНЦЕФАЛОМИЕЛИТЕ У СОБАК

Л. ЛАЗАР, Т. МАРОШ и И. КОВАЧ

Авторы изучали при экспериментальном аллергическом энцефаломиелите у собак отдельные типы физического разрушения миелинового влагалища под микроскопом. Они установили, что на основании начальных признаков демиелинизации можно различать три формы:

1. Первичная демиелинизация, наступающая за счет возникновения миелиновых шариков, где процесс вызывается реакцией антиген-антитело.

2. Вторичная демиелинизация, начинающаяся «выданиями» поверхности набухшей миелиновой оболочки, которая вызывается активировавшимися элементами РЭС в областях, инфильтрированных одноядерными клетками.

3. Вторичная демиелинизация, начинающаяся появлением на поверхности набухшего миелинового влагалища шипов, что может быть связано с поражением и с мобилизацией элементов олигодендроглии.

Вышеперечисленные типы демиелинизации обычно встречаются смешанно и только редко наблюдаются сами по себе. Микроскопическая картина осложняется еще Валлеровским перерождением, возникающим в результате поражения невронов.

Авторы не видят основной разницы между демиелинизацией, наблюдающейся при экспериментальном аллергическом энцефаломиелите собак и человеческой СМ.

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THE MECHANISM OF CORNEAL VASCULARIZATION

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Intracorneal injection of 0.1 per cent lactic acid and pyruvic acid induced early and intensive vascularization in the cornea of rabbits. The injection of 0.1 per cent sodium hydroxide, hydrochloric acid and acetic acid caused corneal intumescence of the same degree as that due to treatment with lactic acid, but vascularization provoked by the latter compound was significantly more intensive than that induced by the other compounds. The accumulation of lactic acid in the course of anaerobic glycolysis in hypoxic tissues plays most probably an important role in vascularization. Impairment of oxygen supply and activated metabolism due to regeneration explain the increased concentration of lactic acid and the resulting corneal vascularization after the injection of substances other than lactic acid. This compound presumably represents the vasoformative factor.

The literature on corneal vascularization has been surveyed by ASHTON [1] and COGAN [4]. COGAN [3] holds that vascularization is connected with corneal swelling, while according to ASHTON [1] it is probably due to a metabolic product arising under hypoxic conditions, and this vessel-forming factor may, owing to the poor oxygen supply of the cornea, be present also in the unimpaired avascular corneal tissue, the reason why a decrease in the density of the cornea may induce the growth of vessels. It is well-known that hypoxia promotes the development of new vessels [2, 18, 23].

The vasoformative factor is presumably lactic acid, a product of anaerobic glycolysis [8]. It was suggested that the reason why vessels are always growing toward the hypoxic tissues is that the lactic acid produced in those tissues is anabolized to glycogen by the cells having a role in vascularization. This is supported by the observation [9, 19] that glycogen is stored in the mesenchymal cells of the vascularizing retina and cornea.

The present experiments were designed to demonstrate the role of lactic acid in corneal vascularization.

Material and method

Intracorneal injections of 0.05 ml volume were made into both eyes of 28 adult rabbits with an average body weight of 2 kg. The puncture was made near the centre of the cornea, and the canula was inserted between the lamellae towards the limbus. As a result, a wheal 4 mm in diameter developed, touching the limbus over a length of 3 to 4 mm.

Usually two injections were made; the second was given 3 days after the first, possibly by the same route so that the injected solution reached the same area on both occasions.

Table I

No. of experiment	Number of animals	Side	Intracorneal injections (0.1%,0.05 ml)			Days of incipient vascularization
			Compound	pH	No	
1.	5	r.	Lactic acid	3.0	2	3—4
		l.	Lactic acid	3.0	2	3—4
2.	5	r.	Lactic acid	3.0	2	—
		l.	Lactic acid	3.0	2	—
3.	5	r.	Lactic acid	3.0	2	2—4
		l.	Sodium hydroxide	12.5	2	3—5
4.	5	r.	Lactic acid	3.0	2	2—4
		l.	Acetic acid	3.5	2	4—6
5.	5	r.	Pyruvic acid	2.5	2	3—4
		l.	Hydrochloric acid	1.5	2	3—7
6.	3	r.	Distilled water		3	8—9
		l.	Puncture only		3	0

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

Experiment No. 1. A 0.1 per cent solution of lactic acid was injected into both corneas of 5 rabbits in order to observe possible discrepancies between the two sides.

Experiment No. 2. After having injected a 0.1 per cent solution of lactic acid into both corneas of 5 rabbits, the eyelids of the left side were united by 5 to 6 stitches. The palpebral fissure was reopened for the second injection and closed again. Closure became incomplete between the 4th and the 10th day when the sutures had cut through the tissues. Although the palpebral fissure was immediately reclosed, air had nevertheless gained access to the cornea in these cases.

Experiment No. 3. A 0.1 per cent solution of lactic acid was injected into the right eye and an equal solution of sodium hydroxide into the left eye of 5 rabbits.

Experiment No. 4. Five rabbits were injected with lactic acid in the right eye, and a 0.1 per cent solution of sterile acetic acid in the left eye.

Experiment No. 5. Five rabbits received a 0.1 per cent solution of pyruvic acid in the right eye and 0.1 per cent solution of hydrochloric acid in the left eye.

Experiment No. 6. The right-side cornea of 3 rabbits was injected with distilled water, while only a puncture was made on the left side. The total number of injections and punctures was 3 per rabbit, at 3-day intervals.

Corneal vascularization, the distance between the limbus and the central rim of the vascularized area were measured daily (except, of course, in experiment No. 2). These data were analysed, the arithmetic mean of the length of the vasularized areas in the identically damaged corneas was computed, its standard deviation determined, and the difference between the two sides analysed statistically by means of Student's *t*-test.

The results of histologic examinations made in connection with the experiments have already been reported (9).

Results

Superficial and deep vascularization was notable in all corneas except those pierced but not injected. New vessel growth originated from a 5 to 7 mm long portion of the limbus, and the vessels formed a dense pannus in most cases (Fig. 1). Numerical results are presented in Table I.

Distance (mm) between vascularized area and limbus (mean value and ± standard deviation)				Notes
4th day	6th day	8th day	10t	
0.38 ± 0.13 0.34 ± 0.18	1.00 ± 0.23 0.98 ± 0.22	1.68 ± 0.44 1.70 ± 0.43	2.28 ± 0.32 2.30 ± 0.36	
0.32 ± 0.13 0.54 ± 0.21	1.26 ± 0.11 —	2.00 ± 0.12 —	3.08 ± 0.46 3.14 ± 0.46	Closed palpebral fissure
0.46 ± 0.22 0.22 ± 0.22**	1.34 ± 0.35 1.00 ± 0.10	1.94 ± 0.35 1.82 ± 0.30	2.54 ± 0.44 2.38 ± 0.36	
0.44 ± 0.32 0.08 ± 0.08**	0.58 ± 0.34 0.58 ± 0.23*	1.88 ± 0.11 1.16 ± 0.47**	2.38 ± 0.35 1.64 ± 0.47	
0.22 ± 0.08 0.06 ± 0.13**	0.94 ± 0.34 0.46 ± 0.23	1.67 ± 0.11 0.94 ± 0.47	2.46 ± 0,23 1.48 ± 0.80	
0 0	0 0	0.10 ± 0.07 0	0.13 ± 0.06 0	

Experiment No. 1. Vascularization was of the same extent on both sides.

Experiment No. 2. On the 4th day of the experiment up to which time the closure of the left palpebral fissure had been practically complete, the vessels of this side were 68 per cent longer and somewhat more densely arranged than those on the right side. The difference was not significant statistically.

Experiments Nos. 3, 4, 5. On the 4th day, vascularization in the corneas treated with lactic or pyruvic acid was more developed than in those treated with sodium hydroxyde, acetic acid or hydrochloric acid. The differences were significant ($p < 0.05$). The difference between the effect of lactic acid and that of acetic acid (Expt. No. 4) was still significant on the 6th and the 8th day ($p < 0.02$ and 0.05 respectively).

Vascularization began on the 2nd to 4th day in the corneas treated with lactic or pyruvic acid, whereas new vessel growth started on the 3rd to 5th day after sodium hydroxyde, on the 4th to 6th day after acetic acid, and on the 3rd to 7th day after hydrochloric acid injections.

Experiment No. 6. Marginal vascularization of 0.1 to 0.3 mm was found on the 8th to 10th day after the injection of distilled water, whereas mere puncturing had no effect.

Discussion

The fact that the injection of lactic acid at a concentration hardly exceeding that of lactic acid present in the cornea under physiological conditions induced early and intensive vascularization does not, in itself, prove the

vessel forming role of the compound since vascularization is generally known to follow corneal swelling. The fact, however, that all injected substances caused a similar degree of intumescence and that vascularization was nevertheless earliest and most pronounced after the injection of lactic acid, argues for a specific enhancing property of this molecule. (Most of the pyruvic acid, employed in Experiment No. 5, must have been converted into lactic acid in the bradytrophic cornea so that its effect was really one of lactic acid.) It should be noted that, while the corneal damage caused by hydrochloric acid, or sodium hydroxide was more serious than, and that caused by acetic acid equal to, the injury due to lactic acid, vascularization was less marked in these cases.

Vascularization induced by lactic acid ceased to be significantly stronger on the 6th day in Experiments 3 and 5 and on the 10th day in Experiment 4. This, however, does not argue against the specific action of lactic acid, since the acceleration of the rate of vascularization in the corneas treated with the other substances may have been due to factors that increase the concentration of lactic acid. One such factor is the impairment of oxidative metabolism which occurs if the cornea is injured. Disturbance of oxidative metabolism is, of course, followed by a rise in the concentration of lactic acid. Another such factor is connected with regeneration. WEIMAR has shown [22] that regeneration means the activation of corneal metabolism. Because of insufficient corneal O_2-supply, such activation gives rise to increased anaerobic glycolysis and so to higher concentrations of lactic acid, a phenomenon demonstrated also by PEÑA-CARRILLO [17].

In case of corneal oedema the physiologically high lactic acid concentration of the cornea [12, 13] may be sufficient in itself to produce corneal vascularization as shown by the vascularization due to distilled water injected in Experiment No. 6.

It is known that continuous wearing of a contact lens may cause corneal swelling and so lead to vascularization [6]. Swelling is due in this case to anoxia [20]. That the protracted wear of a contact lens increases the concentration of lactic acid in the cornea, and that the consecutive erosions promote corneal vascularization, has been shown in numerous animal experiments [5, 6, 16]. Erosions increase the concentration of lactic acid because, synthesized in all corneal layers, this substance is utilized only in the presence of epithelial cells [7]. Alloxan introduced into the anterior chamber has induced marked corneal swelling and vascularization in animal experiments. According to LANGHAM [14], alloxan inhibits glycolysis for 24 hours and reduces the O_2-uptake of the cornea markedly and permanently. The reduced O_2-uptake is, of course, accompanied by a rise in lactic acid concentration. LEVENE et al. [15], by encircling the equator of the globe with an elastic band, induced an increase of intraocular pressure, corneal swelling and vascularization in rabbits. They found that in the unimpaired cornea the lactic acid content amounted (with

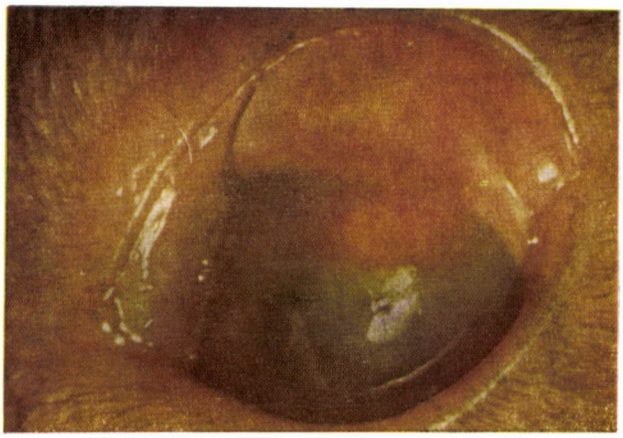

Fig. 1. Dense pannuslike vascularization of rabbit cornea after two intracorneal injections of 0.1 per cent lactic acid., on the 10th day of exp. No. I

reference to dry matter) to 2.9 μg/mg in the peripheral rim and to 5.9 μg/mg in the central disk. While there was no change in this respect in the central disk during the first 12 days, i.e. the initial and active stage of vascularization, the concentration of lactic acid rose to 5.5—6.0 μg/mg in the peripheral rim.

The results of the present experiments as also the quoted literary data make it evident that a rise in lactic acid concentration plays an important role in corneal vascularization, and support the assumption that the vaso-formative factor is also lactic acid. Glycogen, appearing in connection with vascularization, seems to be synthesized from lactic acid by the vessel-forming cells which are activated by the high lactic acid concentration. Accumulation of glycogen supplies the energy required for the proliferation and differentiation of these cells.

Beside the elevated concentration of lactic acid also other factors contribute to corneal vascularization. It has been shown [10, 11] that bradytrophic tissues as well as fragments of brain tissue, soaked in adrenal extract and then grafted on the chorioallantoic membrane of chick embryos, develop an abound-ant vasculature. The prompt vascularization in these cases is probably due to the physico-chemical properties of the explanted hypoxic tissues (e.g. a change in their compactness), but it is likewise possible that mesenchymal stim-ulation by the mineralocorticoids enhances the vascularizing affect of the high lactic acid concentration.

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ÜBER DEN MECHANISMUS DER HORNHAUTVASKULARISATION

GY. IMRE

Intrakorneal injizierte 0,1%ige Milchsäure- und Brenztraubensäurelösung verursachten bei Kaninchen frühe und ausdrückliche Korneavaskularisation. Diese Gefäßbildung war signifikant ausgeprägter als die durch Injizierung von 0,1%iger Salzsäure-, Natronlauge- bzw. Essigsäurelösung herbeigeführte Vaskularisation, obwohl die entstandene Korneaanschwellung im wesentlichen identisch war. Die Experimente sprechen dafür, daß die in den hypoxischen Geweben im Laufe der anäroben Glykose zustandekommende Milchsäureanhäufung in der Gefäßbildung eine bedeutende Rolle zu spielen vermag. In den Fällen, in denen der schädigende Faktor nicht die Milchsäure war, können die Milchsäureanhäufung und die Korneavaskularisation mit der Schädigung des oxydativen Stoffwechsels und der mit der Regeneration verbundenen Stoffwechselaktivierung erklärt werden. Der vasoformative Faktor ist aller Wahrscheinlichkeit nach die Milchsäure selbst.

МЕХАНИЗМ ВАСКУЛЯРИЗАЦИИ РОГОВИЦЫ

Дь. ИМРЕ

Интракорнеальное введение 0,1%-ного раствора молочной и пировиноградной кислот вызывает на роговице кролика раннюю и выраженную васкуляризацию. Эта васкуляризация была статистически достоверно большей, чем таковая, вызванная интракорнеальным введением 0,1%-ного раствора гидроксида натрия, соляной и уксусной кислот, несмотря на то, что набухание роговицы было по сути дела одним и тем же. Опыты подтверждают, что в гипоксических тканях в деле васкуляризации большую роль может играть скопление молочной кислоты, образующейся во время анаэробного гликолиза. В тех случаях, в которых поражение вызывалось не молочной кислотой, скопление последней и васкуляризация роговицы объясняются нарушением окислительного обмена веществ и активацией связанного с регенерацией обмена. Вазоформативным фактором, вероятно, является сама молочная кислота.

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Oncological Institute (Director: Dr. F. VADURA), Praha

SUBMICROSCOPICAL STRUCTURE OF ADENOID CYSTIC CARCINOMA OF SALIVARY GLANDS

V. FRIBORSKÝ

(Received February 23, 1965)

Adenoid cystic carcinoma of the salivary gland has been investigated by polarization and electron microscopy. The polarization microscopical findings revealed that the cylindrical formations are not amorphous but consist of oriented fibres with a characteristic polarization optical appearence. The hyaline cords (spheroids) are built up by two components, *viz.*

(i) acid mucopolysaccharides, chiefly ChSA A and/or C, in great amounts;

(ii) connective tissue fibres with positive Ebner's reaction and electron microscopical periodicity.

In a previous communication we have dealt with the histogenesis, morphological appearence and biological properties of the adenoid cystic carcinoma of salivary glands (FRIBORSKY 1963). The present paper deals with the submicroscopic structure of the cylindrical stroma formations (spheroids), the nature and origin of which is not fully clarified.

Material and methods

Biopsy material has been investigated from adenoid cystic carcinoma by polarization microscopy and electron microscopy. Polarization optical observations were made with Leitz Ortholux polarization microscope equipped with a rotating Köhler-Brace compensator of 30—58 mμ retardation. Concerning the general principles of polarization microscopy, we refer to the papers by SCHMIDT (1938), BENNET (1950) and BREWER (1957). For the special study of the question of the possible collagenous nature as well as of the acid mucopolysaccharide components of the cylindrical hyalinecords, the following topochemical reactions were applied.

1. EBNER's phenol reaction. The positive birefringence of the collagen fibres becomes negative in phenol. This effect is due to the oriented association of the phenol molecules transversally to the micellar collagen structure. Since the reaction depends on the collagens specific molecular structure, it may be considered as one of the most specific morphologic reactions for collagen. The phenol reaction was performed by mounting the slices in a mixture containing equal quantities of phenol and Canada balsam.

2. Anisotropic toluidine blue staining according to ROMHÁNYI (1963), for the study of the submicroscopic structural pattern and relative quantity of the acid mucopolysaccharide components. In a recent study of the submicroscopic structure of the metachromatic staining reaction, ROMHÁNYI (1963) has shown that in connective tissue the acid mucopolysaccharides are present in an orientated macromolecular structural pattern. The metachromatic staining reaction, as an oriented association of the dye molecules on the acidic polysaccharide groups causes characteristic anisotropic changes of the stained structures. By measuring the anisotropy of the metachromasia, quantitative data can be obtained for the acid mucopolysaccharide content in relation to the collagen content estimated by the phenol reaction, and expressed as an anisotropic index of metachromasia (ROMHÁNYI, 1963). Enzymatic treatment: Testicular hyaluronidase (Organon) for 4—6 hours at 37°C; and mg/ml of elastase in carbonate buffer, pH 8.8, for $^1/_2$—2 hours at 37°C.

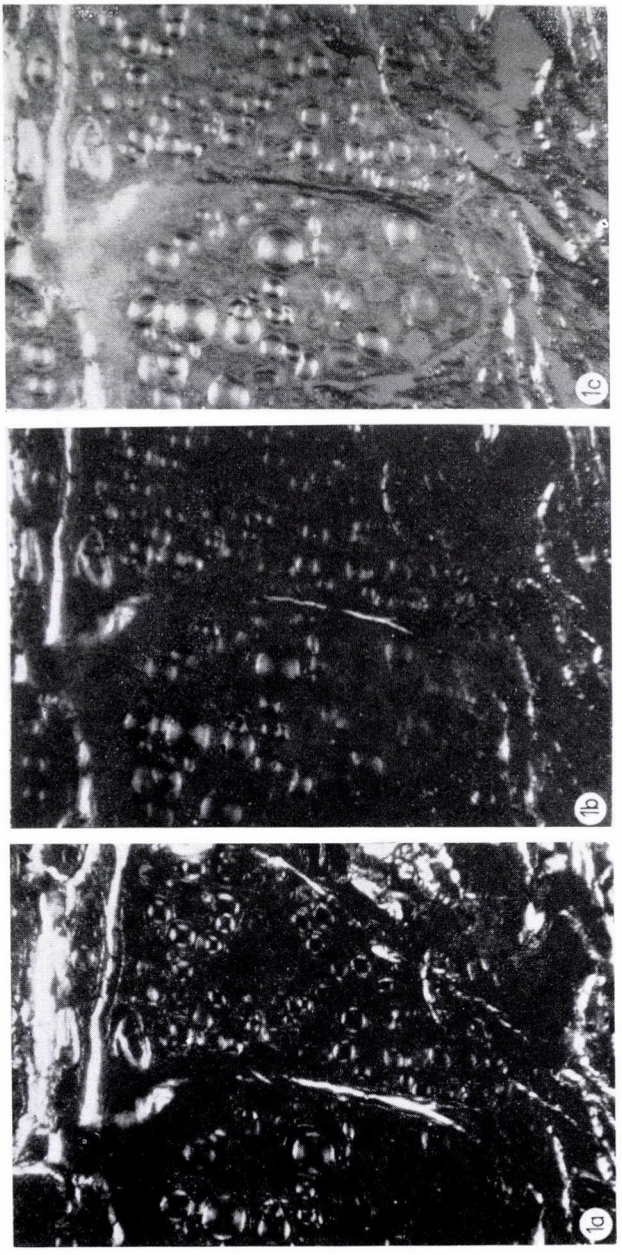


Fig. 1. Strong birefringence of connective tissue and cylindrical spheroids. Negative birefringence of spheroids

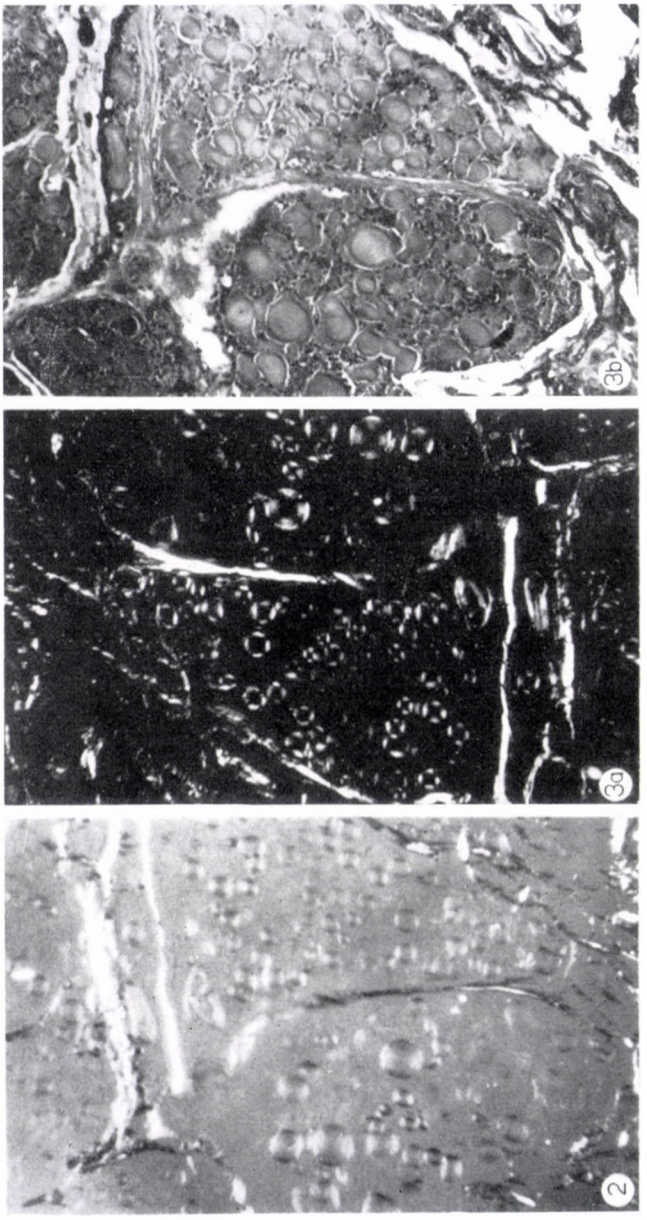


Fig. 3. Toluidine blue staining. A. light microscopy B. polarization microscopy

Fig. 2. Ebner's reaction. Inversion of birefringence

Electron microscopy. Tissue blocks from the neutral formalin-fixed biopsy material were cut into pieces of 1—2 mm, postfixed in 1 per cent buffered isotonic OSO_4, pH 7.2, for 60 minutes, and after dehydration in ethanol they were embedded in a mixture of butyl and methylmethylacrylate, 4 : 1. Sections were cut on a LKB Ultratom type ultramicrotome and examined by a Zeiss ELMI D 2 electron microscope.

Polarization optical findings.

Fig. No. 1. shows the polarisation optical behaviour of the cylindrical cords in a section mounted in water. In Fig. 1/A the microphotograph was taken at crossed polaroids without compensation. A strong birefringence of the connective tissue fibres as well as of the hyalin cords is seen, the latter appearing in the form of spheroids with polarization optical crosses. Using the compensator for establishing the optical character of the spheroids, it was found that their slow axis of transmission was oriented tangentially, corresponding to a negative spheriod.

Fig. 1 B was made at 2.2 mμ compensation. Both lateral sectors of the spheroids are compensated to dark. In Fig. 1 C 7 mμ of the same section is compensated. Here the spheroids are in general overcompensated, but the perpendicularly oriented collagen fibres (in the contre of the picture) are compensated to dark.

The negative character of the form birefringence of the spheroids is indicating the presence of a circulary oriented fibrillary submicroscopic structure. On testing the possible colla-

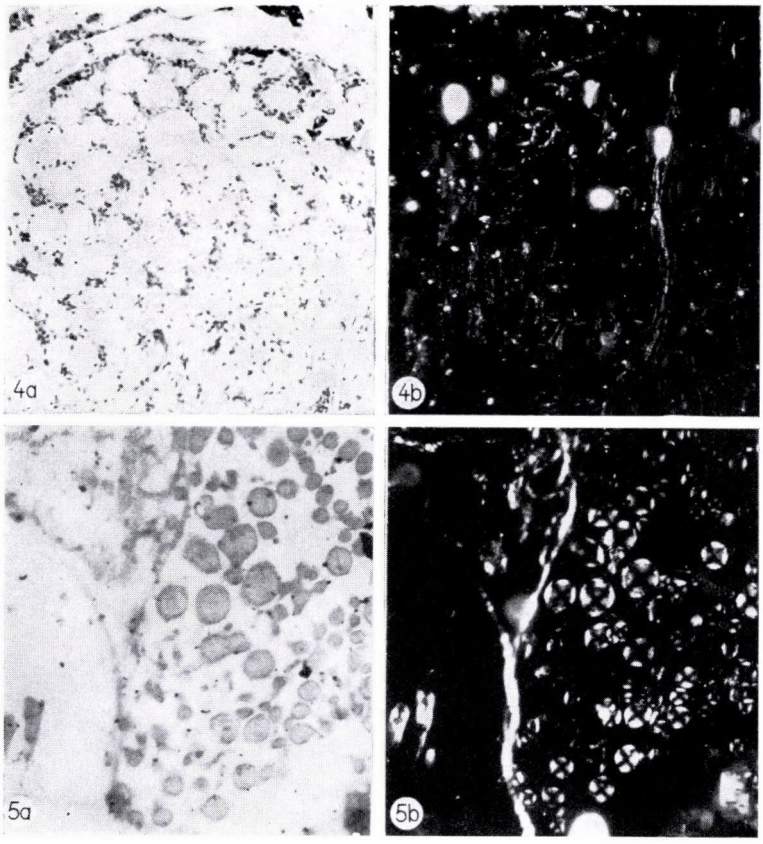
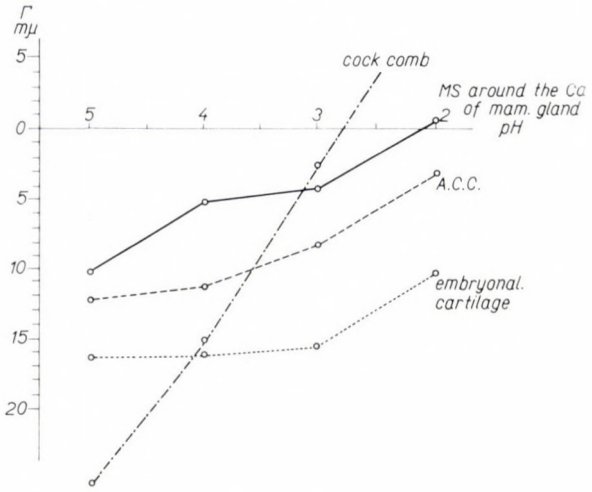


Fig. 4. Digestion with testicular hyalurinidase
A. light microscopy B. polarization microscopy

Fig. 5. Digestion with elastase
A. light microscopy B. polarization microscopy

genous nature of this micellar structure by the phenol reaction the optical character of the anisotropy became reverted, in other words it behaved like collagen (Fig. 2). This microphotograph was taken at 7 mμ compensation, the vertically oriented negative collagen fibres are compensated to dark. In the spheroids the lateral sectors are compensated to dark, their optical character is now positive along the radially oriented slow axis of transmission. Fig. 3 demonstrates the same field as Fig. 1 and 2, but after toluidine blue-ferricyanide staining at pH 4 and mounted in gum arabic. In Fig. 3 A, the typical histological picture of the cylindrical tumour can be seen. In Fig. 3 B, the same field is shown at crossed polaroids, photographed at light of λ 560 mμ. The basophil cylindric-cords appear as strongly birefringent spheroids. The sectors at compensation revealed alternately anomalous colours (blue-red), and when investigated in monochromatic red light, the slow axis of transmission was radially found. Toluidine blue staining at pH 4 indicated the presence of a circularly oriented macromolecular component of acidic nature. Its polysaccharide nature was further established by the eliminaton of this component by hyaluronidase treatment, as demonstrated in Fig. 4 A. Here it can be seen that the basophilia of the cylindrical cords disappeared and therefore nuclear staining



Graph 1

became more distinct. In Fig. 4 B the same field is seen at crossed polaroids. The anisotropic effect of the hyaline cords (as seen in Fig. 3 B) completely disappeared, only the slight anisotropic (metachromatic) effect of the collagen fibres persisted.

The findings demonstrated in Fig. 3 and 4 are indicative of a circularly orientated micellar structure of an acid mucopolysaccharide component sensitive to hyaluronidase. Fig. 5 demonstrates the relative resistence to elastase (contaminated with desoxyribonuclease) of the hyaline cords. In Fig. 5 A the cell nuclei and cytoplasm disappeared but the cylindric cords are resistant and show a strong basophilic staining with toluidine blue and reveal at crossed polaroids strongly birefringent spheroids of positive optical character.

In order further to characterize the acid polysaccharide structural component in the cylindric cords, a comparative study of the quantitative cationic dye binding capacity of some well-known acid mucopolysaccharides and of the cords was made. Graph I shows the findings expressed in mμ of retardation of the metachromatic anisotropy found in sections stained with toluidine blue at different pH. It can be seen that the curve of metachromatic anisotropy of the cock comb, which is known to contain exclusively hyaluronic acid, reaches isotropy at pH 3. This means that its metachromatic anisotropy of embryonal cartilage, containing chiefly chondroitine sulphuric acid, shows small decrease even at pH 2. The dye binding capacity of the acid mucopolysaccharide component of the spheroids of the cylindrical tumour is similar to that of the cartilage. Connective tissue metachromasia around a carcinoma of the breast (biopsy material) was intermediate between those of the cock comb and of adenoid cystic carcinoma.

Electron microscopical findings

The electron microscopic appearence of the hyaline cords was characteristic. Their form is round or ovoid and consists of an outer loose and an inner dense part (Fig. 6). The outer, loose part is a meshwork of thick fibres which connect the hyaline cords or spheroids with the tumor cells and seem to represent a connection with the vacuoles occurring in these cells. The centre of the cords consists of a great number of haphazardly arranged fine fibres. On closer examination, however, at the periphery of the inner part of the cords a circular arrangement of the fine fibres is clearly observable (Fig. 7). When cut longitudinally, the thin fibres may show a periodicity (Fig. 8).

The loose arrangement of the fibres at the periphery of the cords could be explained in different ways. They may be an artefact resulting from shrinkage during fixation, as the formol used was neither buffered nor isotonized. The spaces between the thick fibres seem to be empty. It is possible, however, that they were filled by a material which has been dissolved out during fixation or embedding procedures.

Our electron microscopic observations are in agreement with the polarization optical findings and supply direct evidence of the presence of a circularly zarranged system in the hyaline cords. In some fibres a periodicity is seen indicating their collagen nature, demonstrated also by the phenol reaction in the polarization optical studies. The problem whether or not the thick fibres at the outer part of the spheroids are collagen fibres cannot be answered on the basis of the electron microscopic findings. In these fibres the periodicity might have been obscured, masked by some material, perhaps the hyaluronidase digestible acid mucopolysaccharides demonstrated in the polarization optical studies.

Discussion

It has been shown that the hyaline cylindrical cords of adenoid cystic carcinoma of the salivary gland are not amorphous, as at crossed polaroids they appeared in the form of anisotropic spheroids. Their negative optical character with a tangentially oriented slow axis of transmission suggested the presence of a circularly oriented micellar texture. The reversal by the phenol reaction of their optical character from negative spheroids to optically positive ones with radially oriented slow axis of transmission is indicating the collagenous nature of the micellar structure. Furthermore, the strong anisotropic metachromatic reaction of the cords with toluidine blue staining at pH 4 shows the presence of a circularly oriented acid mucopolysaccharide micellar component which proved to be hyaluronidase-sensitive. Considering the pH curve of its toluidine blue binding capacity and the effect exerted on it by testicular hyaluronidase, the component may be CHSA A and/or C.

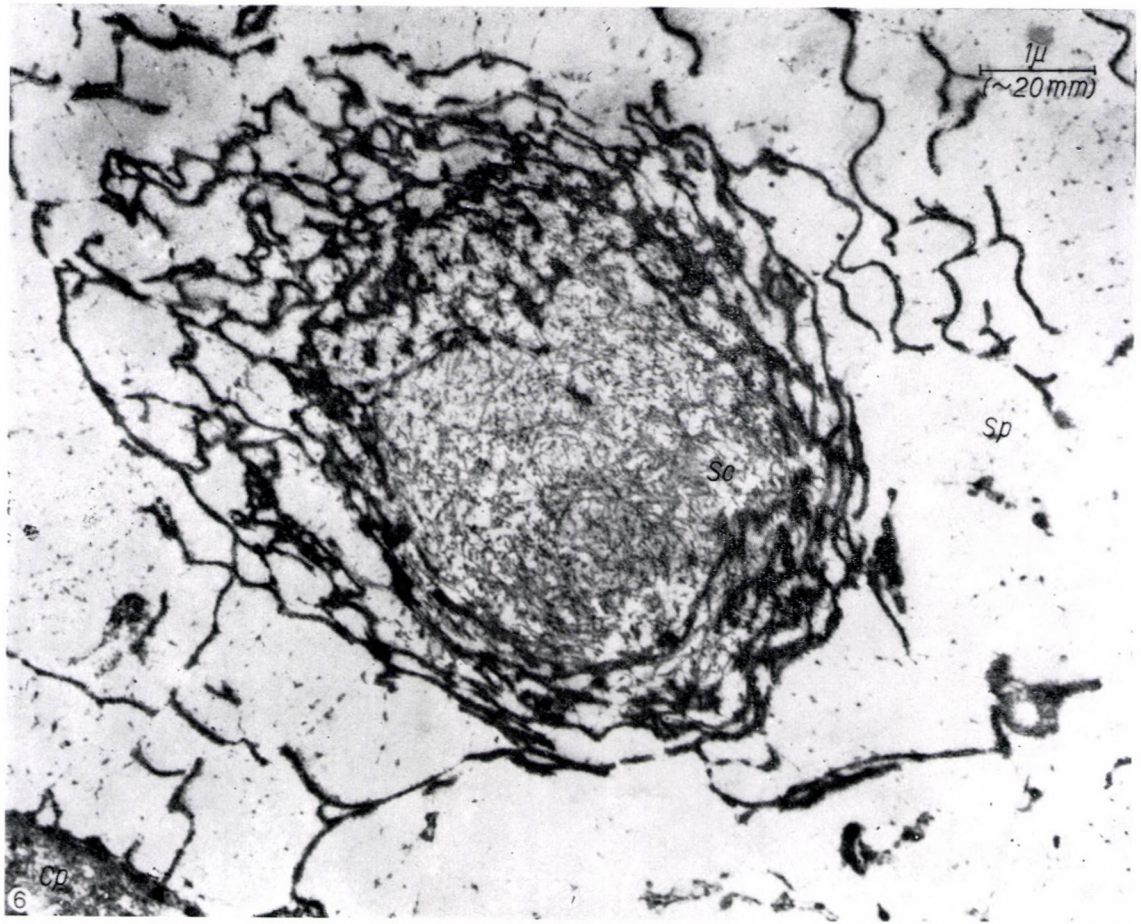


Fig. 6. Sc — centrum of spheroids Sp — periphery of spheroids Magn. × 20,000

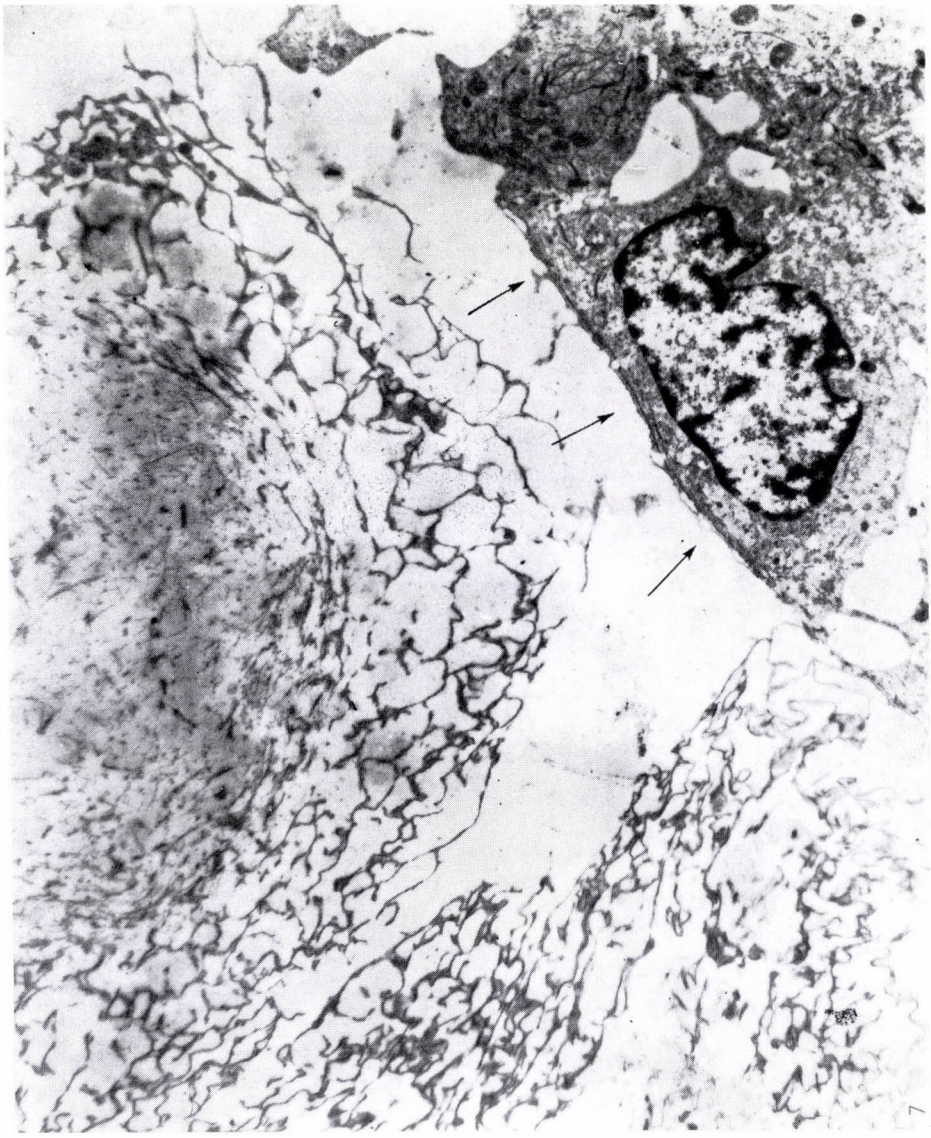


Fig. 7. The arrow shows a tumour cell with vacuoles. The fibres in the spheroid are in connection with them. Magn. × 10,000

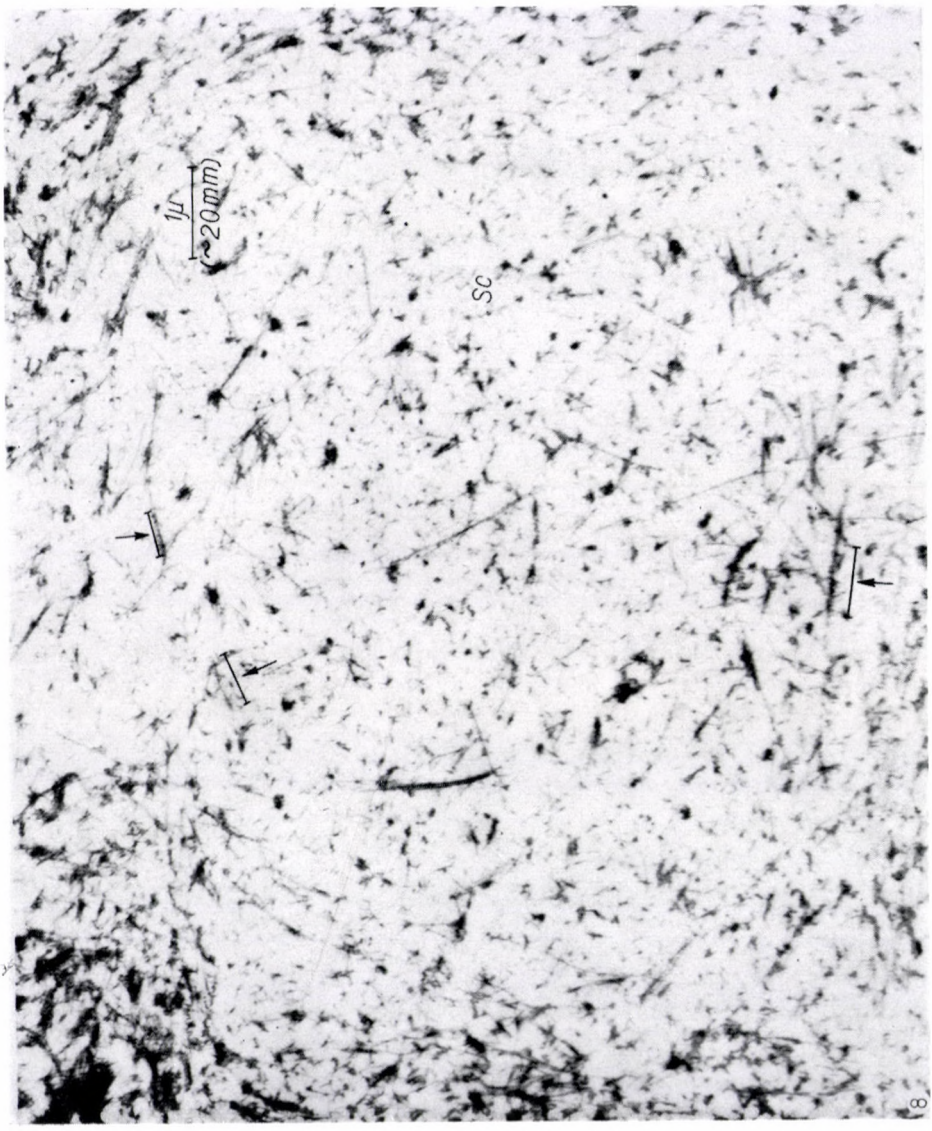


Fig. 8. Fibres with periodicity in the centre of spheroid. Magn. × 20,000

The methods used offered a possibility to compare the cationic dye binding capacity of various tissues in relation to their acid mucopolysaccharide content. The theoretical and practical aspects of the quantitative and qualitative analysis of mucopolysaccharides have been discussed in detail by SZIRMAI and BALÁZS (1958) and ROMHÁNYI (1963).

Electron microscopy supports the observations made by polarization microscopy concerning the circular arrangement of the fibres of hyaline cords. The positivity of Ebner's reaction can be explained by the presence of fibres with periodicity.

The changes in the surrounding tissues during the development or proliferation of neoplasmas are interpreted in various ways. VASSILJEV (1961) supposes that neoplasms need a neostroma for growth and the mucopolysaccharides around the tumour do not originate from decomposed connective tissue but are newly formed to serve for the proliferation of the neoplasm. MALTONI and PRODI (1960) suggest that in the tissue "mucopolysaccharides induce some changes in the medium favouring the nutritional exchange of the tumour cell". Around tumours an increase of mucopolysaccharides and collagen formation usually occurs. According to GULLINO, GRANTHAM and CLARK (1962), and GULLINO and GRANTHAM (1963), this phenomenon depends on the type of the tumour. Different tumours induce different extents of collagen formation.

The relationship between tumour and surrounding connective tissue cannot be solved unambiguously. We have proved that the connective tissue in the spheroids is formed by collagen fibres and acid mucopolysaccharides. This stroma is characteristic of adenoid cystic carcinoma. The specific arrangement of the connective tissue (disorganization of fibrils) and therefore the specific relationship between tumour and stroma is probably one of the factors responsible for the biological behaviour of adenoid cystic carcinoma.

Acknowledgement

This paper was prepared during the author's stay at the Institute of Pathological Anatomy, University Medical School, Pécs. The author is gratefully indebted to Professor G. ROMHÁNYI for guidance and helpful criticism.

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SUBMIKROSKOPISCHE STRUKTUR DES ZYSTISCHEN ADENOKARZINOMS

V. FRIBORSKÝ

Die polarisations- und elektronenmikroskopischen Untersuchungen des zystischen Adenokarzinoms der Speicheldrüse ergaben, daß die zylindrischen Bildungen nicht strukturlose Formationen, sondern orientierte, über charakteristische polarisationsoptische Aktivität verfügende Fasern sind.

Die Hyalinbündel bestehen aus 2 Komponenten:

1. saueren Mukopolysacchariden, hauptsächlich ChSA A und/oder C in großen Mengen, und

2. Ebner-positiven, elektronenmikroskopische Periodizität aufweisenden Bindegewebsfasern.

СУБМИКРОСКОПИЧЕСКАЯ СТРУКТУРА КИСТОЗНОЙ АДЕНОКАРЦИНОМЫ

В. ФРИБОРСКИ

Исследования кистозной аденокарциномы слюнной железы под поляризационным и электронным микроскопами показали, что цилиндрические образования не являются бесструктурными формациями, а волокнами, обладающими характерной поляризационно-оптической активностью.

Гиалиновые пучки состоят из двух конмпонентов:

1. Из кислых мукополисахаридов, главным образом ХСА А и (или) С в большом количестве и из

2. Эбнер-положительных соединительно-тканных волокон, обладающих электронно-микроскопической активностью.

Dr. V. FRIBORSKÝ: Praha 8-Bulovka Czechoslovakia

RECENSIONES

British Medical Bulletin

Transplantation of Tissues and Organs

Vol. 21, Number 2, May 1965. Published by the Medical Department, The British Council.

The present issue of the Bulletin contains the views of prominent scientists on transplantation, a fundamentally important subject in both surgery and biology. It cannot, therefore, fail to command the attention of medical and biological research workers all over the globe.

The introduction of the volume, contributed by P. B. MEDAWAR, emphasizes the great theoretical and practical importance of transplantation. The initial researches in this field were inspired by the progress of surgery. It led to the study of immune-biological relationships and of the problem concerning the possibility of influencing them. In addition to presenting a survey of researches regarding renal, cartilage and corneal transplantations, the volume under review offers information about the intricate problems of tissue immunity and the transplantation of organs. Such information includes, for instance, instructive discussions concerning the transplantation of haemopoietic tissues, especially in cases of radiation injury, and also a highly useful treatise on the transplantation of endocrine organs. The volume includes furthermore information regarding the immune-biological factors of neoplastic growth.

These subjects are contained in the following 15 articles:

J. R. BATCHELOR: Histocompatibility systems.
J. L. GOWANS: Role of lymphocytes in destruction of homografts.
J. F. A. P. MILLER: The thymus and transplantation immunity.
J. F. LOUTIT: Transplantation of haemopoietic tissues.
G. GOWLAND: Induction of transplantation tolerance in adult animals.
M. SIMONSEN: Graft-versus-host reaction in chick embryo.
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T. GIBSON: Cartilage grafts.
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R. Y. CALNE: Supply and preservation of kidneys..
K. A. PORTER: Morphological aspects of renal homograft rejection.
M. F. A. WOODRUFF: Biological and clinical aspects of organ transplantation.

I. TÖRŐ

Pathologie des Ballismus

A. JUBA

Publishing House of the Hungarian Academy of Sciences,
Budapest 1965. 132 pages, 27 illustrations

The monograph under review consists of two parts.

The first part begins with the embryology, anatomical and histological structure, blood supply and the neural connections of Luys' nucleus. There follows a discussion of the morphological changes in cases of hemiballism and paraballism. This discussion is accompanied by a wealth of bibliographic references. The reproduction in animal experiments of nuclear and path

deviations observed in "typical and atypical" human cases is pointed out. The first part covers 58 pages and presents a detailed survey of the pertinent literature. It is instructively supplemented by a number of anatomical drawings representing the supposed or established connections of Luys' nucleus.

The second part contains case reports from the author's clinical material, together with the pathohistological findings concerning the central nervous system. Among four cases of hemiballism there was one in which a softening of the subthalamic nucleus was observed, while symptoms in the other three cases were due to a lesion of the posterior part of the internal capsule. Case No. 9 is interesting from both a pathogenic and a therapeutic angle: although the nucleus had softened, no hemiballism resulted since arterio-sclerosis had induced grave degeneration in other areas of the central nervous system. Location of the degenerated areas makes it possible to determine by histological analysis the sites of inhibition involved in the release of choreic movements.

The case records include two cases of paraballism and two cases of choreoballism. In the latter cases, the interconnection between ballism and chorea of vascular origin is pointed out. The possibilities of conservative and surgical therapy (the latter on the evidence of a case treated by the author) are then discussed.

The morphological descriptions are illustrated by instructive photographs, most of which represent axonal degeneration. The bibliography containing 154 references offers a basis for more detailed studies.

The volume has been edited by S. Dobi; it is a worthy commemoration of the neuro-histological activity of the recently deceased author.

Gy. G.

INDEX

Morphologia Normalis et Experimentalis

Oncologia

Printed in Hungary

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

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ERRATUM

Bd 14, H. 1, pp 82—83. Abbildungen 3, 4 und 5 wurden vertauscht. Die richtigen Abbildungsnummern und -unterschriften sind:

Abb. 3. Kongorotfärbung in der Haut von 5 Monat alten Foeten. (In der Mitteilung: Abb. 5.)

Abb. 4. Die sog. "Übergangszone" wird mit der Bakerschen Reaktion gut sichtbar. (In der Mitteilung: Abb. 3, S. 83.)

Abb. 5. Starke saure Phosphatasereaktion in der Hornschicht. *Vadász*sche Azofarbstoff-Methode. (In der Mitteilung: Abb. 4.)

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First Department of Gynaecology (Director: Prof. B. HORN), Section for Gynaecology and Obstetric (Head: S. FERKÓ) of the Tétényi Hospital, Budapest

HISTOCHEMICAL STUDY OF RAT ORGANS TREATED WITH NORSTEROID AND PROGESTERONE, WITH SPECIAL REFERENCE TO HYDROXYSTEROID DEHYDROGENASES

F. TÓTH and I. SZŐNYI

(Received 21 May, 1965)

A total of 130 white female rats have been treated with progesterone and norsteroid in order to investigate their effect on the lipoid in the ovaries and suprarenal glands and also to study hydroxysteroid, lactic and succinic dehydrogenase, alkaline- and acid phosphatase and non-specific esterase activity in the ovaries, suprarenal glands and the liver. In constrast to norsteroid, progesterone was found to increase the amount of lipoid and enhance the examined reactions. It is suggested that a competition is taking place for the steroid dehydrogenase which converts lynoestrenol into 17-ketosteroid and pregnenolone into progesterone. The latter cannot be formed if lynoestrenol is predominant.

Details of our clinical experience and animal experiments with synthetic progestogens have been reported previously [6, 7]. The present paper deals with the effect of progesterone on the ovary, adrenal gland and liver of rats.

Since our observations concerning the effect of 17-α-methyl-19-nortestosterone and progesterone on the ovary have been reported earlier [8], the present account will be restricted to lynoestrenol (17α-ethynyloestr-4-en-17ß-ol), the most efficacious synthetic progestogen, and progesterone (the Hungarian preparation Progestin).

Material and method

A total of 130 white female rats of identical stock, with body weights between 110 and 120 g, were used. The animals were kept and under identical conditions on a mixed diet and divided of 20 rats each in sex test groups and a control group of 10 rats.

Lynoestrenol (Organon and progesterone were dissolved in sunflower oil and doses of 0.1 mg/g body weight were injected into the crural muscle of the animals every second day.

The first group was treated with lynoestrenol for a monath, the second for 40 days, the third, for 2 months. Animals in the fourth group received lynoestrenol for 45 days and were sacrificed 2 weeks after the last injection, those in the fifth group progesterone for a month and those in the sixth group progesterone for 2 months. The control animals were injected intramuscularly with 0.1 ml doses of sunflower-oil. The experiments were carried out at different periods, and the results were controlled in further experimental groups. Vaginal smears were made daily. The animals were weighed once every week and killed by a blow on the nape, then the parenchymatous organs, endrocrine glands, uterus and diencephalon were immediately removed, weighed and fixed in a 10 per cent solution of neutral formalin or Shabad's flouid which latter contains zink and copper salts for the inhibition of bacterial digestion, and phlorhizin in a mixture of 10 per cent formalin and absolute alcohol for inhibiting glycolytic enzymes. Haematoxylin-eosin, methylgreen pyronine, Feulgen'reaction, oil-red, PAS, Ritter-Oleson's reaction, 0.25 per cent toluidine blue, azan, oil-red and van Gieson's dye were used. The acid and alkaline phosphatase and non-specific esterase reactions were performed with the azocoupl-

ing method, with naphthol As-phosphate substrate. The stains and reactions used for the identification of lipoid were oil red, 0.2 per cent Nile blue, Schultz's method, the phenylhydrazine and phenylhydrazine-formazan reactions. Diastase, hyaluronidase served for digestion.

The incubating solution for the steroid-dehydrogenase reactions, described by LEVY *et al.* [2], had the following composition:

Hormone substrate	0.2 mg	
Propylene glycol	0.5 ml	1 Mol.
Neotetrazolium chloride, 1 mg/ml	1.0 ,,	,,
DPN solution, 3 mg/ml	0.8 ,,	,,
Nicotinamide solution, 1.6 mg/ml	0.7 ,,	,,
Phosphate buffer 0.1 Mol, pH 7.1 to 7.4	4.0 ,,	,,
Total	7.0 ml	

The following hormone substrates were employed: dehydroepiandrosterone (DHA), androstenedione, androsterone, testosterone, pregnenolone, progesterone, oestradiol, oestrone, oestriol, lynoestrenol, corticosterone, cortisone and hydrocortisone. Some incubating solutions contained, instead of hormone, 50 mM sodium lactate or 1.4 per cent sodium succinate as substrate. Incubation was carried out at 37°C for 2 hours. After dissolving the hormones in 0.5 ml of acetone, the latter was evaporated at 56°C, replaced by 0.5 ml propylene glycol and the incubating solution was then added to it. The reactions were first performed on cryostat sections, later on frozen one previously fixed in cold acetone for 30 minutes. Recently, the specimens are fixed in glacial neutral formalin for one half hour, and the sections are made by means of a freezing microtome. Since there is no essential difference between the results obtained by these three methods, the third has been adopted as the routine procedure because it provides material suitable for phosphatase reactions and lipid staining as well. The pH of the incubating solution, having been found to be of decisive importance, is repeatedly checked. The frozen sections are carefully washed in Ringer's solution and distilled water. After incubation, the preparations are fixed in 10 per cent neutral formalin and mounted in glycerinated gelatin.

Results

It is clear from Table 1 that animals treated with progesterone (groups 5 and 6) gained most, and those treated with lynoestrenol least, in weight.

The greatest loss of uterine and ovarian weight occurred in group 3, i.e. in the animals treated longest with lynoestrenol. The ovary of animals of

Table 1

Effect of lynoestrenol and progesterone on uterine, ovarian and adrenal weight in rats

Groups	Body weight g	Uterine weight mg	Ovarian weight mg	Adrenal weight mg
	average			
Group 1	136	338	60	68
Group 2	140	350	62	70
Group 3	120	257	39	60
Group 4	140	297	86	61
Group 5	162	403	85	65
Group 6	170	364	94	71
Group 7 control	150	340	85	80

group 4 (i.e. those killed 2 weeks after the last lynoestrenol injection) contained many more corpora lutea than that of the controls. Adrenal weight decreased in every group, most markedly in groups 3 and 4.

None of the examined organs exhibited pathological changes.

Lynoestrenol induced protracted oestrus, while prolonged periods of dioestrus alternated with prolonged oestrus (8 to 10 days) in the progesterone-treated animals.

Uterus. Uterine changes were best demonstrable by van Gieson's dye and azan. By progesterone-treated animals the uterine became thinner and there was collection of fluid in the lumen. The glandular ducts were dilated and lined with high columnar epithelium with bright cytoplasm. The stroma contained a network of delicate collagen fibres. Norsteroid caused a thickening of the uterine wall and a constriction of the lumen. Coarse, fibrous hyaline connective tissue accumulated in the stroma, the epithelial cell lining the glandular ducts became flatter, and their cytoplasm stained dark.

Ovary. A great number of persistent corpora lutea were found in the ovary of progesterone-treated animals, and a large amount of Schultz-negative and carbonyl-positive birefringent lipoid had accumulated in the ripe corpora lutea (Fig. 1). The amount of lipoid was highest in the cells of the follicular theca interna of freshly organizing corpora lutea. Corpora lutea undergoing involution displayed coarse, irregular carbonyl-negative lipoid granules (Fig. 2). Staining with methylgreen pyronine revealed a large amount of pyroninophilic matter in the cytoplasm of postovulatory granulosa cells. The positive pyroninophilic and PAS reactions of the zona striata and the liquor folliculi turned negative after digestion with ribonuclease and diastase.

The active cells of the stroma, rich in lipoid, exhibited intense pyroninophilia and could therefore be well distinguished from the inactive, hardly pyroninophilic interstitial fibroblasts. Degenerating granulosa cells in the centre of follicles were markedly PAS-positive. The reaction became weaker after diastase treatment, indicating that these cells contain glycoproteins in addition to glycogen.

The ovary of animals treated with norsteorid showed signs of atrophy. It exhibited follicles at different stages of development, fibrous connective tissue with dilated vessels, shrunk and some degenerated corpore lutea. The amount of lipoid was unchanged in the tunica interna of the theca folliculi and in the interstitial cells (Fig. 3). Most of the lipoid granules were, however, Schultz-positive, carbonyl-negative and not birefrigent.

Adrenal glands. A large amount of lipoid was observed in the zona fasciculata and in the adjacent cells of the zona reticularis of progesterone-treated animals (Fig. 4). The lipoid was Schultz-negative, carbonyl-positive and birefringent (Fig. 5). The adrenal of animals treated with norsteorid contained much Schultz-positive, carbonyl-negative and nonbirefringent lipoid (Fig. 6).

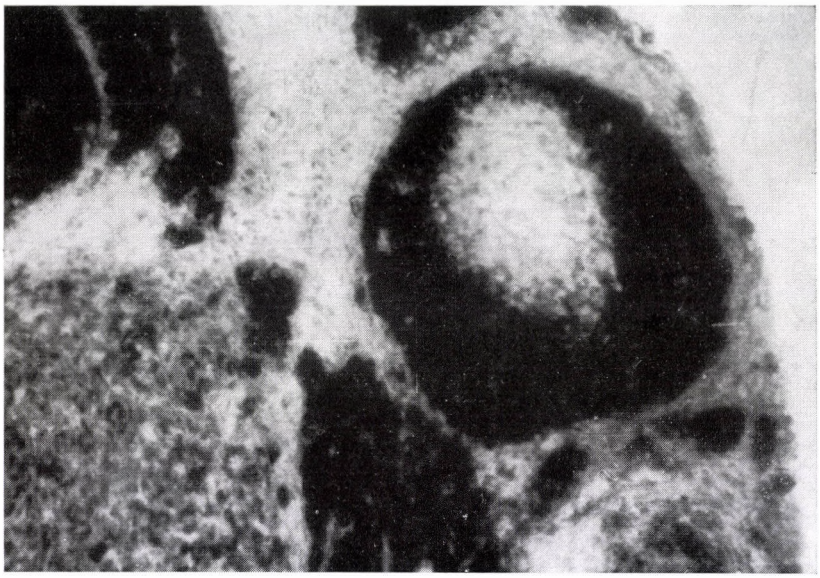

Fig. 1. Large amount of birefringent, Schultz-negative, carbonyl-positive lipoid in ripe corpus luteum, × 128

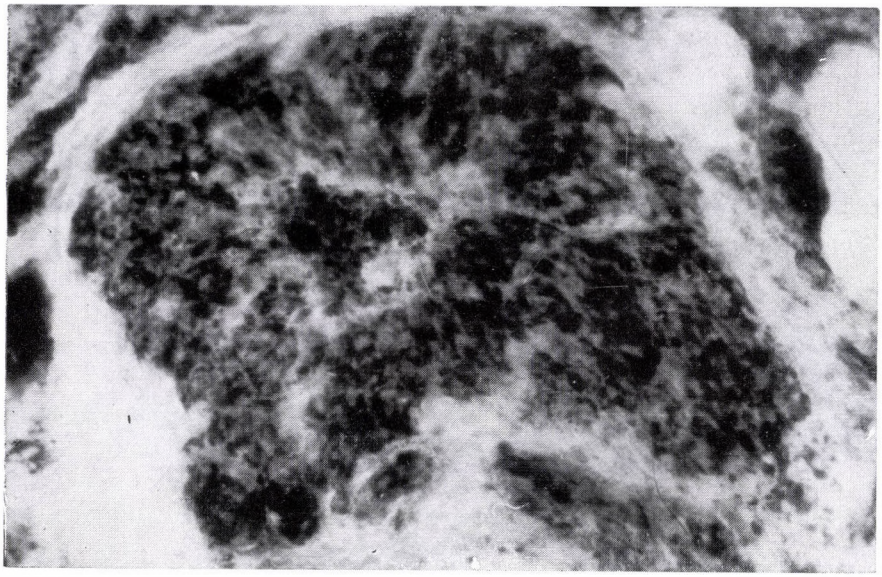

Fig. 2. Schultz-positive, carbonyl-negative, non-birefringent, coarse lipoid granules in degenerating corpus luteum. Fat. red, × 128

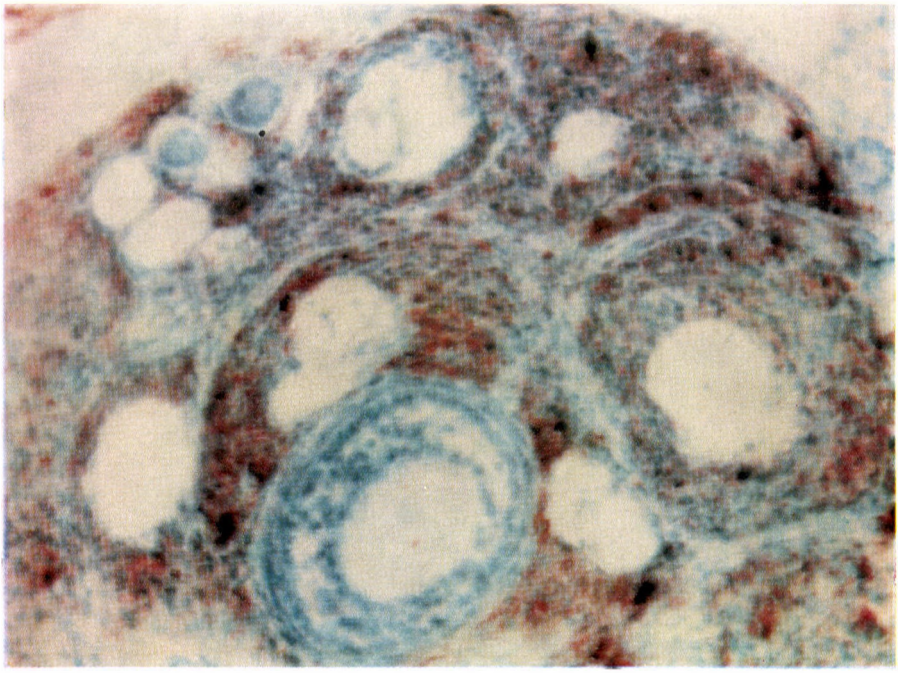

Fig. 3. Mostly Schultz-positive, carbonyl-negative, non-birefringent lipoid in the ovary of lynoestrenol-treated rat. Fat red, × 80

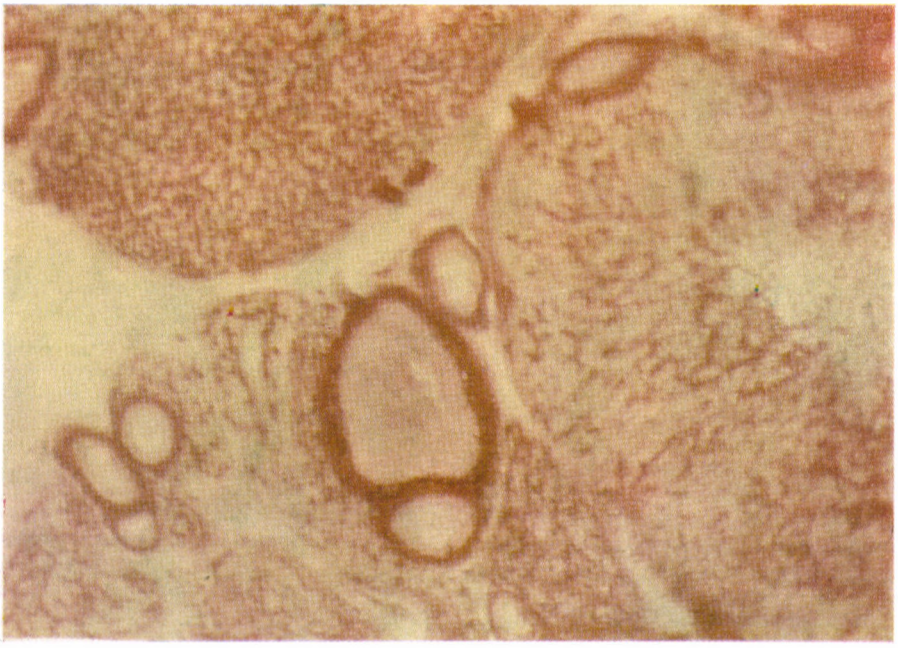

Fig. 8. Intensive alkaline phosphatase activity in corpora lutea after progesterone treatment. Azo-coupling method with naphthol-As-phosphate as substrate, × 80

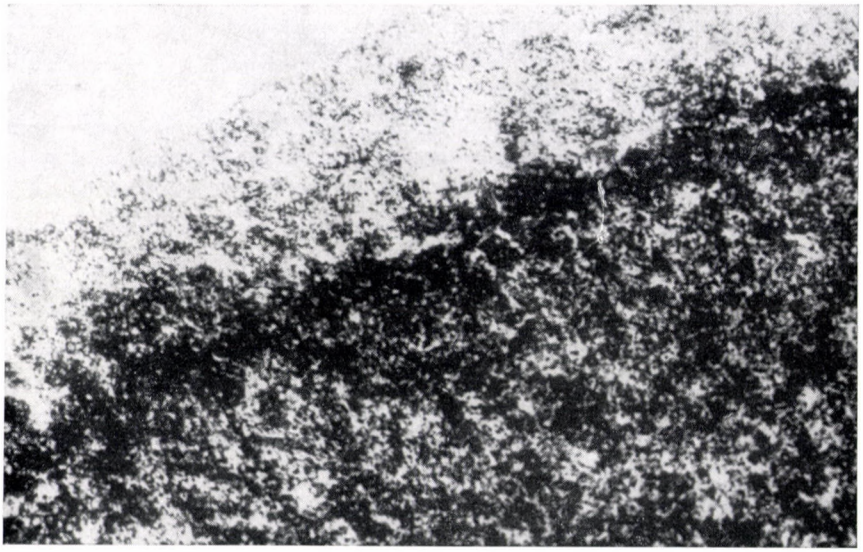

Fig. 4. Large amount of lipoid in the outer layer of the zona fasciculata and zona reticularis of progresterone-treated rat. Fat red, × 128

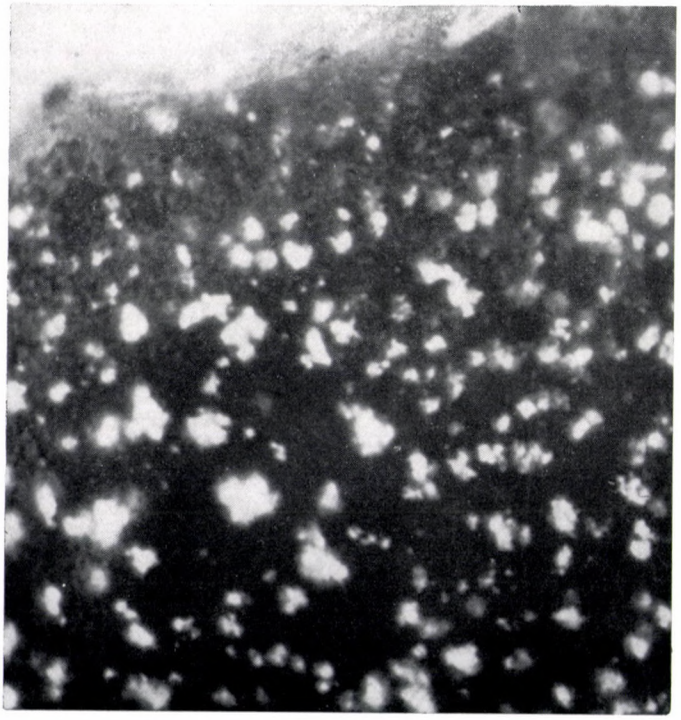

Fig. 5. Large amount of birefringent lipoid in the zona fasciculata after progesterone treatment. Fat red. Polarization microscopy, × 128

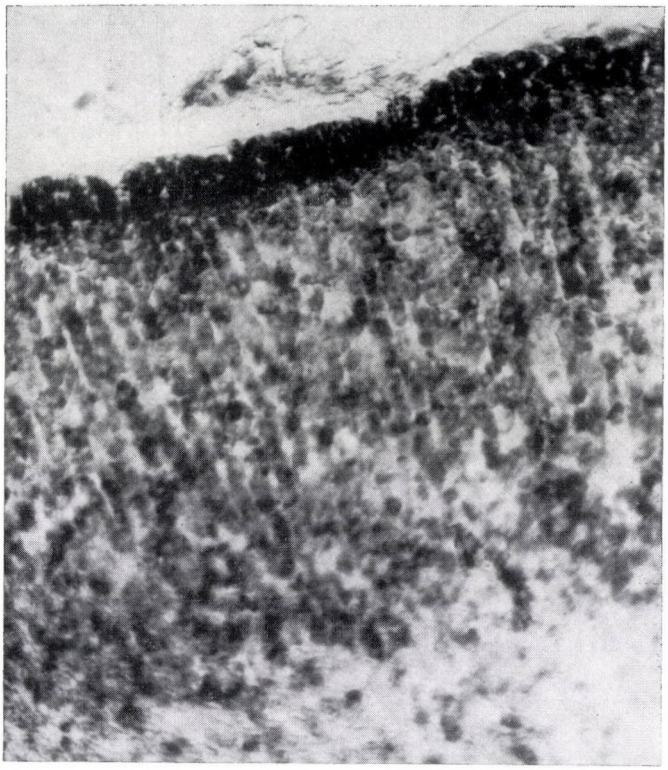

Fig. 6. Mostly Schultz-positive, carbonyl-negative, non-birefringent lipoid in suprarenal gland of lynoestrenol-treated rat. Schultz's reaction, × 128

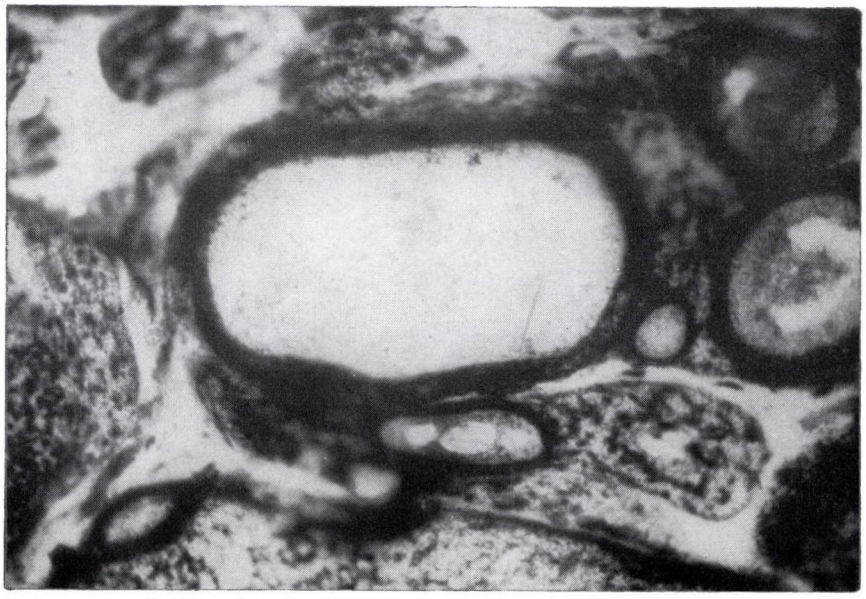

Fig. 7. Intensive alkaline phosphatase activity in theca interna. Azo-coupling method with naphthol-As-phosphate as substrate. × 128

Hydrolytic enzymes and dehydrogenases

(1) *Ovary*

(a) *Alkaline phosphatase.* Reaction was most pronounced in the follicular theca interna (Fig. 7) and the ripe corpora lutea. In the latter even the small capillaries were sharply outlined. The reaction was somewhat weaker in the interstitial cells and the vessels of the connective tissue. An intensive reaction

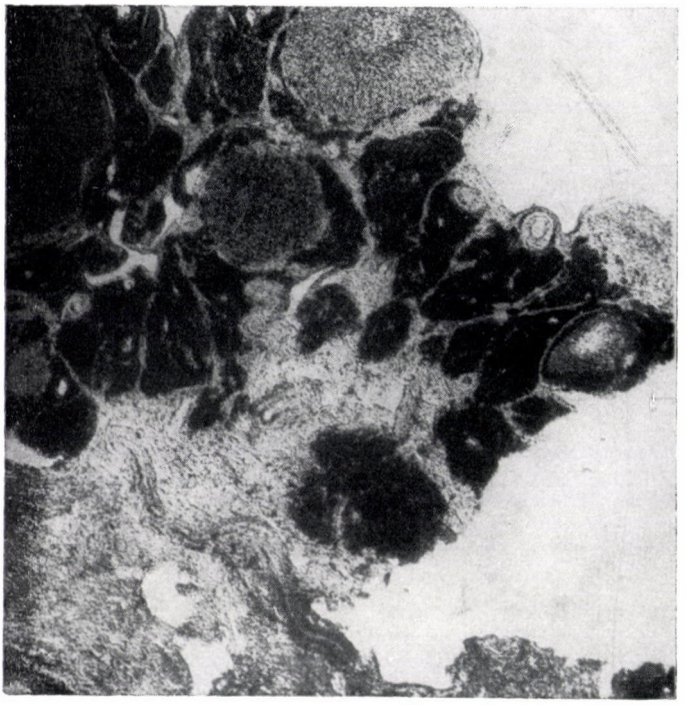

Fig. 9. Intensive lactic dehydrogenase reaction (with neotetrazolium chloride) in interstitia theca cells and theca interna. × 80

was obtained in the endothelium of the capillaries running in the theca interna of secondary and tertiary follicles, and a slightly weaker one in the theca cells. Granulosa cells gave no reaction. Activity seemed to decrease in the degenerating corpora lutea (Fig. 8). The ova were sharply outlined. An intensive reaction was noted in the nuclear membranes; this became less pronounced under the effect of lynoestrenol. In contrast to the controls and the progesterone-treated animals, the reaction became positive in the granulosa cells of primary and tertiary follicles, without, however, having reached the intensity observed in the theca.

(b) *Acid phosphatase.* Activity of this enzyme was weaker than that of alkaline phosphatase. It was most marked in the theca interna, and less so in

the interstitial luteal cells and the endothelium of capillaries running in the corpora lutea. Progesterone increased and norsteroid diminished the activity.

(c) *Non-specific esterase.* Reaction was most marked in the theca interna and the corpora lutea. Involuting corpora lutea, too, showed intensive activity, and even the granulosa cells gave a moderate reaction. Activity was increased by progesterone and decreased by norsteroid.

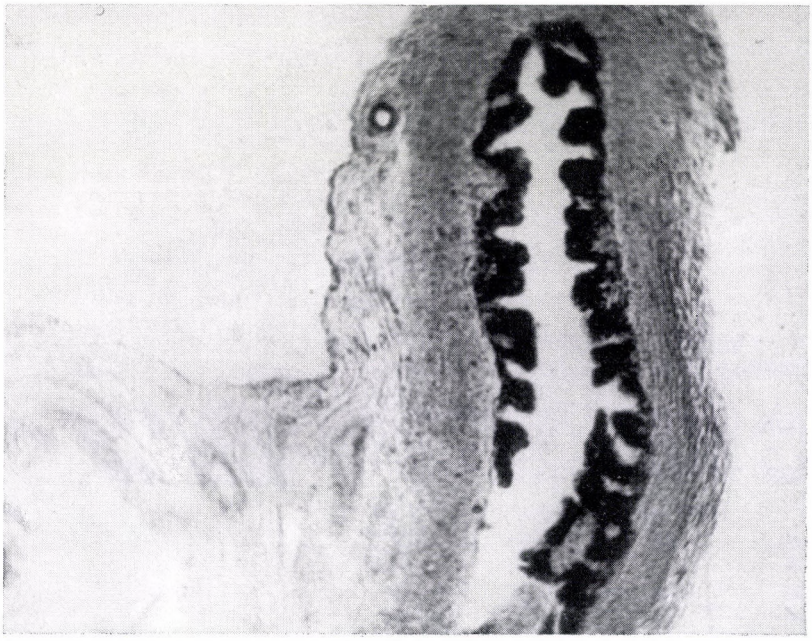

Fig. 10. Intensive hydroxysteroid dehydrogenase reaction in epithelial cells of the uterine tube, with lynoestrenol as substrate. × 80

(d) *Dehydrogenases.* The hydroxysteroid, lactic, and succinic dehydrogenase reactions were most pronounced in the interstitial luteal cells and the theca interna (Fig. 9), somewhat less marked in the granulosa cells of atretic follicles and in the corpora lutea. Primary follicles and vessels failed to react with hydroxysteroid dehydrogenase, while reaction in the endothelium of the uterine tube was positive (Fig. 10). The lactic-dehydrogenase reaction was intensive in the vessels. Sharper than alkaline phosphatase did the precipitating formozan outline the endothelium, media and adventitia of the vessels (Fig. 11).

Progesterone intensified all the three reactions, especially in the corpora lutea (Fig. 12). Norsteroid induced no change in the reactions. As regards differences due to the hormone substrates, the reaction was negative in lynoestrenol-treated animals if the incubating medium contained pregnenolone, whereas — in comparison with the control animal — activity was marked if

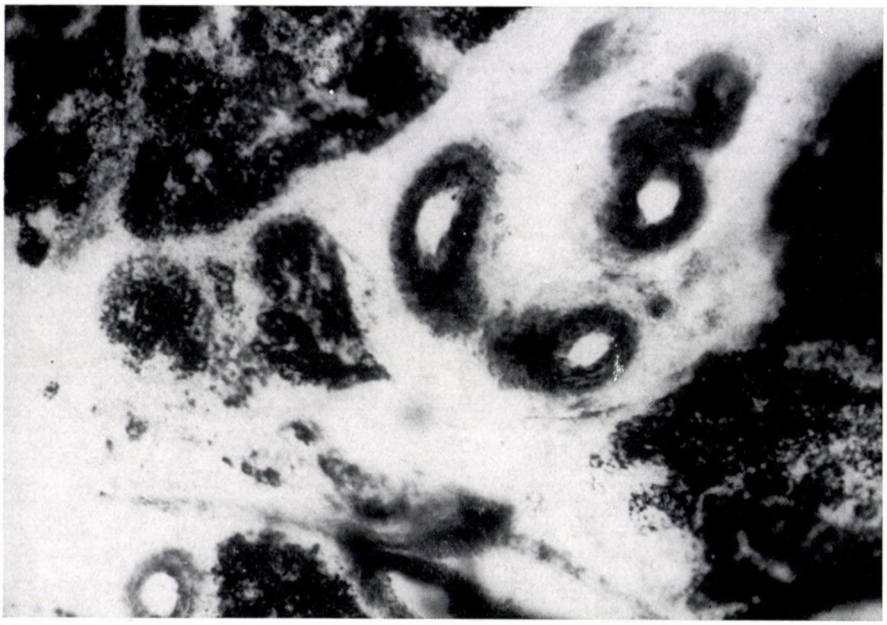

Fig. 11. Intensive lactic dehydrogenase reaction in vessels of the ovary. × 128

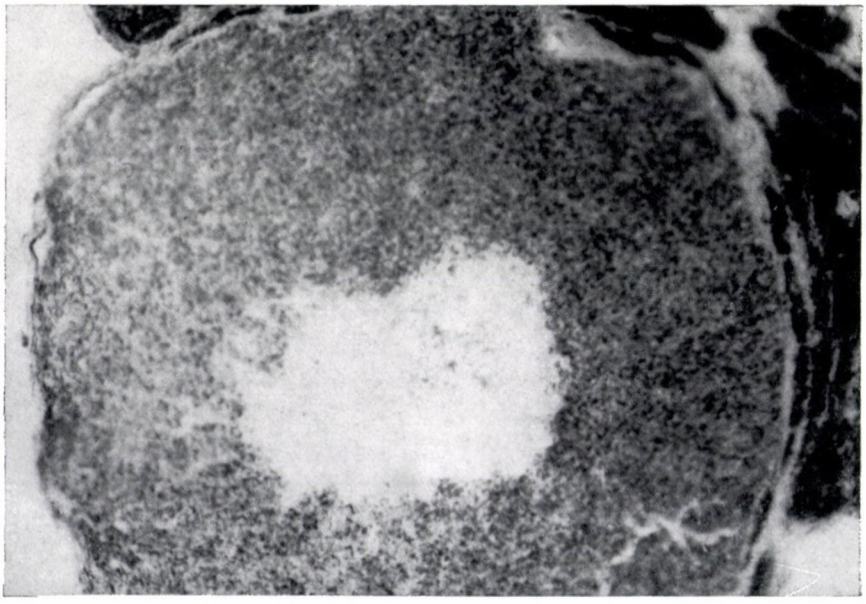

Fig. 12. Hydroxysteroid dehydrogenase reaction in corpus luteum, with pregnenolone as substrate, after treatment with progesterone. × 128

Table 2

Steroid dehydrogenase activity in the ovary, adrenal and liver of untreated (control) rats and rats treated with lynoestrenol

Hormone substrate	Control animals			Animals treated with lynoestrenol		
	Ovary	Adrenal	Liver	Ovary	Adrenal	Liver
Dehydroepiandrosterone (DHA)	++++	++++	++++	++++	++++	+++
Androstenedione	0	0	0	0	0	0
Androsterone	0	0	0	0	0	0
Testosterone	++	++	++	++	++	++
Pregnenolone	++	++	++	0	0	+
Progesterone	0	0	0	0	0	0
Oestradiol	0	0	0	0	0	0
Oestrone	0	0	0	0	0	0
Oestriol	0	0	0	0	0	+
Lynoestrenol	+	+	++	+++	+++	+++
Corticosterone	+	+	++	+	+	++
Cortisone	0	0	0	0	0	0
Hydrocortisone	0	+	++	0	+	++
Controls	0	0	0	0	0	0

the incubating medium contained lynoestrenol (Fig. 13). DHA, testosterone and corticosterone did not affect the reactions (Table 2).

(2) *Adrenal*

Fig. 17 presents a schematic illustration of the structure of this organ. Quadrant 2 shows the three zones, the other three quadrants illustrate the intensity and distribution of enzymatic activities in the different zones of the suprarenal cortex in animals treated with lynoestrenol. The hydroxysteroid reaction was strongest in all groups when DHA served as substrate. Activity was most intensive at the boundary of the zona fasciculata and the zona reticularis (Fig. 14). The lactic dehydrogenase and alkaline phosphatase reactions were most marked in the zona fasciculata (Fig. 15), less pronounced in the zona reticularis and still less so in the zona glomerulosa. Succinic dehydrogenase, acid phosphatase and non-specific esterase gave weak reactions in all zones. All activities were slightly enhanced by progesterone and somewhat decreased by norsteroid.

(3) *Liver*

Fig. 18 presents a schematic illustration of a hepatic lobule. Quadrant 2 shows the normal structure of the liver: d = liver cells, e = sinusoids, f =

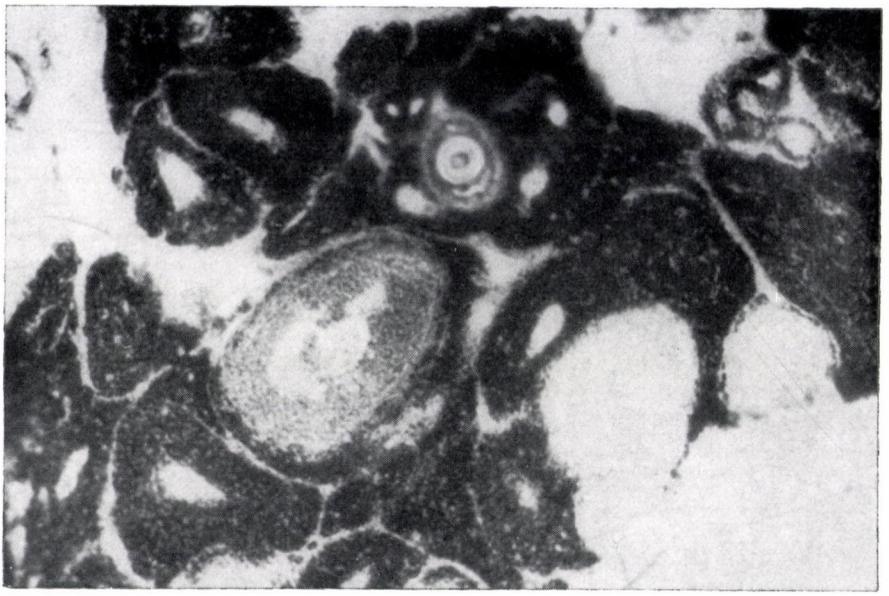

Fig. 13. Hydroxysteroid dehydrogenase reaction in the ovary, with lynoestrenol as substrate. × 128

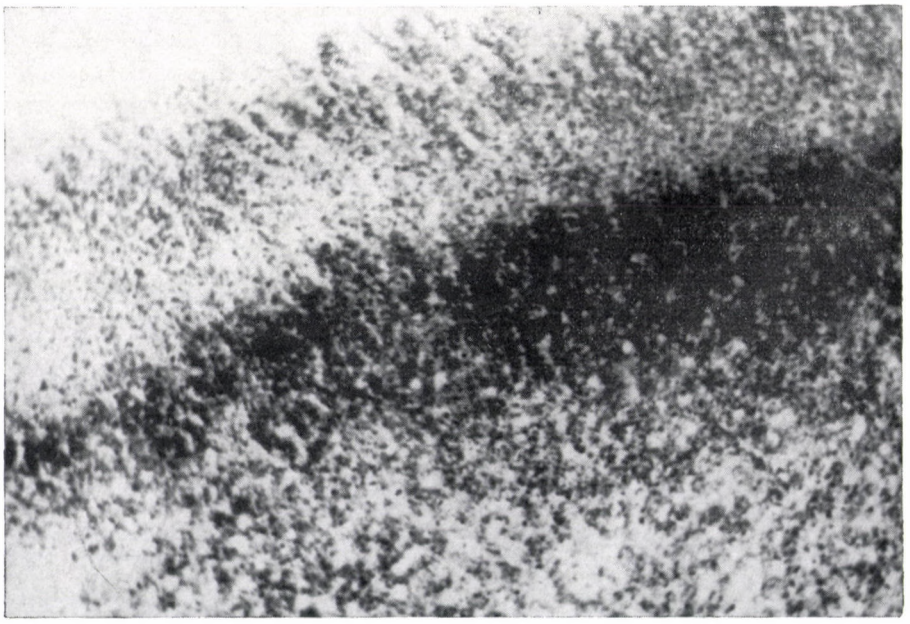

Fig. 14. Intensive hydroxysteroid dehydrogenase reaction at the boundary of the zona fasciculata and zona reticularis, after lynoestrenol treatment, with DHA as substrate. × 128

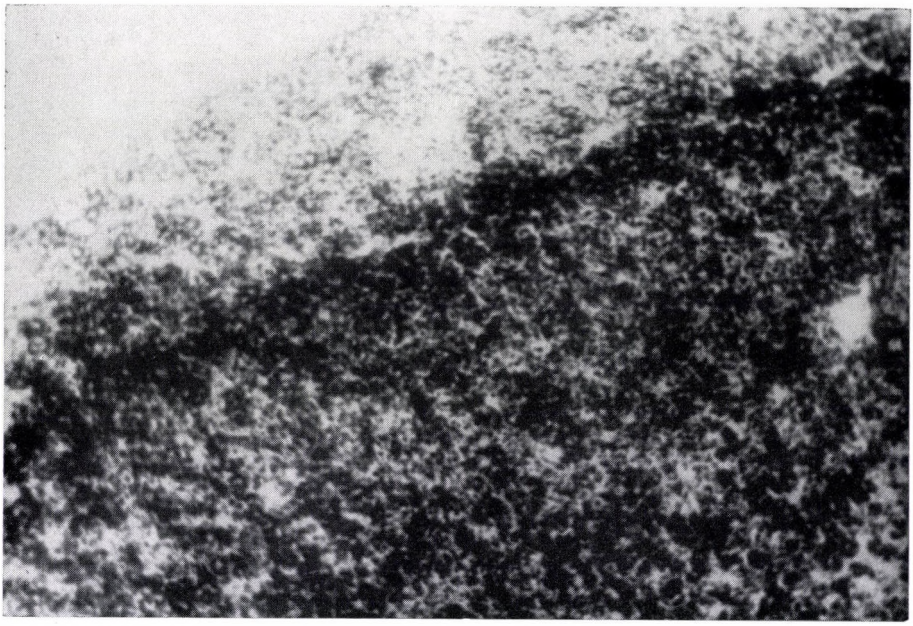

Fig. 15. Intensive lactic dehydrogenase reaction in zona fasciculata, after progesterone treatment. × 128

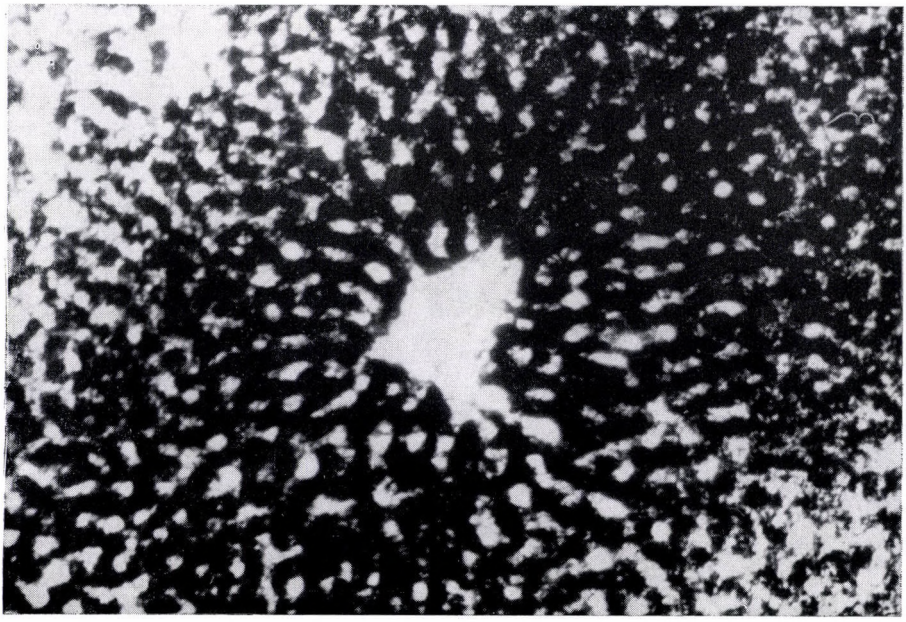

Fig. 16. Intensive, mostly centrolobular, lactic dehydrogenase reaction in liver of progesterone treated rat. × 128

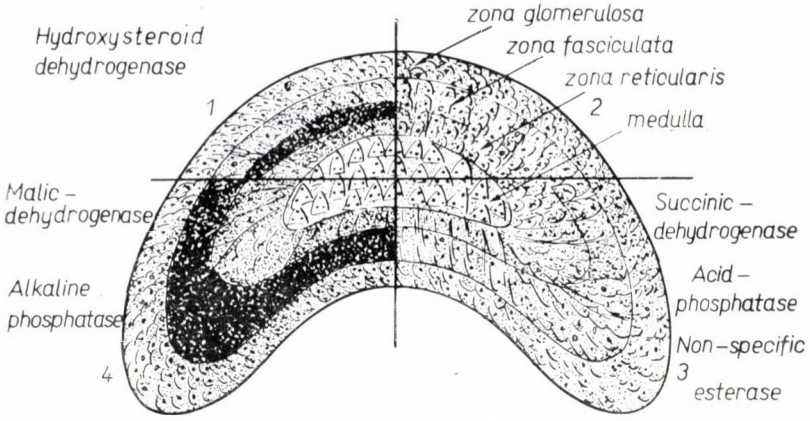

Fig. 17. Intensity of different enzymatic activities in the suprarenal gland of the rat

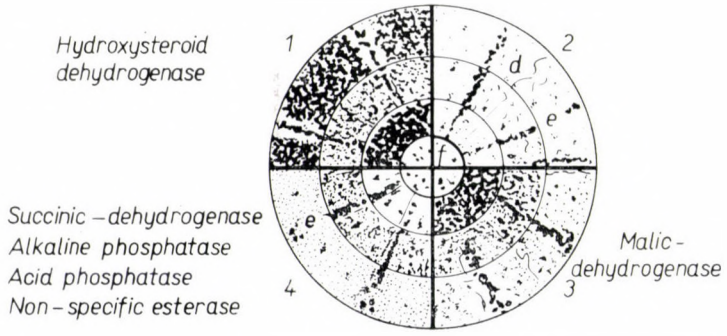

Fig. 18. Intensity of different enzymatic activities in a hepatic lobule of the rat

central vein. The lobule is divided into three zones; that surrounding the central vein is the centrolobular zone, the outermost is the peripheral zone, with the midzone between them. Lactic dehydrogenase activities was strongest in the centrolobular zone (Fig. 16); succinic dehydrogenase activity was negative here, strong in the midzone and moderate in the peripheral zone. The same distribution applies to alkaline and acid phosphatase and non-specific esterase as well. Hydroxy-steroid dehydrogenase activity was most intensive in the central and peripheral zones, less so in the midzone. The reactions were most marked in all groups when the incubation fluid contained DHA. Treatment

with lynoestrenol left this reaction unaffected, nor did it change under the effect of testosterone, but was considerably weakened when pregnenolone served as substrate. Lynoestrenol as substrate increased the activity in comparison to the controls. Alkaline and acid phosphatase and non-specific esterase activites diminished during treatment with lynoestrenol. All enzymatic activities were enhanced by the administration of progesterone.

Discussion

Our intention was to describe the observed changes and, in addition, to present a correlated evaluation of the histochemically accessible processes occurring in the ovary, adrenal and liver, i.e. the organs that play a decisive role in the synthesis and conversion of hormones. In order to make comparisons possible it was necessary to present physiological conditions also, since — for example — literature does not contain a comprehensive report of this kind on the intensity of enzymatic activity in the various parts of the adrenal gland or the liver. Deviations from the normal state, as observed under the microscope and revealed by photomicrographs, are presented in Tables II and III as also by way of illustrations. Changes observed in the kidney are not discussed in this paper; the hydroxysteroid dehydrogenase activity was invariably negative in this organ, while other enzymatic reactions have already been described [9].

In the present experiments progesterone increased the amount of lipoid and intensified the examined enzymatic reactions. Lynoestrenol, administered for 2 months to rats with active ovary, decreased the weight of the uterus, ovary and adrenal, and weakened all the examined enzymatic reactions.

Table 3

Localization of hydroxysteroid-, lactic and succinic dehydrogenase, alkaline and acid phosphatase, and non-specific esterase reactions in the ovary of rats

	Hydroxy-steroid dehydro-genase	Lactic dehydro-genase	Succinic dehydro-genase	Alkaline phospha-tase	Acid phospha-tase	Nonspeci-fic esterase
Primary follicle	0	++	++	+	+	0
Ripe follicle	++	++	++	++	+	+
Atretic follicle	+++	++	++	+	+	0
Interstitial luteal cells	++++	++++	++++	+++	++	0
Ripe corpus luteum	+++	+++	++	++++	++	+++
Involuting corpus luteum	++	+++	+++	+	+	++
Connective-tissue vessels	0	++	++	+++	+	+
Theca cells	++++	++++	++++	++++	+++	+++

However, these changes were but temporary. Organs of the animals in group 4 regenerated completely after 2 weeks, regained their original weight, and a large amount of Schultz-negative, carbonyl-positive birefringent lipoid appeared in the ovary and adrenal, the reactions were also enhanced. These phenomena point to a liberation of the hypothalamo-hypophyseal system from the inhibition caused by peripheral steroids, and to an increased production of gonadotrophic hormone (rebound effect). It is this effect of the norsteroids that is utilized in the treatment of sterility due to hypoplasia. It should be noted that norsteroids show this effect only in animals with intact ovary, whereas — according to experiments now in progress — the effect is reversed in ovariectomized animals. This phenomenon and the length of the oestrus point to a peripheral effect of norsteroids. These observations may be useful when prescribing norsteroid to patients in the menopause.

Prolonged oestrus and dioestrus following progesterone treatment point to a disturbance of the hormonal equilibrium, i.e. an upset of the harmony between the ovary, adrenal and the hypothalamo-hypophyseal apparatus a desynchronization of their rhythmicity.

Nothing is so far known about the conversion of norsteroids in the organism. Observations made in the present experiments in connection with hydroxysteroid dehydrogenase reactions may be a step towards the solution of this problem.

SAMUELS *et al.* [4] described a 3B-ol steroid dehydrogenase which was dependent on DPN and which oxidized hydroxysteroid to unsaturated α and β ketones. The reaction in vitro requires the presence of an OH group on C_{17} or C_{20}. WATTENBERG [10], PEARSON and GROSE [3] were the first to attempt the histochemical demonstration of dehydrogenases capable of oxidizing hormones. It was in 1959 that LEVY *et al.* [2] described the histochemical reaction used in this study.

DPN-diaphorase can be coupled with tetrazolium in the enzymatic reactions in which DPN serves as the coenzyme. Nicotinic acid and its amide, together with adenine and pentosephosphoric acid, form the enzymes which transfer H_2, i.e. co-zymaze I and co-dehydrogenase II. In order to demonstrate steroid dehydrogenases histochemically, we must employ some hormone substrate to be reduced, DPN, tetrazolium salt and nicotinamide in 0.1 Mol. phosphate buffer at a pH of 7.1 to 7.4. The enzymatically liberated H_2 is taken up by DPN which is thus converted into DPNH. This hydrogen is then transferred by the codehydrogenase to the colourless tetrazolium salt. Tetrazolium so reduced (formazan) appears in the tissues as a coloured insoluble dye-precipitate. In order to prevent aspecific reactions, the acetone in which the hormone substrate is dissolved has to be evaporated completely.

According to TALALAY and MARCUS [5], β-hydroxysteroid dehydrogenase catalyzes the reversible DPN-linked oxidation of 3 β-, 16 β and 17 β-hydro-

xysteroids. It catalyzes, according to FUHRMANN [1], steroids with a keto or OH group at C_{17} or C_{20} to the corresponding ketosteroids. Its catalytic action is most pronounced when DHA is converted into androstenedione, or pregnenolone into progesterone. Intensive precipitation of formazan has been observed in the ovary and adrenal of lynoestrenoltreated animals when this same hormone served as substrate, while the reaction was practically negative if the incubating fluid contained pregnenolone. We assume that the OH group on the C_{17} of lynoestrenol is oxidized and converted into ketosteroid by hydroxysteroid dehydrogenase. A competition seems to take place for the steroid dehydrogenase which changes lynoestrenol to 17-ketosteroid, or pregnenolone to progesterone. The latter cannot be formed if lynoestrenol is predominant. This theory seems to be supported by the clinical observation that patients treated with norsteroids excreted practically no pregnanediol. It is noteworthy that norsteroids do not interfere with the conversion of DHA into androstenedione. According to the foregoing, norsteroids — apart from inhibiting the luteinizing hormone — produce a direct effect on the enzymes of the ovary, adrenal glands and the liver. Hydroxysteroid-dehydrogenase activity in the Fallopian tube permits of the supposition that a local metabolism of steroid hormones is occurring there.

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HISTOCHEMISCHE UNTERSUCHUNGEN AN DEN ORGANEN VON MIT NORSTEROID UND PROGESTERON BEHANDELTEN RATTEN MIT BESONDERER RÜCKSICHT AUF DIE HYDROXYSTEROID-DEHYDROGENASEN

F. TÓTH und I. SZŐNYI

An 130 mit Progestin und Norsteroid behandelten weiblichen Albinoratten wurden die auf Wirkung der Behandlung in den Ovarien und Nebennieren zustandekommenden Lipoidveränderungen untersucht; in den Ovarien, Nebennieren und in der Leber wurden außerdem Hydroxysteroid-, Lakto-, Sukzinodehydrogenase, alkalische und sauere Phosphatase

und aspezifische Esterase-Reaktionen durchgeführt. Es wurde festgestellt, daß das Progestin sowohl die Menge der Lipoiden, als auch die Intensität der angeführten Enzymreaktionen steigert. Das Norsteroid setzt die dem Steroid entsprechende Lipoidmenge und die Enzymreaktionen herab. Es wird angenommen, daß um die Steroiddehydrogenase, die das Pregnenolon in Progesteron und das Lynöstrenol in 17-Ketosteroid umwandelt, zwischen dem Lynöstrenol und dem Pregnenolon eine Kompetition besteht, woraus folgt, daß im Falle von Lynöstrenol-Übergewicht kein Progesteron produziert wird.

ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ ОРГАНОВ КРЫС, ПОДВЕРЖЕННЫХ ВОЗДЕЙСТВИЮ НОРСТЕРОИДА И ПРОГЕСТЕРОНА С ОСОБЫМ УЧЕТОМ ДЕГИДРОГЕНАЗ ГИДРОКСИСТЕРОИДОВ

Ф. ТОТ и И. СЕНЕИ

Авторы проводили исследования на 130 белых крысах-самках, подверженных воздействию прогестина и норстероида. Они исследовали липоидные изменения, возникающие под влиянием воздействия в яичниках и в надпочечниках, далее реакции дегидрогеназы гидроксистероидов, лакто-, сукцинодегидрогеназ, щелочной и кислой фосфатаз и неспецифических эстераз в яичниках, надпочечниках и в печени. Согласно их установлениям прогестин повышает как количество липоидов, так и силу рассмотренных энзимных реакций. Норстероид уменьшает количество соответствующих стероиду липоидов и энзимные реакции. По предположению авторов между линэстренолом и прегненолоном существует соперничество за стероидную дегидрогеназу, превращающую прегненолон в прогестерон и линэстренол в 17-кетостероид. В случае преобладания линэстренола прогестерон и поэтому не может образовываться.

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NEUERE UNTERSUCHUNGEN AN DEN MESENTERIALEN LYMPHGEFÄSSEN

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(Eingegangen am 28. Mai, 1965)

Die Bedeutung der Lymphkapillaren neben dem im postmuralen Gekröseabschnitt befindlichen Lymphgefäß-Anastomosensystem und ihre Wichtigkeit bei der Lymphspeicherung wird betont. Neben den Einzel- und Doppelblattklappen wird die Rolle der Invaginationen sowohl in der Lenkung der Strömung, wie auch im Aufrechterhalten des Kapillarkreislaufs hervorgehoben. Die Morphologie der Doppelblattklappen der Lymphgefäße wird erörtet und festgestellt, daß diese, solange ihre Blätter unversehrt sind, nicht insuffizient werden können. Die Frage des mit der retrograden Pflanzung der Lymphstauung zustandekommenden Ödems wird besprochen.

In früheren Untersuchungen wurde festgestellt, daß sich am darmnahen Abschnitt des Gekröses ein mächtiges Anastomosensystem von Lymphgefäßen befindet. In den anastomisierenden Lymphgefäßen sind zahlreiche Doppelblattklappen, in den dünneren auch Eizelblattklappen, oder beide Typen abwechselnd in ein und demselben Gefäß zu beobachten (VAJDA u. TÖMBÖL, 1964).

Nach HORSTMANN (1951) fällt in der Speicherung der aus dem Darm in die Lymphgefäße geratenen Flüssigkeit dem ersten, postmuralen Abschnitt der mesenterialen Lymphgefäße eine bedeutende Rolle zu. Diese Flüssigkeit wird bei der Kontraktion des Darmes aus den Lymphgefäßen der Darmwand in jene des Gekröses sozusagen eingeschleudert. Die Transportkapazität der Letzteren reicht jedoch nicht aus, diese Saftmenge weiterbefördern zu können, und ein Teil der Lymphe wird in dem Anastomosensystem des darmnahen Abschnittes gespeichert. Diese Annahme wurde von ZHDANOW (1952) bestätigt. Die Speicherungsfähigkeit der Lymphknoten wurde von ROUVIÈRE u. VALETTE (1933) aufgeworfen und 1957 von RUSZNYÁK, FÖLDI u. SZABÓ bewiesen. WENT (1958) ist ebenfalls der Meinung, daß die gesamte Menge der in die Lymphgefäße geratenen Flüssigkeit nicht in den Ductus thoracicus gelangt, sondern zum Teil in der Peripherie bzw. den Lymphknoten gespeichert wird. An diesem speichernden Abschnitt konnte HORSTMANN 1951 Erweiterungen der Lymphgefäße mit elastischem Fasernetz in der Wandung nachweisen. In der uns zugänglichen Literatur über diesen Abschnitt fanden wir keinen Hinweis in bezug auf die Lymphkapillaren vor. Laut ZHDANOW (1952) und YAMAGISHI (1960, 1961) besitzen die Lymphkapillaren keine Basalmembran, und es fehlen auch die perivaskulären Zellen. Somit kommt die Zugwirkung der

auseinandergedrängten Bindegewebefasern auf die Lymphgefäße besser zur Geltung, wie z. B. bei der Ödembildung. Die Fasern hängen nämlich mit der Wand der Lymphgefäße eng zusammen. Die Lymphkapillaren können im Mangel einer Basalmembran, sogar auf das Dreifache ihres ursprünglichen Kalibers gedehnt werden. PULLINGER u. FLOREY (1935) kamen zu dem Schluß, daß Adrenalin bzw. Pituitrin keine Wirkung auf die Lymphkapillaren ausüben. Dehnung und Verengung der Lymphkapillaren stehen also nicht unter neuraler Steuerung. Nach KRAUS (1959) ist die Lumenänderung bei Lymphkapillaren mehr passiven, bei Blutkapillaren mehr aktiven Charakters, da letztere innerviert sind. Der Umstand, daß das Gesamtvolumen der Lymphkapillaren die Transportfähigkeit des zugeordneten ableitenden Lymphgefäßes wesentlich übertrifft, bedingt beim Lymphabfluß eine solche Einengung, die auf das Einkonzentrieren der Primärlymphe fördernd wirkt. Der Eindickung während der länger anhaltenden Speicherung im Kapillargebiet dürfte ein osmotischer Mechanismus oder der hydrodynamische Effekt der retrograden Stauung zugrunde liegen.

Material und Methode

Als Untersuchungsmaterial diente das Mesenterium von Katzen und in einigen Fällen von Hunden. Bei der Entnahme des Materials wurde darauf geachtet, daß der mesenteriale Rand unversehrt bleibe. Die histologischen Schnitten wurden mit Hämatoxylin-Eosin gefärbt oder mit Silber imprägniert.

Ergebnisse

An den darmnahen Partien befinden sich zahlreiche Lymphkapillaren. Die meisten dieser blind anfangenden Endothelröhrchen sind neben den Blutkapillaren, im Verzweigungswinkel derselben (Abb. 1), und auch im blutgefäßfreien Bereich des Gekröses aufzufinden. Diese blinden Säcke sind in sämtlichen Fällen erweitert (Abb. 2, 3, 4). Die Kapillaren zeigen alle Übergänge vom ganz weiten bis zum fadenförmig zusammengefallenen Lumen. Diese Anfangsabschnitte der Lymphkapillaren münden nach kurzem Verlauf in eine teichartige Erweiterung, zumal bildet sich sogar eine Insel aus (Abb. 5, 6). An den Stellen, wo die dünnwandigen Endothelkapillaren von Blutkapillaren überkreuzt werden, kommen mäßige Einengungen vor. Die Lymphkapillaren verfügen zunächst noch über gar kein Gebilde zur Lenkung oder Beeinflussung der Strömung; solche erscheinen erst im weiteren Abschnitt. Diese sind keine Klappen, sondern Invaginationen. Der periphere Lymphkapillarabschnitt ragt gleichsam in die Erweiterung des mehr zentral Gelegenen ein, und endet in diesem mit einer engeren, schlauchförmigen Mündung (Abb. 7). Manchmal sieht man mehrere Invaginationen an einer Erweiterung (Abb. 8). Die Erweiterungen weisen ebenfalls große Lumenverschiedenheiten auf. In der Nähe des Anastomosensystems von Lymphgefäßen sind die Lymphkapillaren bereits

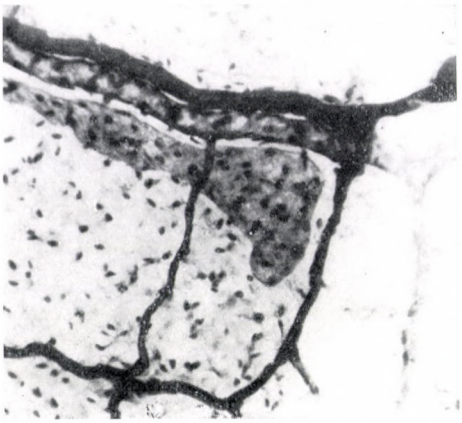

Abb. 1. Blind anfangendes Lymphkapillargefäß im Blutgefäßwinkel

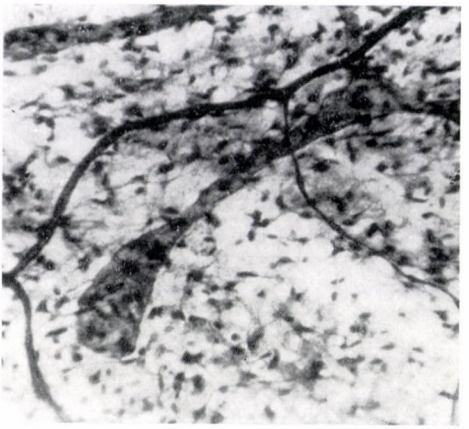

Abb. 2. Blind anfangendes Lymphkapillargefäß mit Einengung

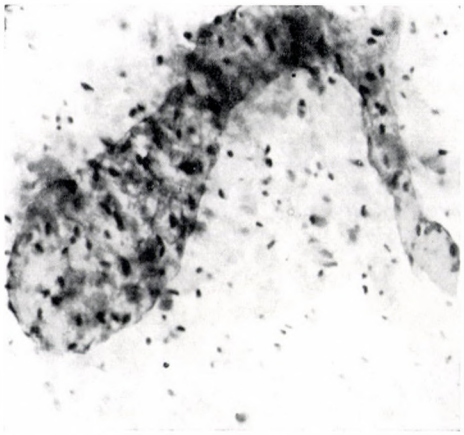

Abb. 3. Blinde Lymphsäcke verschiedenen Kalibers

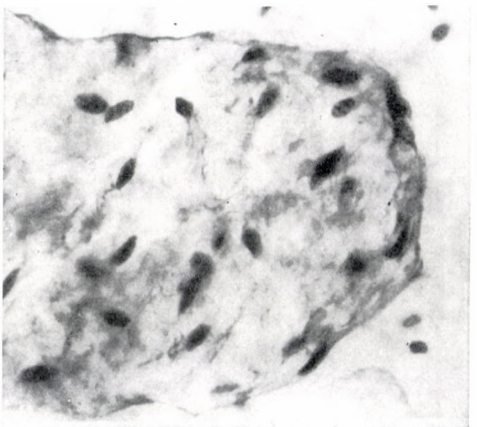

Abb. 4. Blinder Anfangsabschnitt unter starker Vergrößerung

mit Einzelblattklappen versehen (Abb. 9) und folgen nicht mehr den Verlauf der Blutgefäße, sondern ziehen selbständig dem Anastomosensystem zu (Abb. 10). Am Endabschnitt befinden sich auch Doppelblattklappen neben den Einblattklappen (Abb. 11, 12). Der morphologische Aufbau der einander nahe gelagerten Doppelblattklappen läßt gar keinen Rückfluß in die Kapillaren zu. Diese Klappen bestehen aus zwei halbkreisförmigen Blättern, welche sich im spitzen Winkel zur Längsachse des Gefäßes in der Stromrichtung in das Lumen hineinragen (Abb. 13, 14). Die beiden Blätter ergeben zusammen ein ovales Gebilde; die spaltförmige Öffnung befindet sich bei dem kurzen Durchmesser. Die gebogenen Insertionslinien der Klappenblätter treffen sich in je einem an

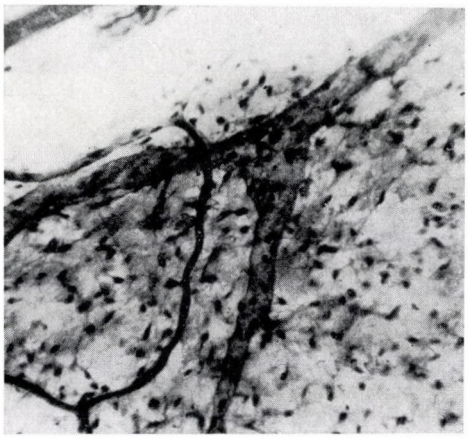

Abb. 5. Zwei zusammenfließende Lymphkapillaren mit Erweiterung

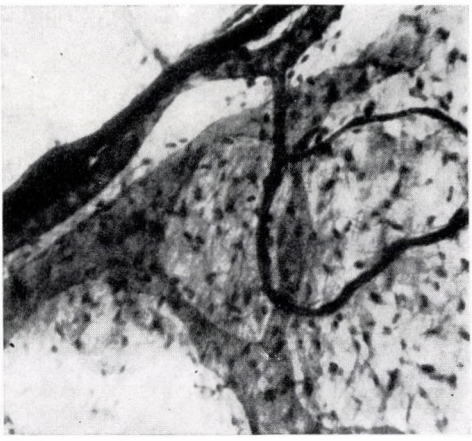

Abb. 6. Ausbildung einer Insel beim Zusammenfluß, Einengung bei der arteriellen Überkreuzung

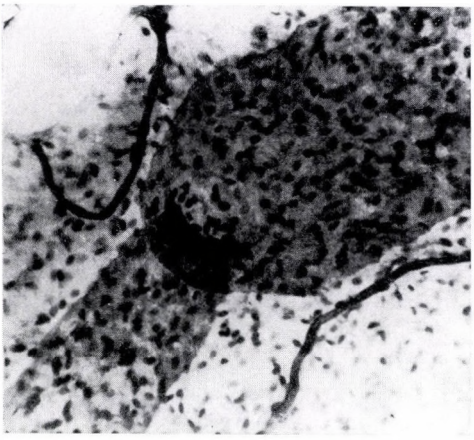

Abb. 7. Invagination

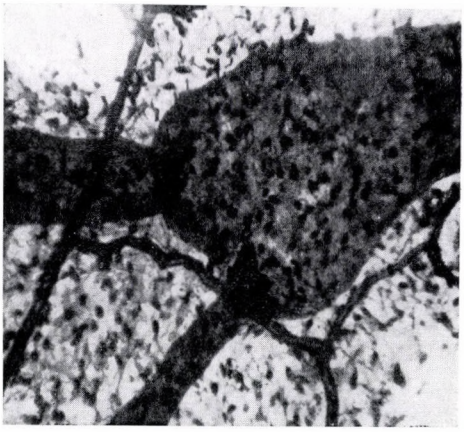

Abb. 8. Zwei Invaginationen in derselben Erweiterung

der Gefäßwand einander gegenüber liegenden Punkt (Abb. 15). Ihre freien Ränder überbrücken saitenartig das Lumen; die Flüssigkeit kann nur durch die zwischen ihnen gelegene Längsspalte strömen (Abb. 16.) Ohne Verletzung der Struktur ist hier kein Rückstrom möglich. Bei Druckzunahme im weiteren Abschnitt spannt die gedehnte Wand die gemeinsam inserierenden straffen Ränder der Blätter an, die dann, ähnlich der Stimmritze, die Stromspalte noch mehr verschließen. Wegen der gemeinsamen Insertionspunkte der Klappenränder vermag selbst eine hochgradige Dehnung keine Insuffizienz dieser Klappen hervorzurufen.

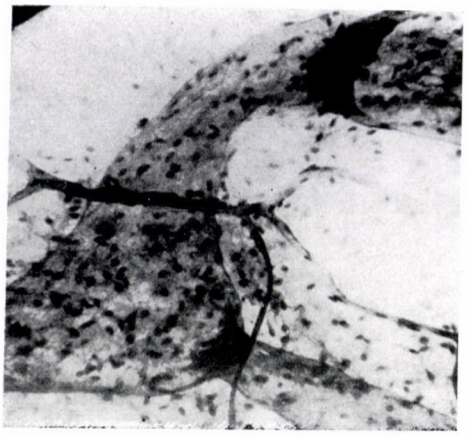

Abb. 9. Invagination und Einzelblattklappe beim Zusammenfluß

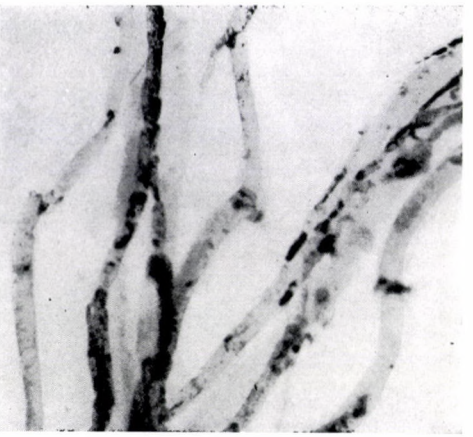

Abb. 10. Anastomosensystem im postmuralen Abschnitt

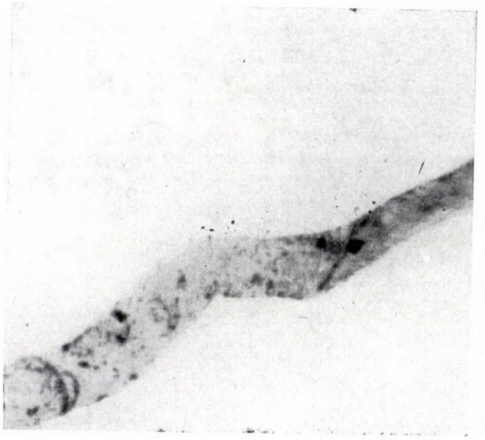

Abb. 11. Einzel- und Doppelblattklappe desselben Lymphgefäßes

Abb. 12. Doppelblattklappen

Die Einzelblattklappen sind ebenfalls oval, inserieren aber nur mit einem Rande an der Gefäßwand. Es ist offensichtlich, daß es bei Druckanstieg durch Dehnung des Gefäßes zum Rückbeugen des nicht fixierten freien Randes der bogenförmigen Platte kommen kann. Somit kann hier, in Abhängigkeit von den Druckverhältnissen im nach der Klappe folgenden Abschnitt ein Rückstrom zustande kommen.

Hinsichtlich der Lenkung der Lymphströmung sind auch die Invaginationen von Belang; hier kommt jedoch ein Rückfluß schon bei geringer Druckerhöhung zustande, was durch die zwar engere, aber offene schlauchartige Mündung auch ermöglicht wird. Die Erweiterung nach der Invagination übt wahr-

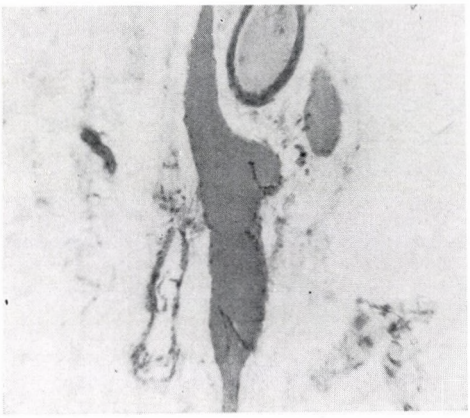

Abb. 13. Einzel- und Doppelblattklappen im Lymphgefäß

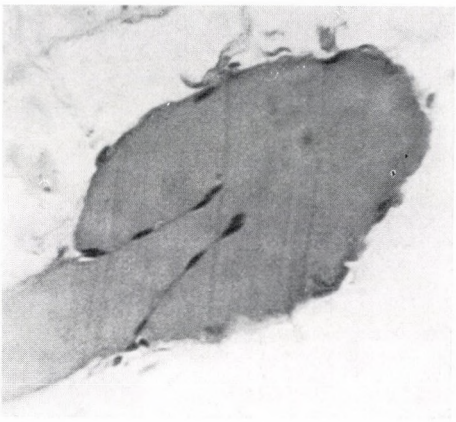

Abb. 14. Längsschnitt einer Doppelblattklappe

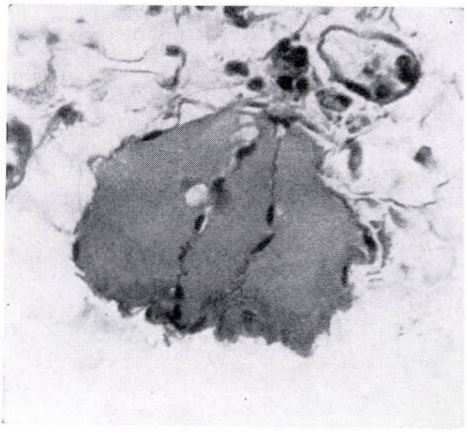

Abb. 15. Querschnitt einer Doppelblattklappe

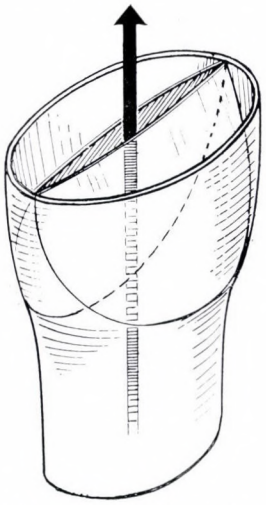

Abb. 16. Schematische Darstellung einer Doppelblattklappe

scheinlich eine Saugwirkung auf den vor ihr gelegenen Abschnitt aus und dürfte somit in der Aufrechterhaltung der Strömung eine Rolle spielen.

Im Hinblick auf die aus der Schilderung mehrerer Autoren bekannten Tatsache, daß die Lymphkapillaren selbst auf das Dreifache ihrer ursprünglichen Lumina gedehnt werden können, ist die Lymphmenge nicht zu vernachlässigen, die in diesem reichlichen Kapillarnetz bei Hemmung des Abflusses oder beim die Transportkapazität überschreitenden peripheren Angebot gespeichert werden kann.

Die aus dem morphologischen Aufbau gezogenen Schlüsse konnten wir auch experimentell bestätigen. Nach Tuschegaben in die Darmwand von

Katzen konnte eine Auffüllung des Anastomosensystem und der ableitenden Haupt-Lymphgefäße beobachtet werden. Der Abfluß wurde dann durch Unterbindung der ableitenden Stämme unterbrochen. Das periphere Angebot führte zur stetigen Zunahme des Lymphdruckes im Sammelgebiet. Nach kurzer Zeit begann die Tusche über die den arteriellen Bogen begleitende Anastomose zum tributären Gebiet des benachbarten Hauptstammes hinzufliessen, was zwar zu einer Verdünnung der Tusche am Rand des Netzes führte, den durch Verbleiben des peripheren Angebotes entstandenen Überschuß jedoch nicht abzuleiten vermochte. Nach vierstündiger Abklemmung kam bereits eine mäßige Schwellung des zwischen den benachbarten Sammelgebieten liegenden Gekröseabschnittes zustande und es erschienen Lymphstränge von verschiedenem Kaliber. Den Weg der Tuschekörnchen verfolgend, strömte die Lymphe neben dem Anastomosensystem zurück zum geschwollenen Gebiet hin. Der Bereich, wo die Tusche verdünnt wurde, endete mit einer fast scharfen Grenze.

Die obigen Befunde lassen die Feststellung zu, daß eine Behinderung des Abflusses durch Lymphknoten- oder Lymphgefäßentzündung, Abschnürung, Obstruktion, usw. oder die Steigerung des peripheren Angebotes zunächst eine Speicherung der Lymphe im Anastomosensystem bewirken. Aus diesem Bereich ist ein Weiterfließen über die den arteriellen Bogen begleitenden Verbindungsäste zu den benachbarten tributären Gebieten noch möglich. Bei weiterem Druckanstieg reicht diese Verbindung nicht mehr aus und es kommt zu einer Stauung in den Lymphgefäßen. Nach dem Insuffizientwerden der Einzelblattklappen beginnt die Strömung zu den Kapillaren hin; das Speicherungsvermögen derselben nimmt auf das Mehrfache des ursprünglichen zu. Da gibt es offensichtlich keine Resorption, sondern die Flüssigkeit diffundiert aus den mächtig geschwollenen Kapillaren und den blinden Anfangssäckchen, und es kommt zur Ödembildung in dem Sammelgebiet des unterbundenen Lymphgefäßes. Bei länger anhaltender Abflußhinderung kann die Stauung auf die Darmwand übergreifen und auch hier eine Schwellung verursachen.

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FURTHER INVESTIGATIONS CONCERNING MESENTERIC LYMPH VESSELS

J. VAJDA and M. TOMCSIK

Besides the system of lymphatic anastomoses in the postmural portion of the mesentery, the lymphatic capillaries, too, contain a significant volume of lymph. Not only the simple — pocket and double-pocket valves but also the invaginations play an important role in directing the flow of lymph and maintaining capillary circulation. The morphology of double-pocket valves is discussed in detail, and it is emphasized that these valves do not become insufficient provided their constituent membranes remain intact. The problem of oedemata developing in connexion with the retrograde spread of lymph stasis is also discussed.

НОВЫЕ ИССЛЕДОВАНИЯ НА ЛИМФАТИЧЕСКИХ СОСУДАХ БРЫЖЕЙКИ

Я. ВАЙДА и М. ТОМЧИК

Авторы подчеркивают наряду с системой анастомозов лимфатических сосудов на постмуральном этапе брыжейки значение лимфатических капилляров с точки зрения хранения лимфы. Наряду с одно- и двулистковыми клапанами они подчеркивают роль инвагинации как в отношении направления лимфотока, так и в деле поддерживания капиллярного лимфообращения. Они специально рассматривают морфологию двулистковых клапанов и устанавливают, что при интактности листков не может наступать их недостаточность. Авторы рассматривают далее вопрос отека, возникающего в связи с ретроградным распространением застоя лимфы.

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GROSSER INTRAMEDULLÄRER RÜCKENMARKSNERV BEIM MENSCHEN

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Das Rückenmark von 18 Verstorbenen wurde untersucht. Die Schnitte wurden laut Cajal imprägniert, bzw. mit Hämatoxylin-Eosin gefärbt. Im oberen Drittel des ersten Lendensegments, im zwölften und elften sowie im unteren Teil des zehnten Brustsegments wurde ein 3,5 cm langer und 0,25 bis 0,51 mm dicker Nerv nachgewiesen. Er nimmt seinen Ausgang vom oberen Teil des ersten Lumbalsegments, tritt in die vorderen Stränge der linken und rechten Seite des Rückenmarks ein, um von dort in kranialer Richtung medial von der Clarkeschen Säule unter die Basis des Hinterhorns zu ziehen. Im Nervenbestand finden sich dicke sensible und dünne vasomotorische Nerven. Es wird angenommen, daß die Fasern dieses Nerven die intramedullären Blutgefäße auf dem Gebiet des Lendenabschnittes und in einem großen Teil des Thorakalabschnittes innervieren. Es wird vorgeschlagen, diesen Nerv Nervus intramedullaris perpetuus major medullae spinalis (großer, konstanter intramedullärer Rückenmarksnerv) zu nennen, zwecks seiner Unterscheidung von den kleinen Gefäßnerven, die äußerst veränderlich und nicht immer nachweisbar sind.

Bei der Untersuchung der die Blutgefäße des Rückenmarks versorgenden Nerven konnte einer von nur [8, 9] in den unteren Segmenten des thorakalen Abschnittes einen großen intramedullären Nerv nachweisen. Das eingehende Studium der diesbezüglichen Literatur [10] ergab, daß den Neuromorphologen nichts über diesen Nerven bekannt ist. Deshalb beschreiben wir ihn in der gegenwärtigen Arbeit als ein neues Strukturelement des Rückenmarks, das mit vollem Recht als selbständige Einheit aufgefaßt werden darf.

Untersucht wurde das Rückenmark von 18 Personen beiderlei Geschlechts im Alter von 24 bis 60 Jahren, die verschiedenen zufälligen Todesursachen erlagen. Wir zergliederten das Rückenmark zweier Verstorbener segmentweise und untersuchten es an Schnitten von je 50 μ, die alle 0,5 mm entnommen wurden. Die mikroskopische Analyse dieses Materials ermöglichte die approximative Lokalisation des Nerven, den Ausschluß ähnlicher Gebilde, und beschränkte die weitere Arbeit auf einen bestimmten Abschnitt des Rückenmarks. Deshalb wurde aus dem Rückenmark der anderen 16 Verstorbenen der sich vom siebenten Thorakalsegment bis zum dritten Lumbalsegment erstreckende Teil herausgeschnitten und in Stücke zergliedert, die dem 8., 9., 10., 11. und 12. Brustsegment sowie dem ersten und zweiten Lendensegment entsprachen. Das Rückenmark von 15 Verstorbenen wurde in seiner gesamten Länge in einer wässerig-alkoholischen Chloralhydratlösung, in Ammonia-

Alkohol fixiert und laut Cajal imprägniert. Das sechzehnte Rückenmark wurde in Zelloidin eingebettet und die 15 bis 30 μ dicken Schnitte wurden mit Hämatoxylin-Eosin gefärbt, unverzüglich untersucht und nötigenfalls, etwa alle 0,3—0,3 mm, unter Glas eingeschlossen.

Auf Grund der folgerichtigen Untersuchung der Serienschnitte ließ sich feststellen, wo der Nerv seinen Ausgang nimmt und wo er endet. Seine Länge

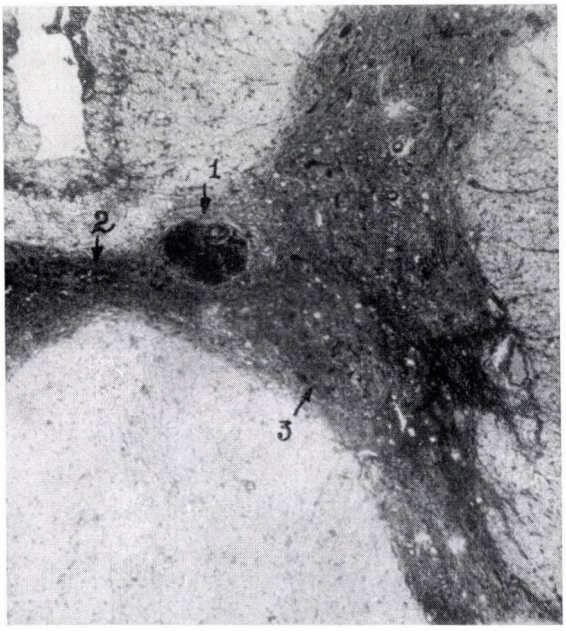

Abb. 1. Querschnitt des oberen Teils des 12. Thorakalsegments. 1 = Der Nerv mit einem Durchmesser von 0.38 mm. 2 = Der zentrale Kanal. 3 = Clarkesche Säule. Imprägnation. 45fache Vergrößerung

und Dicke, seine Beziehungen zu den anderen Gebilden des Rückenmarks, die Struktur des Nervenstammes, die Natur der zu seinem Bestand gehörenden Fasern konnten geklärt werden, soweit dies die Imprägnationsverfahren erlauben. Wir können auch auf die Frage eine vorläufige Antwort geben, ob dieser Nerv eine obligatorische und konstante morphologische Einheit des Rückenmarks bildet.

Der Nerv beginnt zumeist im oberen Drittel des ersten Lumbalsegments. Er tritt — zusammen mit der im Sulcus intermedius medullae spinalis verlaufenden Arterie in die linke oder in die rechte Seite des Rückenmarks ein (mit dieser Arterie zieht er der ganzen vorderen Furche entlang) und lagert in der weißen Substanz des dorso-medialen Teils des Vorderstranges, unter der Basis der vorderen Commissura alba. In den unteren zwei Dritteln des zwölften Thorakalseg-

ments findet er sich am medialen Rand der Intermediärzone und liegt ventromedial der Clarkeschen Säule bzw. ventrolateral des zentralen Kanals (Abb. 1). Im oberen Drittel des zwölften und im gesamten elften Thorakelsegment zieht der Nerv in dorsaler Richtung, umgeht die mediale Seite der Clarkeschen Säule in einem Bogen von etwa 180° und erscheint im Anfangsteil des zehnten Brustsegments unter der Basis des Hinterhorns. Ein geringer Teil des Nerven liegt im Bereich der weißen Substanz der hinteren Stränge. An dieser Stelle finden wir ihn im unteren Drittel des zehnten Brustsegments. In drei Fällen nahm der Nerv seinen Ursprung aus der Mitte des zwölften und endete im oberen Teil des elften Brustsegments. Die allmähliche Verlagerung des Nerven von vorne nach hinten weist darauf hin, daß seine Achse nicht streng vertikal, sondern etwas der Horizontalebene zugeneigt ist. Die Länge des Nerven wechselt zwischen 1,3 und 3,5 cm.

Bei vier Personen erschien der Nerv als paariges Organ, d. h. er ließ sich sowohl in der linken als auch in der rechten Seite des Rückenmarks gleichzeitig nachweisen. Sein Stamm war in diesen Fällen auf der einen Seite zwei — bis dreimal so dick und in der Regel auch länger als auf der gegenüberliegenden Seite.

Zusammen mit dem Nerven erscheint ein Blutgefäß, dessen Querschnitt etwa 55 bis 60 μ beträgt. Seine Wand besteht aus drei Schichten: einer äußeren, einer mittleren und einer inneren Schicht, woraus gefolgert werden darf, daß es sich um eine Arterie handelt. Im oberen Drittel des zwölften und im unteren Drittel des elften Thorakalsegments liegt der Nerv und die Arterie neben der längsverlaufenden Hauptvene. Sie bilden gemeinsam ein neurovaskuläres Bündel, das auch makroskopisch gut zu erkennen ist. Im Anfangsteil des zehnten Brustsegmentes und bisweilen auch niedriger trennen sich Nerv und Gefäße und ziehen in verschiedenen Richtungen.

Der Nerv verschwindet nicht spurlos. Schon an der Stelle wo er in die Marksubstanz eintritt, gibt er ein bis zwei Bündel von je 20 bis 25 μ ab, die zusammen mit den Abzweigungen der im Sulcus intermedius verlaufenden Arterie zum Lendenabschnitt ziehen. Dem gesamten Verlauf des Nerven entlang sieht man andererseits die Wand der Blutgefäße innervierende kleine Nervenfasernbündel. Besonders mächtige Stämme sendet der Nerv zu den längsverlaufenden Hauptvenen und -arterien, die über die intermediäre Zone zum Hinterhorn und zu der Clarkeschen Säule ziehen. Im unteren Drittel des zehnten Segments teilt sich der Nerv in kleine Stämme, die längs der Gefäße auseinanderlaufen, und in den höherliegenden Thorakelsegmenten erscheinen. Der Nerv versorgt nicht nur die gleichseitigen, sondern auch die gegenseitigen Gefäße mit Fasern. Er entsendet kleine Nervenstämme entlang der Gefäße der Commissura alba und grisea zur gegenüberliegenden Seite des Rückenmarks. Der Nerv gibt aber nicht nur Fasernbündel ab, er nimmt solche auch auf. Diese kommen mit der im Sulcus intermedius verlaufenden Arterie aus den

Segmenten, die höher liegen als das Segment, in dem der Nerv seinen Ausgang nimmt. Deshalb ist seine Dicke im Verlauf des zwölften und elften Thorakalsegments bei ein und derselben Person fast konstant. Bei verschiedenen Personen schwankt sein Querschnitt von 0,25 bis 0,51 mm.

Der Nerv selbst, aber auch das neurovaskuläre Bündel sind von einer Glia- und Bindegewebshülle umgeben und heben sich von der Marksubstanz scharf ab. (Abb. 2). Es läßt sich nicht bezweifeln, daß das Bindegewebsgerüst

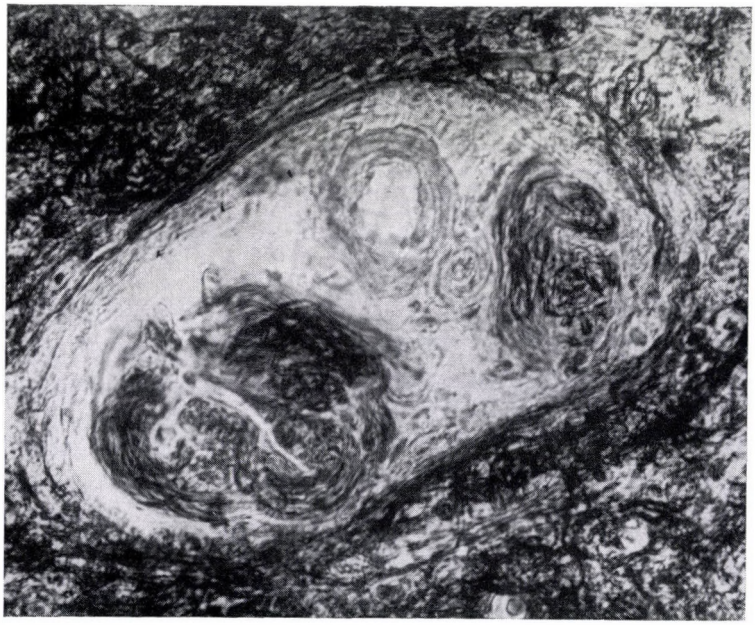

Abb. 2. Querschnitt des mittleren Teils des 11. Thorakalsegments. Arterie und der aus zwei Bündeln bestehende Nerv. Imprägnation. 400fache Vergrößerung

dieser Hülle, wie auch das gesamte Bindegewebe, von dem es im Nervenbestand sehr viel gibt, die Fortsetzung der weichen Hirnhaut im Inneren der Rückenmarksubstanz darstellt. Dank dieses Reichtums an Bindegewebe läßt sich der Nerv mit den gewöhnlichen histologischen Methoden leicht nachweisen, beispielsweise auch mit Hämatoxylin-Eosin-Färbung (Abb. 3). Im Nervenbestand oder außerhalb der Nervenhülle finden sich kleine Arteriolen von 12 bis 15 μ, die offenbar die Ernährung der Nervenfasern besorgen.

In seltenen Fällen bildet der Nerv einen einzigen Stamm mit undeutlicher Aufbündelung. Häufiger besteht er aus zwei bis sieben Bündeln, deren jedes von sichtbaren Bindegewebsschichten umgeben ist (Abb. 2, 4). Die Achsen dieser Nervenbündel nehmen einen stark gewundenen Verlauf, die zu ihrem Bestand gehörenden Fasern sind intensiv verflochten, was an den Längsschnitten des Nerven besonders anschaulich zutage tritt. Die Nervenbündel enthalten

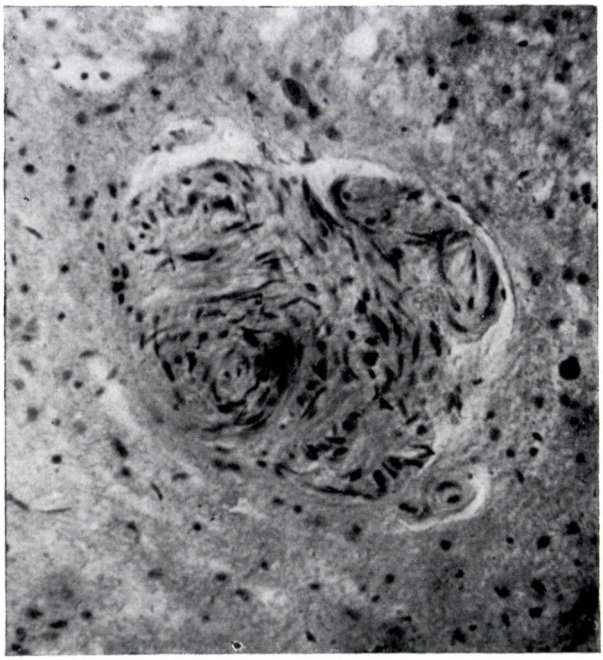

Abb. 3. Querschnitt des Anfangsteils des 10. Thorakalsegments. Der Nerv und zwei kleine Arteriolen. Die eine ist innerhalb der Nervenhülle, die andere außerhalb der Hülle zu sehen. Hämatoxylin-Eosin. 400fache Vergrößerung

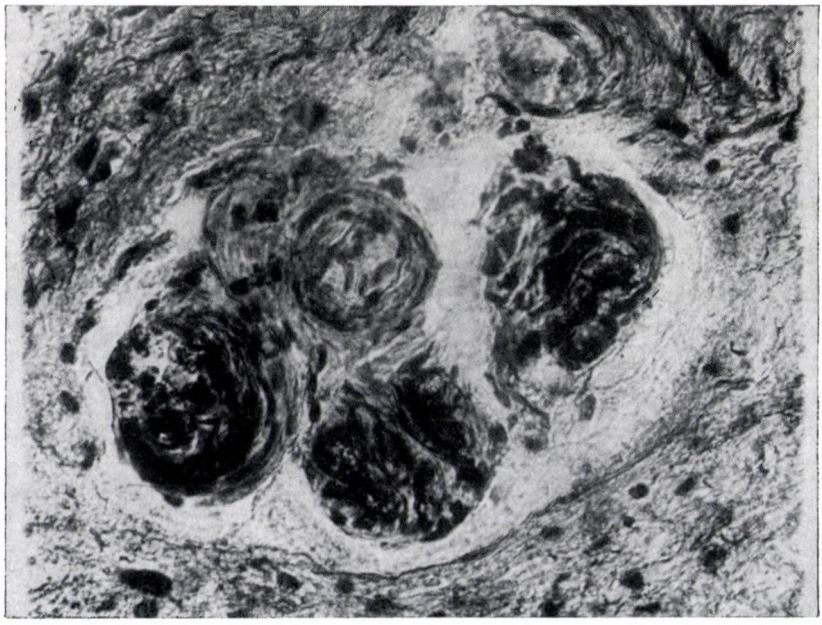

Abb. 4. Qerschnitt des oberen Teils des 1. Lumbalsegments. Arterie und der aus drei großen Bündeln bestehende Nerv. Imprägnation. 400fache Vergrößerung

Fasern vornehmlich mittleren Kalibers mit einem Querschnitt von 1,5 bis 2,5 μ. Vereinzelt finden sich auch dickere Nervenfasern (3 bis 3,5 μ). Äußerst gering ist auch die Zahl der Nervenfasern mit dünnen, deutlich sichtbaren Axonen. Die ersten zwei Faserntypen gelten als sensible Nerven [6, 3, 4], die dritte Art wird der Regel den vasomotorischen zugeordnet, was in bezug auf die Blutgefäße des Rückenmarks auch experimentelle Bestätigung fand [11, 12].

Zusammenfassend läßt sich sagen: Der im obigen beschriebene Nerv nimmt seinen Ausgang meistens vom oberen Drittel des ersten Lumbalsegmentes, tritt in die weiße Substanz der vorderen Stränge der linken und rechten Seite des Rückenmarks ein, zieht dann in kranialer Richtung und geht in die Zona intermedia. Von dort zieht der Nerv medial von der Clarkeschen Säule unter die Basis des Hinterhorns, wo er im Niveau des unteren Drittels des zehnten Thorakalsegments in kleine Bündel zerfällt und endigt. Die maximale Länge des Nerven beträgt 3,5 cm, sein Querschnitt schwankt zwischen 0,25 und 0,51 mm. Die Struktur des Nerven ist innerhalb des Stammes variabel. Er kann als kompakter, undeutlich aufgebündelter Stamm auftreten, oder er besteht aus zwei bis sieben einzelnen Bündeln, die von deutlich erkennbaren Bindegewebsschichten umgeben sind. Im Bestand des Nerven finden sich vornehmlich Fasern mittleren Kalibers und eine geringe Anzahl dicker und dünner Nervenfasern. Die Funktion des Nerven besteht vermutlich in der Innervation der intraorganischen Blutgefäße, zumindest im Abschnitt der Lumbalsegmente und in einem bedeutenden Teil des thorakalen Teil des Rückenmarks. Es läßt sich annehmen, daß die Fasern dieses Nerven in kaudaler Richtung bis zum Kreuzbein vordringen und in kranialer Richtung das fünfte bis sechste Thorakalsegment erreichen. Die genauere Feststellung des Ausbreitungsgebietes der Nervenfasern bedarf indessen weiterer Beweise, die weniger morphologisch als experimentell zu erbringen wären. Bedauerlicherweise fanden wir unter den zu neurohistologischen Experimenten herangezogenen Versuchstieren (Hund, Katze, Kaninchen) keines, bei dem ein dem beschriebenen Nerven entsprechendes Gebilde nachzuweisen wäre.

Die Tatsache, daß wir den charakteristisch lokalisierten Nerven bei jedem von den 18 untersuchten Individuen nachweisen konnten, dürfte als Beweis betrachtet werden, daß der Nerv eine ziemlich konstante Struktureinheit des Rückenmarks darstellt, und als erstmalig beschriebenes Gebilde einer Benennung bedarf. Zwecks seiner Unterscheidung von den kleinen Gefäßnerven, die äußerst veränderlich und nicht immer nachweisbar sind [10], schlagen wir vor, diesen Nerv Nervus intramedullaris perpetuus major medullae spinalis (großer, konstanter intramedullärer Rückenmarksnerv) zu nennen.

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A MAJOR INTRAMEDULLARY SPINAL NERVE IN HUMANS

P. A. MOTAWKIN, A. A. BELIKOV and L. N. SLOBODINA

The spinal cords of 18 cadavers have been examined. The sections were stained according to Cajal's silver method and/or with haematoxylineosin. The existence of a nerve, 3.5 cm long and 0.25 to 0.51 mm thick, was demonstrated in the upper third of the first lumbar segment, further in the 12th and 11th, as also in the lower part of the 10th thoracic segment. It originates in the upper part of the first lumbar segment, enters the anterior fascicles on both sides of the spinal cord, runs cranial medially to Clarke's column and terminates beneath the base of the posterior horn. The neural substance contains thick sensory and thin vasomotor nerves. The fibres of the nerve presumably innervate the intramedullary blood vessels in the lumbar and in the greater part of the thoracic segment. In order to distinguish the nerve in question from minor vascular nerves that are extremely variable and not always demonstrable, the name "Nervus intramedullaris perpetuus major medullae spinalis" is suggested.

БОЛЬШОЙ ИНТРАМЕДУЛЛЯРНЫЙ НЕРВ СПИННОГО МОЗГА ЧЕЛОВЕКА

П. А. МОТАВКИН, А. А. БЕЛИКОВ и Л. Н. СЛОБОДИНА

Авторы изучили 18 спинных мозгов человека, используя импрегнацию по Кахалю и окраску срезов гематоксилинэозином. На протяжении верхней трети первого поясничного, 12,11 и нижней части 10 грудных сегментов ими обнаружен внутримозговой нерв длиною до 3,5 см и толщиною от 0,25 мм до 0,51 мм. Он начинается на уровне верхней части первого поясничного сегмента, вступает в передние канатики левой или правой половины спинного мозга и, следуя в краниальном направлении, перемещается по медиальной стороне столба Кларка под основание заднего рога. В составе нерва обнаружены толстые — чувствительные волокна и тонкие проводники с вазомоторной функцией. Авторы считают, что волокна нерва иннервируют внутримозговые кровеносные сосуды на протяжении поясничного и большей части грудного отдела спинного мозга. Они предлагают этот нерв (в отличие от мелких сосудистых нервов крайне вариабельных и наблюдающихся не постоянно) назвать большим и постоянным внутримозговым нервом — Nervus intramedullaris perpetuus major medullae spinalis.

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DIE FEINSTRUKTUR DER GALLENKAPILLAREN NACH SUBTOTALER HEPATEKTOMIE

I. Bartók und Sz. Virágh

(Eingegangen am 2. August, 1965)

An Ratten wurde nach subtotaler Hepatektomie die Feinstruktur der Gallenkapillaren untersucht. Am 2—10. postoperativen Tage waren eine Vermehrung und Erweiterung, sowie intrazelluläre Einstülpungen der Gallenkapillaren und Veränderungen der Mikrovilli zu beobachten. Zu den gleichen Zeitpunkten war der Golgi-Apparat hypertrophisch und es konnte ein »heller« und ein »dunkler« Leberzellentyp unterschieden werden. Die submikroskopischen Veränderungen werden mit den Aktivitätsänderungen der alkalischen Phosphatase und der ATPase in der regenerierenden Leber in Parallele gestellt. Die im Frühstadium der Regeneration feststellbaren kanalikulären Veränderungen werden mit der gesteigerten Sekretionsaktivität der Restleber in Zusammenhang gebracht. In den späteren Phasen der Regeneration entwickeln sich die Veränderungen der Gallenkapillaren allmählich zurück.

Der histochemische Nachweis der alkalischen Phosphatase (Gömöri, 1941, Wachstein und Zak, 1949, 1964) und noch mehr der der Adenosintriphosphatase (ATPase) (Wachstein und Meisel, 1957, Wachstein, 1959, Novikoff und Mitarb., 1958, Wachstein, Meisel und Falcon, 1962) erwies sich als ein vorzüglich geeignetes Verfahren zur Darstellung der Gallenkapillaren. Bekanntlich zeigen die beiden kanalikulären Enzyme nach subtotaler Hepatektomie eine erheblich gesteigerte Aktivität (Wachstein und Meisel, 1958, Wachstein, 1959).

Nach früheren Untersuchungen ist nach subtotaler Leberresektion die alkalische Phosphataseaktivität der Gallenkapillaren in den ersten 48 Stunden und die der ATPase in den ersten 96 Stunden gesteigert: das Reaktionsprodukt zeigt zu den genannten Zeitpunkten eine Erweiterung und reiche Verzweigung der Gallenkapillaren an. 10 Tage nach der Operation wird die Aktivität der Enzyme jener der normalen Leber ähnlich (Bartók und Mitarb., 1962, 1963).

Auf Grund der histochemischen Befunde war zu erwarten, daß die Gallenkapillaren des regenerierenden Leberparenchyms elektronenmikroskopisch augenfällige Veränderungen aufweisen würden. Frühere elektronenmikroskopische Versuche bezüglich der Leberregeneration (Bernhard und Rouiller 1956, Gansler und Rouiller 1956) hatten bereits die Aufmerksamkeit auf einige Veränderungen der Gallenkapillaren gelenkt, doch hatten sich die Autoren mit der Frage der Entstehung und der genauen morphologischen Charakterisierung der Veränderungen nicht eingehender befaßt.

Material und Methoden

Es wurde bei männlichen Albinoratten von 150—200 g Gewicht der mittlere und der linke Leberlappen, d. h. 60—65% des Leberparenchyms, entfernt. Nach der Operation erhielten die Tiere 48 Stunden lang täglich 3 × 5 ml 20%-iger Dextroselösung und dann — bis zu ihrer Tötung — wieder die Standarddiät (s. BARTÓK und Mitarb., 1962). 24, 48, 72 und 96 Stunden, bzw. 10 und 20 Tage nach der Operation wurden stets zur gleichen Tageszeit je 2 Tiere durch Nackenschlag getötet. aus den Lebern sofort kleine Stückchen exzindiert, diese in 1%-iger — nach PALADE gepufferter — OsO_4-Lösung fixiert und nach Alkoholdehydrierung in Butyl-Methyl-metacrylat eingebettet. Zur phasenkontrastoptischen Untersuchung wurden aus den Blöcken halbdünne Schnitte hergestellt. Die dünnen Schnitte wurden nach MILLONIG (1961) mit Bleizitrat kontrastiert und ein Teil derselben vor der Untersuchung mit einer Kohlenmembran zugedeckt. Die elektronenmikroskopischen Untersuchungen erfolgten in dem Zeiss-Jena 180 1D und JEM 6C Apparat.

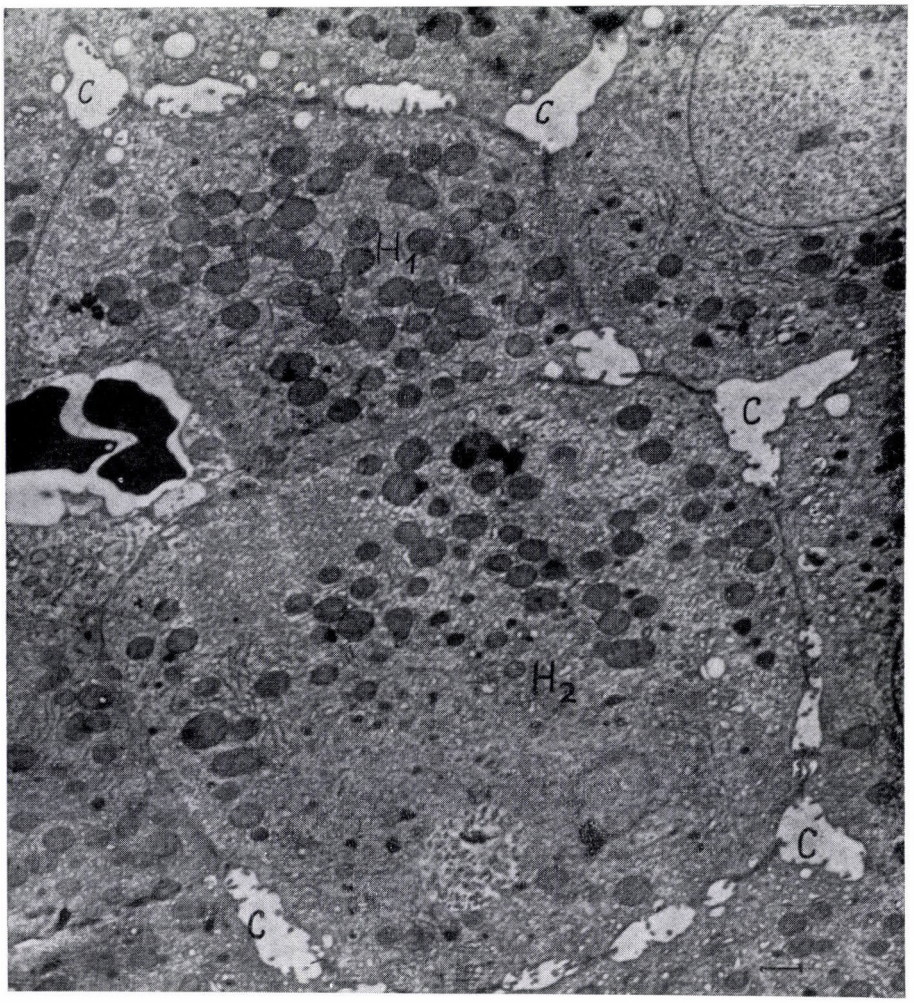

Abb. 1. 48 Stunden nach der Entfernung von $^2/_3$ der Leber sind mehr Gallenkapillaren anzutreffen, als in der normalen Leber. Um die mit H bezeichnete Leberzelle sind 5, und um die mit H_2 bezeichnete 10 Gallenkapillaren (c) sichtbar. 5.200 ×

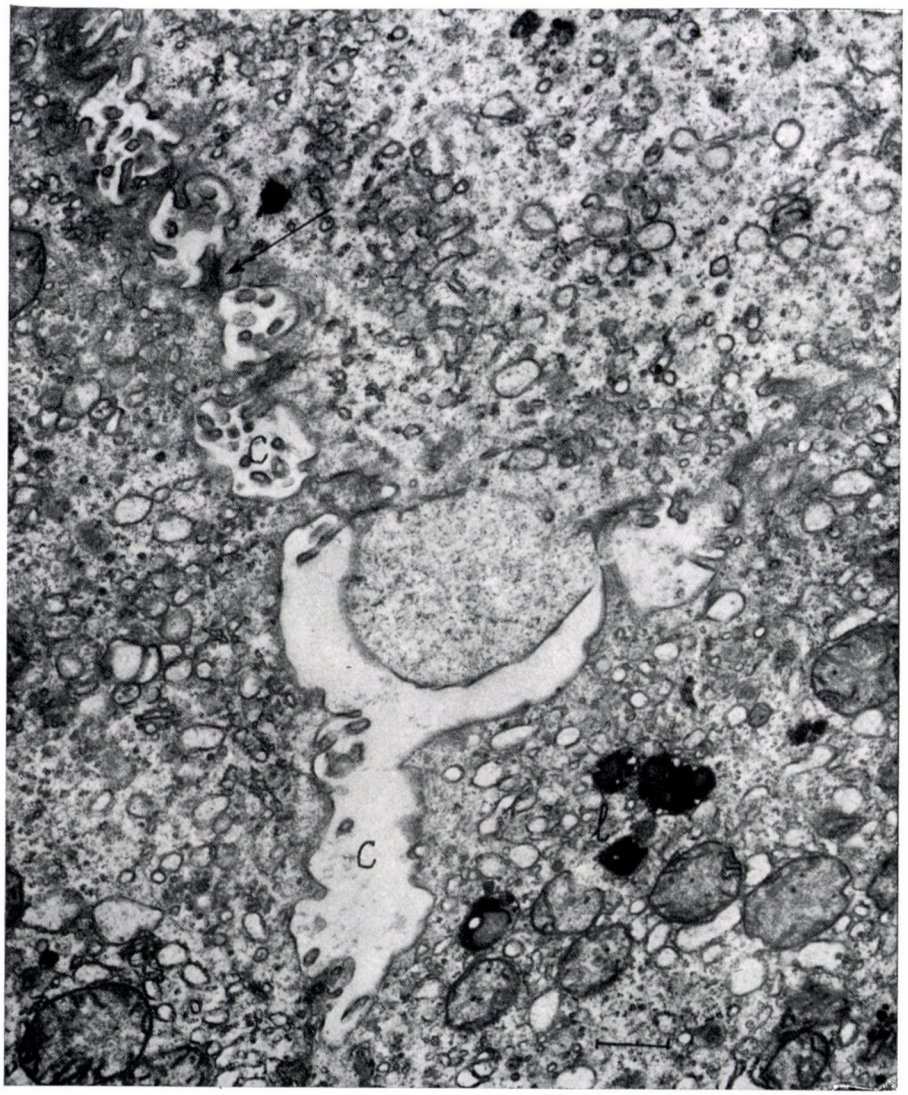

Abb. 2. Die obere Leberzelle ist von 7 Gallenkapillaren (c) umgeben. Die Lumina der linksseitigen sind nur durch die zellverbindenden Strukturen (Pfeil) voneinander getrennt. In die mittlere, erweiterte Gallenkapillare ist ein ödematöser, keine Organellen enthaltender Zellanteil eingestülpt. In der rechten unteren Zelle werden peribiliäre Lysosome (l) sichtbar. 9.300 ×

Ergebnisse

24 Stunden nach der subtotalen Leberresektion waren einzelne Gallenkapillaren mäßig dilatiert, die Mikrovilli etwas geschwollen und verkürzt. Nach diesen relativ geringfügigen Veränderungen konnten während des 2—10. Tages der Regeneration die folgenden wesentlicheren beobachtet werden:

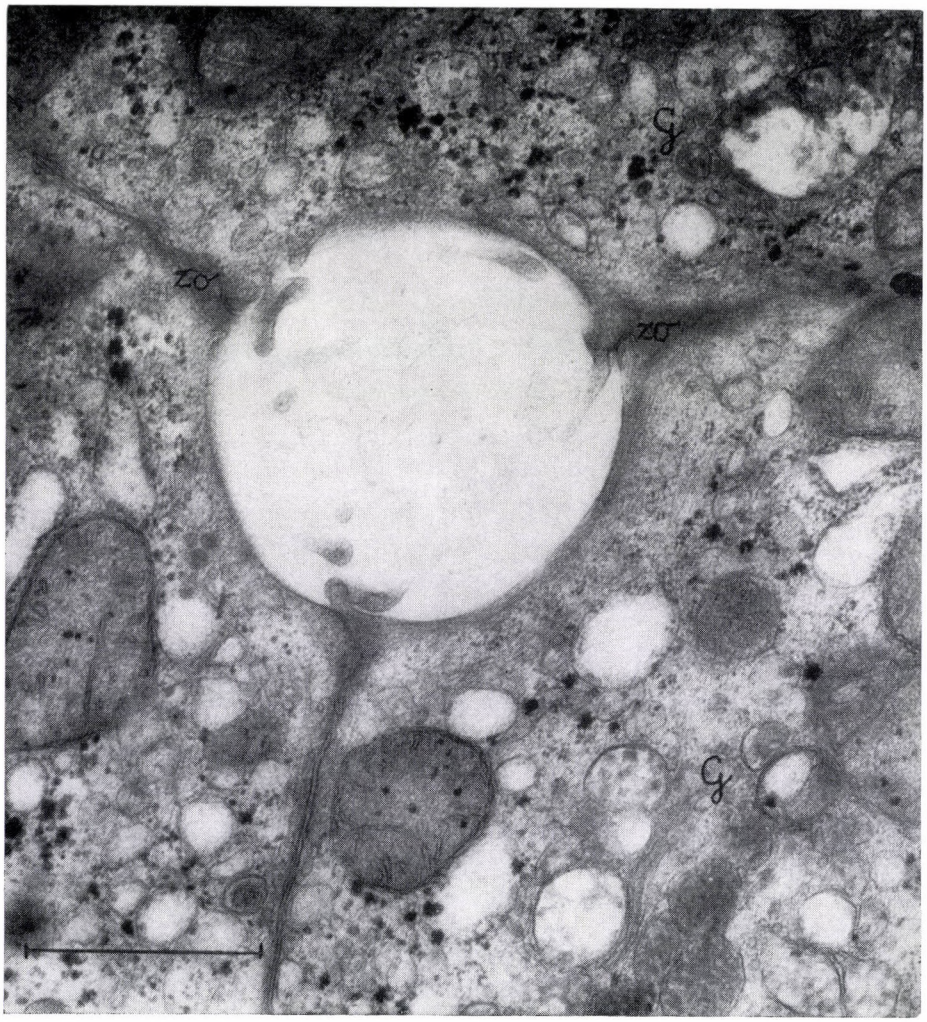

Abb. 3. Konzentrisch erweiterte Gallenkapillare. Die Mikrovilli sind nur in der Nachbarschaft der Zonula occludens (zo) erhalten geblieben. Der Golgi-Apparat ist hypertrophisch (G), in seinen Vesikeln befinden sich Granula. 30.500 ×

Vermehrung der Gallenkapillaren. Im ganzen Bereich der Leberläppchen waren — praktisch in jedem Gesichtsfeld — mehr Gallenkapillaren anzutreffen als im normalen Lebergewebe. Die Vermehrung der Gallenkapillaren ist im Frühstadium der Regeneration (2—4. Tag) hochgradiger als später. Um die Leberzellen liegen normalerweise 2—4 (SCHAFFNER und POPPER 1959, STEINER und Mitarbeiter, 1963), in der regenerierenden Leber aber wesentlich mehr, stellenweise auch 10 Kanälchenquerschnitte (Abb. 1 und 2). Die Entfernung zwischen den Kapillarquerschnitten ist verschieden: während sie stellenweise

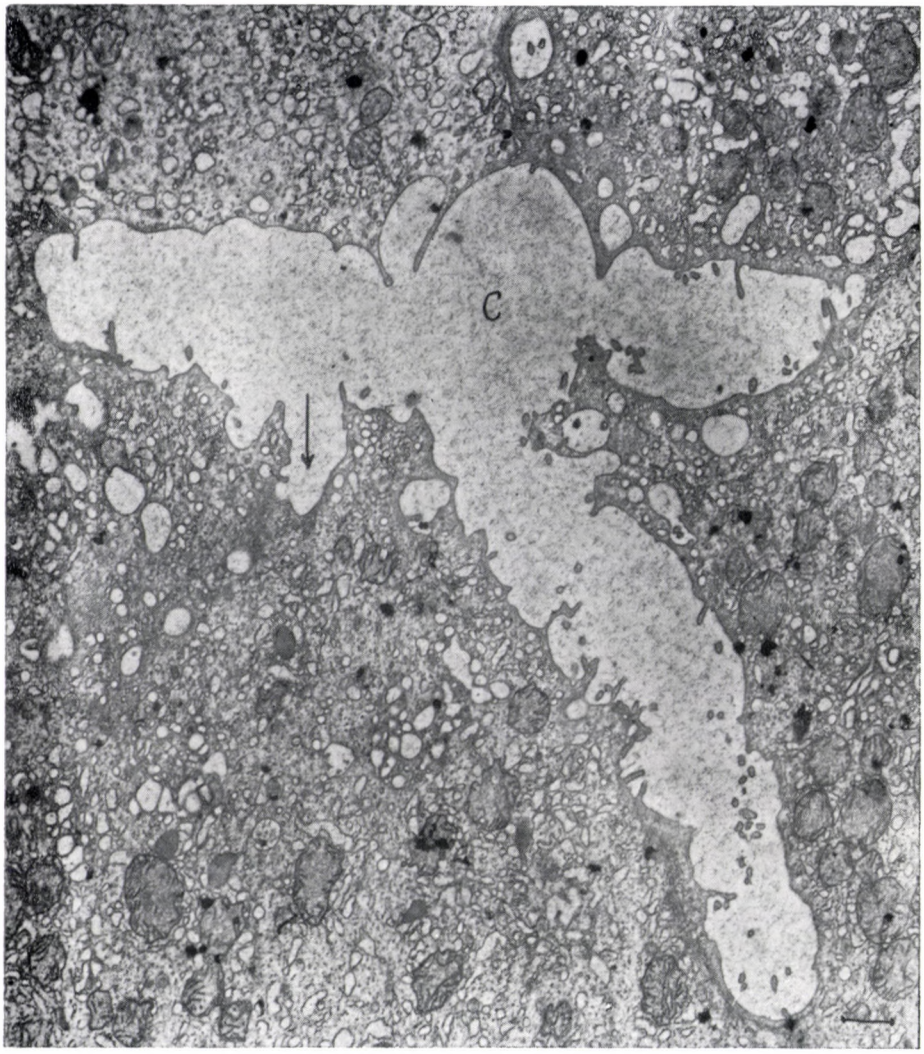

Abb. 4. Erweiterte Gallenkapillare (c), deren unterer Rand sich in die rechtsseitige Zelle einstülpt. Die Mikrovilli sind zahlenmäßig auffallend vermindert, besonders wenige finden sich in der linken oberen »hellen« Leberzelle. Bei dem Pfeil ist das Lumen der Gallenkapillare sackartig in das Zytoplasma gestülpt 5.700 ×

durch eine relativ lange Strecke der Zellmembran voneinander getrennt sind, bilden im anderen Bereichen nur die Schlußleisten eine Grenze. Die einander naheliegenden Gallenkapillaren sind oft perlkettenartig aneinandergereiht (Abb. 2). Das Lumen enthält wechselnde Mengen filamentär-granulärer Substanz (Abb. 3, 4).

Dilatation. Das Lumen der Gallenkapillaren ist am 2—4. postoperativen Tage an zahlreichen Stellen auf mehrere Mikron erweitert. Wie bei der extra-

hepatischen Cholostase (KALIFAT und Mitarb., 1962), läßt sich auch in der regenerierenden Leber eine konzentrische und eine exzentrische Dilatation erkennen. Bei der konzentrischen Dilatation bilden die symmetrisch erweiterten Lumina gleichmäßige Einstülpungen in den benachbarten Leberzellen (Abb. 3), während bei der exzentrischen Dilatation das Lumen assymmetrisch in die eine Zelle weiter vordringt als in die andere (Abb. 4). Man sieht in den

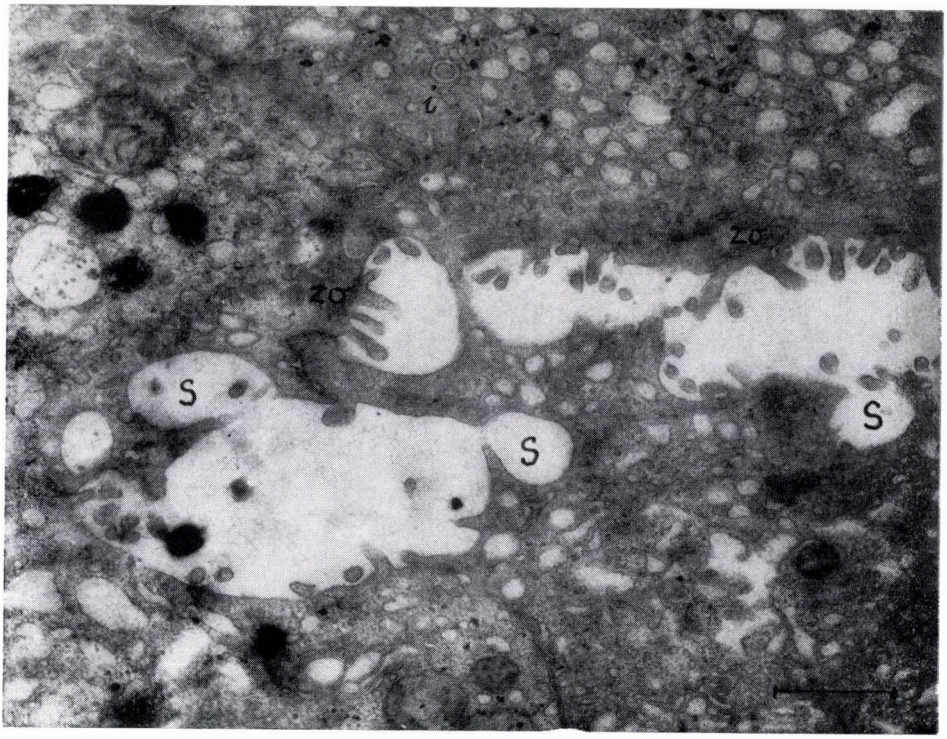

Abb. 5. An der Gallenkapillare sind zahlreiche Sakkulationen erkennbar (s). Die Mikrovilli sind der Zonula occludens (zo) entlang zahlreicher als anderwärts. Oben in der Mitte sind invaginierte Zellmembranen sichtbar (i). 14.800 ×

längsgetroffenen Kapillaren, daß die Lumenerweiterung nicht immer gleichförmig, sondern an der einen Stelle enger und an der anderen weiter ist.

Die ungleichmäßige Erweiterung der Gallenkapillaren kann zur Folge haben, daß sich das Lumen an einer Stelle sackförmig in das Zytoplasma der Leberzellen einstülpt (Abb. 5) (Sakkulation, s). Wenn der sackartige Teil nicht vollkommen in die Schnittebene fällt, kann das Bild eine, vom Lumen der Gallenkapillare isolierte, intrazytoplasmatische Vakuole zeigen (Abb. 6). Da letztere bis zum Golgi-Apparat reichen können, lassen sie sich an manchen Bildern schwer von den großen Golgi-Vakuolen unterscheiden (Abb. 6). Eine

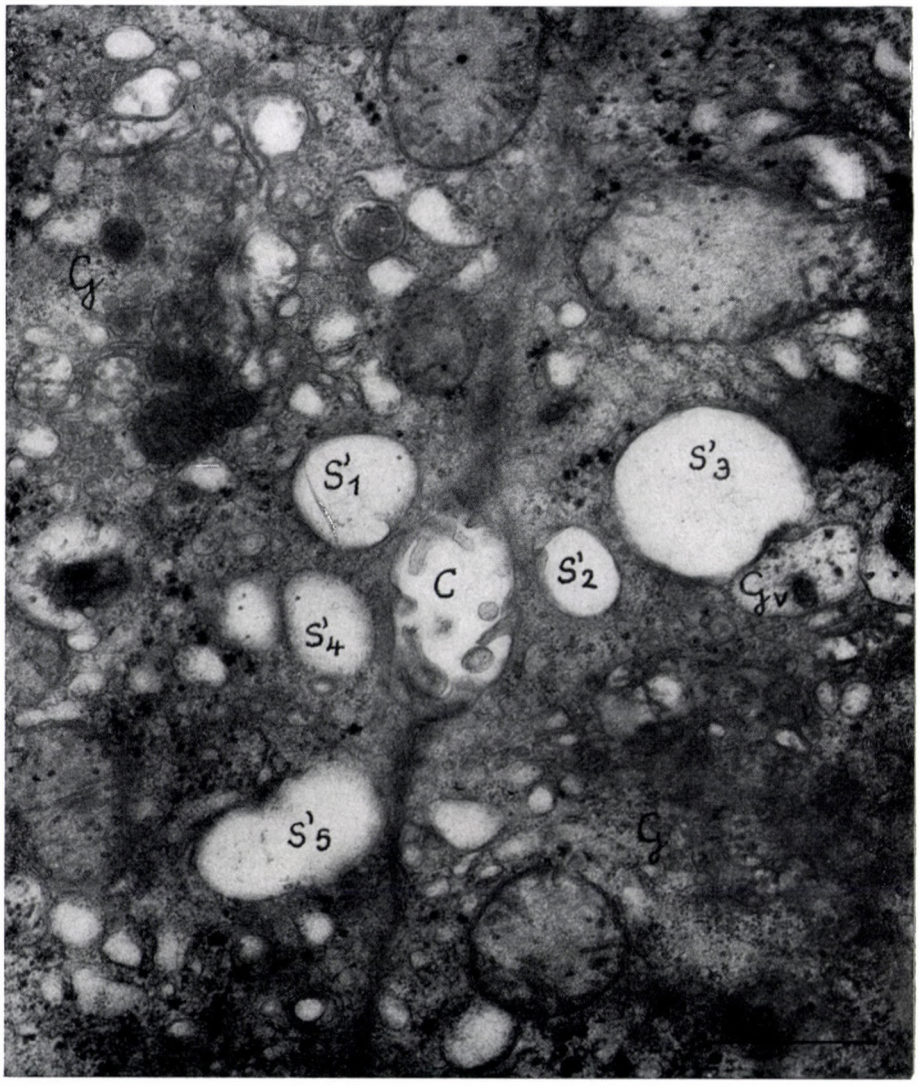

Abb. 6. Nicht erweiterte Gallenkapillare (c), von zahlreichen großen Hohlräumen umgeben die sakkulierten Gallenwegsquerschnitten entsprechen (s'1—s'5). Der Golgi-Apparat (G) ist in beiden Zellen hypertrophisch, seine Vakuolen (Gv) enthalten Granula. 22.000 ×

Hilfe bedeutet hier, daß die Sakkulationen die bereits erwähnte fein-filamentäre, granuläre Substanz enthalten, während die Golgi-Vakuolen entweder »leer« sind oder aber Granula von 20 mμ Durchmesser enthalten (Abb. 6). Um die Sakkulationen ist außerdem das Hyaloplasma randartig verdichtet (Abb. 6). Sakkulationen wurden gewöhnlich an beträchtlich dilatierten Gallenkapillaren gefunden, vereinzelt konnten sie aber auch in der Nähe von nicht erweiterten Gallenkapillaren nachgewiesen werden (Abb. 6).

Aus früheren Arbeiten ist bekannt, daß die Verbindung der benachbarten Leberzellen durch spezifische Strukturen — nach der Terminologie von FARQUHAR und PALADE (1963): Zonula occludens, Zonula adhaerens, Desmosomen und Membranverzahnungen (Abb. 5) — gewährleistet wird. In unserem

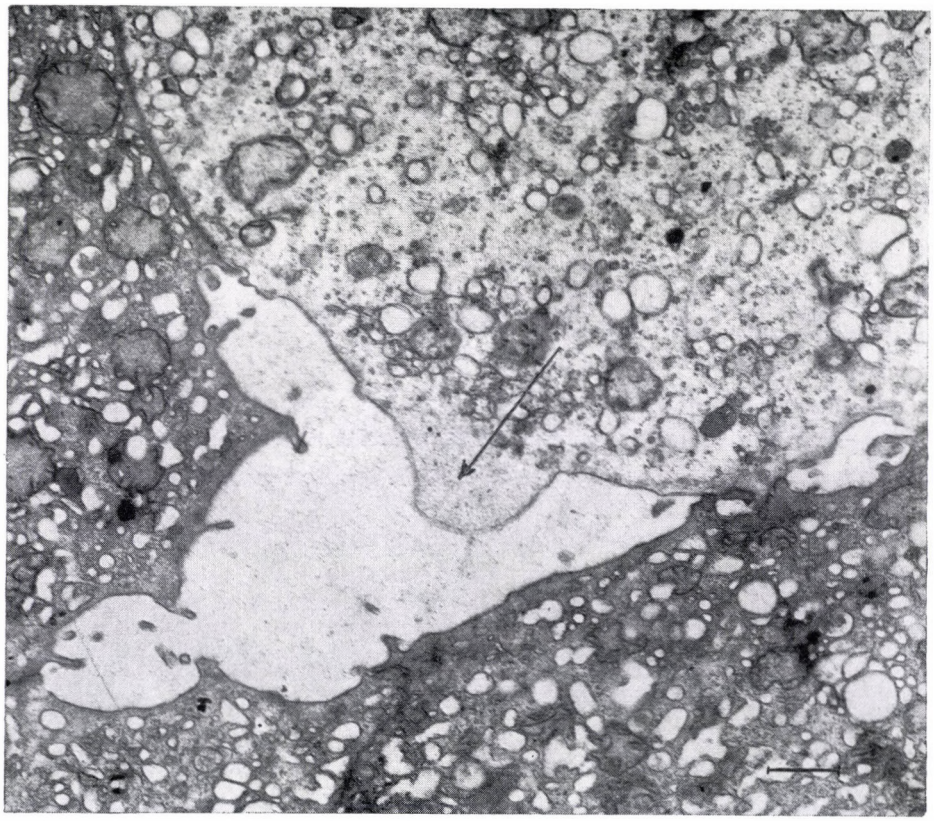

Abb. 7. Der ödematöse, keine Zellorganellen enthaltende Teil der »hellen« Leberzellen stülpt sich in die Gallenkapillare ein (Pfeil), in der sich auffallend wenige Mikrovilli befinden. 9.100 ×

Material hat die Erweiterung der Gallenkapillaren nie eine Abtrennung dieser zellverbindenden Strukturen bewirkt, obwohl sie um die erweiterten Gallenkapillaren oft in irgendeiner Richtung verschoben (Abb. 5) oder mitunter in das Lumen der Gallenkapillare eingestülpt waren.

Zytoplasmaveränderungen: An anderer Stelle (VIRÁGH und BARTÓK, im Druck) haben wir ausführlich darüber berichtet, daß in der frühen Phase der Regeneration ein »heller« und ein »dunkler« Typ von Leberzellen zu unterscheiden ist (Abb. 4 und 7). Die »hellen« Leberzellen enthalten weniger Zellorganellen als normalerweise, sie sind geschwollen, abgerundet und pressen die Sinusoide und benachbarten »dunklen« Leberzellen zusammen. Die »dunk-

len« Leberzellen stehen hinsichtlich des Gehaltes an Zellorganellen, Dichte, Gestalt und Größe den normalen Zellen näher, als die »hellen« Zellen.

Kurz nach Entfernung von $^2/_3$ der Leber hypertrophiert der Golgi-Apparat in den Leberparenchymzellen, und in seiner Umgebung erscheinen viele multivesikuläre Körper, winzige Vesikeln und zahlreiche Lysosome (Abb. 2—3 und 5—6).

Veränderungen der Mikrovilli. In den meisten erweiterten Gallenkapillaren sind die Mikrovilli vermindert und verkürzt. An der kanalikulären Oberfläche der »hellen« Zellen sind in der Regel weniger Mikrovilli zu beobachten als an der der »dunklen« Zellen (Abb. 4 und 7). Die Abnahme der Zahl der Mikrovilli bezieht sich nur ausnahmweise auf den ganzen Umkreis der Gallenkapillaren, denn in der Nachbarschaft der zellverbindenden Strukturen finden sich auch dann noch mehr oder weniger Mikrovilli, wenn sonst die Zellmembran vollkommen glatt ist (Abb. 3, 5).

In zahlreichen Gallenkapillaren sind die Mikrovilli geschwollen, ödematös. Das Ödem kann das distale Ende der Zotten oder aber ihr Ganzes betreffen. Im letzteren Falle bewirken sie eine mehr oder minder starke Einengung des Lumens der Kapillaren (Abb. 2 und 7). Erhebliche Ödeme der Mikrovilli fanden sich vorwiegend an den »hellen« Zellen. Die ödematösen Mikrovilli sind mit hellem feingranulärem Hyaloplasma ausgefüllt, welches keine Zellorganellen aufweist. An der Basis der verbreiterten, ödematösen Mikrovilli sind stellenweise reihenförmig geordnete Bläschen wahrnehmbar, welche das die Zellorganellen enthaltende Zytoplasma von dem in den Zotten befindlichen Hyaloplasma trennen.

Die beschriebenen Veränderungen sind 48—72 Stunden nach der partiellen Leberresektion am ausgeprägtesten, aber auch am 10. Tage noch beträchtlich. Am 20. Tage der Regeneration weisen die Gallenkapillaren eine vorwiegend normale Struktur auf, doch sind vereinzelt auch dann noch mäßigere Erweiterungen und intrazelluläre Einstülpungen feststellbar. Die Mikrovilli sind am 20. Tage wieder intakt.

Besprechung

Auf Grund der vorliegenden Untersuchungen sind in der frühen Phase der Leberregeneration die Aktivitätsänderungen der gallenkanalikulären Enzyme (alkalische Phosphatase und ATPase) mit den submikroskopischen Veränderungen der Gallenkapillaren in Beziehung zu bringen. Mit den erwähnten Enzymreaktionen ist eine Intensivierung der kanalikulären Zeichnung sowie eine reiche Verzweigung der Gallenkapillaren feststellbar. Das submikroskopische Äquivalent dieser Veränderungen ist die Vermehrung der Gallenkapillaren, ihre Dilatation und häufig ihre tiefe Einstülpung in die Zelle.

Kalifat und Mitarb. (1962) haben bei der extrahepatischen Cholostase im wesentlichen gleiche Gallenkapillarenveränderungen gefunden wie wir im

regenerierenden Lebergewebe. Während aber bei der Cholostase die Veränderungen eine Folge der Anreicherung der abflußbehinderten Galle sind, dürften sie nach der subtotalen Hepatektomie eher mit der gesteigerten sekretorischen Aktivität im Zusammenhang stehen. Die Untersuchungen von SCHALM und Mitarb. (1952) beweisen nämlich, daß nach Blockierung des Gallenabflusses des übrigen Parenchyms selbst 1/4 des Lebergewebes imstande ist, die Gesamtgallenmenge ohne Funktionsstörungen auszuscheiden. Dabei ist in der funktionierenden Restleber eine Hypertrophie nachzuweisen. Es muß also angenommen werden, daß nach der subtotalen Hepatektomie das beträchtlich verringerte Lebergewebe relativ bedeutend größere Mengen Galle ausscheidet. Dies erklärt sowohl die Vergrößerung des Gesamtquerschnittes der Gallenableitung, welche den Abfluß der gesteigert produzierten Galle ermöglicht, als auch die Steigerung der Enzymaktivität (ATPase, alkalische Phosphatase) der Zellmembran. Es ist nämlich bekannt, daß die ATPase eine wesentliche Rolle im transzellulären Transport spielt (ESSNER und Mitarb., 1958, ERNSTER und Mitarb., 1962, RONDEZ und RÜTTNER, 1963). Möglicherweise steht auch die Steigerung der alkalischen Phosphatase-Aktivität mit einer gesteigerten Funktion der Zellmembran im Zusammenhang. Eine Stütze für diese Annahme stellen jene Befunde dar, wonach die in dem transzellulären Transport eine wichtige Rolle spielende Zellmembran der Nierenepithelzellen (SPATER und Mitarb., 1958, MÖLBERT und Mitarb., 1960) und Dünndarmepithelzellen (CLARK, 1961) über eine hohe alkalische Phosphatase-Aktivität verfügt.

Die Vermehrung der Gallenkapillaren kann auf verschiedene Weise zustandekommen. Nach unseren Befunden kann die starke Erweiterung der präexistierenden Lumina und ihre Schlängelung den Anschein einer Vermehrung erwecken. Es ist aber auch vorstellbar, daß die unter normalen Umständen teilweise oder vollkommen geschlossenen Kanälchen sich öffnen (ROUILLER, 1956) oder auf die zunehmende Belastung zwischen den einander anliegenden Zellmembranen auch neue Lumina entstehen. Nach Unterbindung des Ductus choledochus, wenn der Abfluß der Galle gehemmt und auch seine Richtung geändert ist, können Gallenkapillaren auch an abnormalen Zelloberflächen entstehen (STEINER und CARRUTHERS, 1961).

Die exzentrische Dilatation der Gallenkapillaren führt häufig zur Sakkulation des kanalikulären Lumens. Diese sackartigen Einstülpungen sind auf Grund von lichtmikroskopischen Untersuchungen als intrazelluläre Gallengänge beschrieben worden (WACHSTEIN und ZAK, 1949, NOVIKOFF und NOE, 1955, HAMMERBECK, 1958). Die Sakkulationen können tief in das Zytoplasma vordringen und in der frühen Phase der Regeneration in die Nähe des stark hypertrophischen Golgi-Apparates gelangen. Dem Golgi-Apparat wird eine Rolle in der Sekretionstätigkeit der Leberzellen zugesprochen (STEINER, PHILLIPS und BAGLIO, 1963). Auf Grund der obigen Angaben erweitern die Sakkulationen nicht nur die Lumina der Gallenkapillaren, sondern verkürzen auch

den Weg für die Zellprodukte, die von den Leberzellen in die Gallenkapillaren sezerniert werden.

Von HIGGINS und ANDERSON (1931) wurde beschrieben — und später auch von anderen bestätigt —, daß während der Regeneration die Hydratation der Leberzellen gesteigert ist. Hiermit dürfte das Ödem der Mikrovilli in Beziehung stehen. Auf Grund der elektronenmikroskopischen Bilder ist besonders im Falle der »hellen« Zellen an eine gesteigerte Hydratation zu denken. Diese Zellart is nämlich nicht nur wegen der Schwellung der Mikrovilli, sondern auch in anderer Hinsicht als ödematös zu betrachten (VIRÁGH und BARTÓK, im Druck). Diese Veränderungen deuten darauf hin, daß die Mikromorphologie der Gallenkapillaren nicht nur durch die Gallenausscheidung, sondern auch durch andere Faktoren beeinflußt wird.

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THE MICROSCOPIC STRUCTURE OF BILE CANALICULI AFTER SUBTOTAL HEPATECTOMY

I. BARTÓK and S. VIRÁGH

The microscopic structure of bile canaliculi has been studied in subtotally hepatectomized rats. Between the 2nd and 10th postoperative day, increase in the number and the diameter of bile canaliculi, intracellular invagination of the bile paths and various changes in the microvilli were observed. At the same time, the Golgi apparatus became hypertrophic, and the liver cells differentiated into a "light" and a "dark" variety. The submicroscopic changes are compared to changes in the activity of alkaline phosphatase and ATPase seen in regenerating livers. It is suggested that the observed changes in the structure of bile canaliculi during the early phase of regeneration are due to the increased task to be performed by the surviving one third of the liver parenchyma. The structural changes of the bile canaliculi gradually disappear with advancing regeneration.

ТОНКАЯ СТРУКТУРА ЖЕЛЧНЫХ КАНАЛЬЦЕВ ПОСЛЕ СУБТОТАЛЬНОЙ ГЕПАТЕКТОМИИ

И. БАРТОК и С. ВИРАГ

Авторы изучали тонкую структуру желчных канальцев после субтотальной гепатектомии у крыс. На 2—10 день после операции они наблюдали увеличение числа желчных канальцев, их расширение, внутриклеточное впячивание желчных путей и различные изменения микроворсинок. В это же время аппарат Гольджи гипертрофированный и отличаются «светлый» и «темный» варианты печеночных клеток. Авторы устанавливают параллель между субмикроскопическими изменениями и изменениями щелочной фосфатазы и АТФ-азы в регенерирующейся печени. Возникающие в ранней стадии регенерации изменения желчных канальцев авторы приводят в связь с усиленной нагрузкой, которую представляет выделение желчи и других веществ на печеночную паренхиму, уменьшенной до одной трети. В более поздних стадиях регенерации изменения желчных канальцев постепенно прекращаются.

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EFFECT OF PAINTING WITH ACID ON MUSCULAR TYPE SMALL ARTERIES

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(Received 9 September, 1965)

Elastic and muscular type blood vessels have been found to react in a different way to painting with acid. Fibrinoid necrosis develops only in the small arteries of the muscular type. Muscle necrosis, one of the components of the vascular wall fibrinoid can readily be differentiated from the other components by Mallory's phosphotungstic acid haematoxylin, since the necrosed muscle cells bind more dye.

In previous experiments [1, 2] the regeneration of the aortic wall damaged by painting with acid has been studied. The method employed was as follows: — Albino rats were laparatomised and the exposed infrarenal part of the aorta was painted for a few seconds with concentrated hydrochloric acid. It was found that the cellular elements disappeared and the framework of regeneration was constituted by the remaining fibre network.

In most cases the small blood vessels of the muscular type running near the aorta were also damaged. The characteristic histological changes displayed by them are illustrated by presenting typical case.

The vascular wall is homogeneous, eosinophilic, stains a homogeneous yellow with Van Gieson's dye and flame-red with Azan (Fig. 1), and contains only at sites a few muscle cells with elongated nuclei. Foot's silver impregnation shows an intact fibre network. On treatment with pH 4.5 toluidine blue the vascular wall shows no metachromasia (Fig. 2), the Ritter-Oleson method reveals a homogeneous PAS-positivity. In the vascular wall staining red with Azan, Mallory's phosphotungstic acid haematoxylin reveals strongly staining bluish-black islets corresponding to necrosing muscle cells in the reticular network (Fig. 3).

When survival is longer, in the necrosed vessels intimal proliferation a strong metachromasia begins (Fig 2), and later on the necrosis undergoes hyaline transformation.

From the findings two conclusions have been drawn.

(i) Blood vessels of the elastic and of the muscular type react in a different way to the same injury. Whereas fibrinoid necrosis failed to develop in the aortic wall, in the small arteries of the muscular type a change showing all the characteristic features of fibrinoid necrosis was observed.

(ii) By Mallory's phosphotungstic acid haematoxylin, the homogeneous fibrinoid was broken up into its components. Whereas the plasma components and necrosed muscle cells stained in the same way with Azan by the PAS reac-

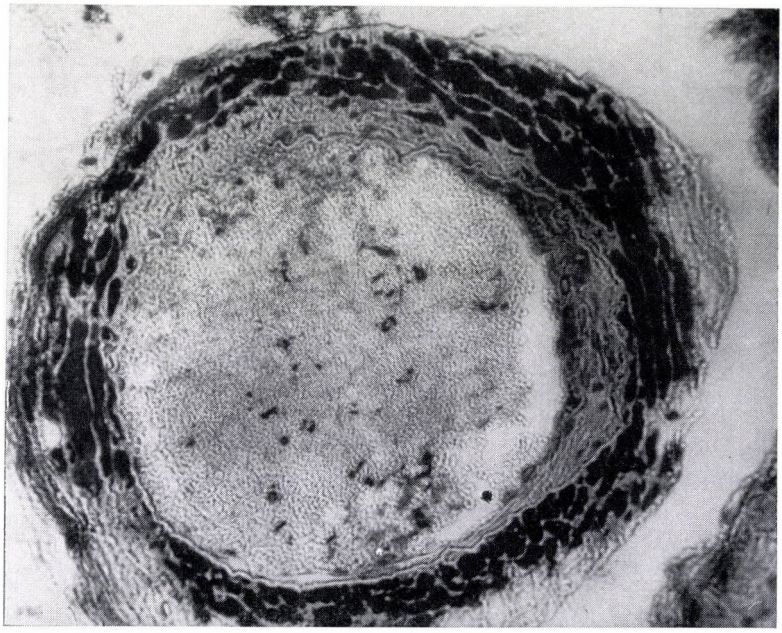

Fig. 3. Necrosed muscle cells staining well with Mallory's phosphotungstic acid haematoxylin are visible in the fibre network of the vascular wall

tion which showed a homogeneous vascular wall fibrinoid, with Mallory's phosphotungstic acid haematoxylin the necrosing muscular elements of the vascular wall, by virtue of their enhanced dye-binding ability, can clearly be differentiated from the plasma in the media.

*

Acknowledgement:

We are indebted to Mr. S. Kiss for the photographs.

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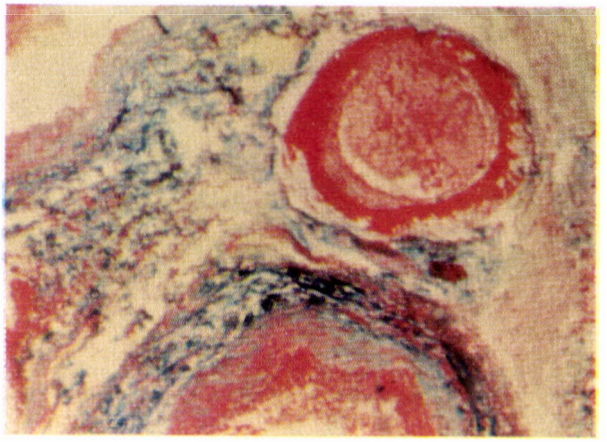

Fig. 1. Small artery of the muscular type characteristic of fibrinoid necrosis, homogeneous staining red with Azan, alongside acid-painted large vessels of the elastic type. There is no fibrinoid necrosis in the wall of the elastic vessels. Intimal proliferation in the upper vessel (Azan)

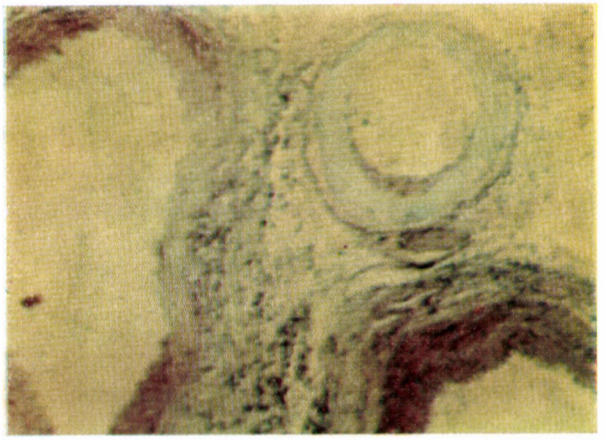

Fig. 2. The small blood vessel and part of the adjacent elastic vascular wall have lost metachromasia, which appears exclusively in the area of intimal proliferation (Toluidinie blue, pH 4.5)

ÜBER DIE NACH SÄUREPINSELUNG BEOBACHTETEN VERÄNDERUNGEN DER KLEINARTERIEN MUSKULÄREN TYPS

H. JELLINEK, KLÁRA SZEMENYEI, T. KERÉNYI und I. HÜTTNER

Die nach Säurepinselung entstehenden Veränderungen der Gefäße elastischen und muskulären Typs sind grundlegend unterschiedlich. Fibrinoidnekrose entwickelt sich lediglich in den Kleinarterien muskulären Typs. Die, einen Bestandteil des Gefäßwandfibrinoids bildende Muskelnekrose kann den übrigen Komponenten gegenüber mit Hilfe der Malloryschen Phosphor-wolframsäure-Hämatoxylin-Färbung — infolge der gesteigerten Farbenbindung der nekrotisierten Muskelzellen — deutlich abgesondert werden.

ИЗМЕНЕНИЯ В МАЛЫХ АРТЕРИЯХ МЫШЕЧНОГО ТИПА, НАБЛЮДАВШИЕСЯ ПОСЛЕ СМАЗЫВАНИЯ КИСЛОТОЙ

Х. ЙЕЛЛИНЕК, К. СЕМЕНЬЕИ, Т. КЕРЕНЬИ и И. ХЮТТНЕР

В связи со смазыванием кислотой авторы установили, что реакции сосудов эластического и мускулярного типа существенным образом отличаются друг от друга. Фибриноидный некроз возникает только в малых артериях мышечного типа. Представляющий составную часть фибриноида сосудистой стенки мышечный некроз может быть хорошо дифференцирован за счет повышенного связывания краски некротизированными мышечными клетками, если проводить окраску с фосфорвольфрамокислым гематоксилином по Маллори.

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FIBRINOID NECROSIS OF THE VASCULAR WALL INDUCED BY PAINTING WITH ACID

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(Received 9 September, 1965)

Fibrinoid necrosis of the muscular arterioles of the rat's uterus has been induced by painting with hidrochloric acid. The first change observable by light microscopy was a necrosis of muscle cells. A plasma imbition of such a rate the result of which was a development of typical fibrinoid of the media and which caused a precipitation of fibrin in severe cases was visible only after the necrosis of muscle cells. In addition, in the larger uterine arteries possessing a resistant internal elastic membrane, subendothelial fibrinoid, originating mostly from a precipitation of fibrin, was observed.

In earlier studies we often observed fibrinoid necrosis of the muscular arterioles in the environment of the aorta damaged by painting with acid in order to investigate the process of regeneration [4]. As fibrinoid necrosis of the vascular wall could reliably be evoked by painting with acid and the extent of the lesion appeared to be controllable, the process was subjected to detailed analysis.

In 40 albino rats of 200 g average weight the uterus as an organ rich in small blood vessels, was painted with different dilutions of hydrochloric acid. The animals were sacrificed at intervals from the 1st to the 60th day following operation. The uterus was removed, fixed in formalin and Carnoy's fluid, embedded in paraffin, then were examined after staining with haematoxylin-eosin, Azan, Mallory's phosphotungstic acid haematoxylin, PAS-reaction (after diastase-digestion), by fluorescence and polarisation optical methods (phenol, aniline-reaction), and in some instances following digestion with trypsin.

The vascular changes of the uterus were different depending on the concentration of the acid and on the distance of the effect, i.e. on the intensity of acid action.

In response to direct treatment with acid the small blood vessels showed dilatation, stasis, the wall was thinned, the connective tissue and elastic fibres were elongated, the muscular elements could not be differentiated. These blood vessels have not been used in the studies.

Various stages of fibrinoid necrosis were present in the wall of the small blood vessels lying in an intact environment somewhat distant from the site of treatment. The changes which depended on the length of survival and the intensity of acid action, will be described below in the sequence of their development.

In response to weak acid, the endothelial and muscle cells showed vacuolisation, hyperchrome, sometimes deformed nuclei, but no necrosis in the vascular wall (Fig. 1). Later, proliferation of muscle cells and the endothelium without fibrinoid necrosis was visible in these slightly damaged arterioles (Fig. 2).

In response to strong acid, some muscle cells underwent early necrosis; they stained flame-red with Azan, bound phosphotungstic acid haematoxylin more firmly than did the intact cells, gave a diastase-resistant PAS-reaction (Fig. 3), showed increased birefringence and an intense greenish-yellow fluorescence with coriphosphin as well as with the combination of euchrysin and thiazine red, giving the characteristic reactions of muscle cell necrosis. Later on, more and more of the muscle cells become necrosed, the necrotic areas show first a segmental, then a circular confluence, and the typical picture of fibrinoid necrosis arises (Fig. 4, 5). In the homogeneous, eosinophilic, PAS-positive fibrinoid which stains red with Azan, the necrosed muscle cells can for a while be differentiated by Mallory's phosphotungstic acid haematoxylin, but later, with the further progress of the process, this method shows a homogeneous fibrinoid, too (Fig. 6). The confluence of muscle cell necroses, and later the excessive increase in diameter of the vascular wall (Fig. 7) indicate an increased influx of plasma components. This is confirmed by the decrease, then cessation of the birefringence of the necrosed muscle cells.

When the damage is more severe and the influx of plasma is more copious, the process extends to the adventitia and the perivascular tissue, and birefringente fibrin appears in it. The damaged blood vessels are surrounded by granulation tissue composed of lymphocytes, histiocytes, fibroblasts, fibrocytes and collagen fibres, making the pattern reminiscent of periarteriitis nodosa (Fig. 8).

In the larger arteries of the acid-painted rat uterus, possessing a more resistant elastica interna, there is a homogeneous, eosinophilic material also between the membrana elastica interna and the endothelial cells, displaying the characteristic appearance of fibrinoid. While in these instances the entire vascular wall stains a homogeneous red with Azan (Fig. 9), phosphotungstic acid haematoxylin readily differentiates the subendothelial fibrinoid, the necrosed muscle cells of the media, as well as the smoothened-out elastica interna between the two (Fig. 10). In polarised light the subendothelial fibrinoid shows the birefringence and topochemical reactions characteristic of fibrin. Under Canada balsam the birefringence varies from $+35$ to 48 mμ, and this value is not effected by the aniline or the phenol reaction (Fig. 11, A, B, C). The likewise positive birefringence of the necrosed muscle cells of the media reach a maximum of 12 mμ. An 0.3 per cent trypsin solution took 60 minutes to digest the subendothelial fibrinoid in the Carnoy-fixed preparation (Fig. 11, D).

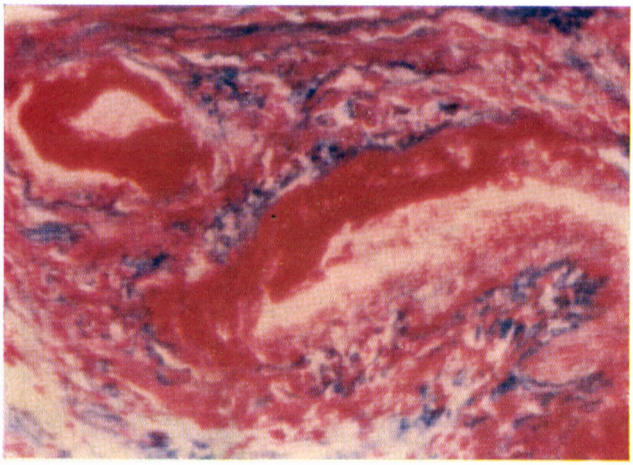

Fig. 9. A large uterine artery 7 days after treatment, with a fibrinoid necrosis staining homogeneous red with Azan

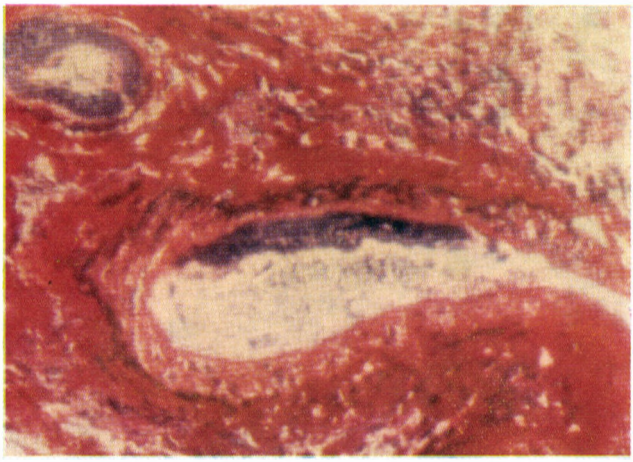

Fig. 10. In the same vascular wall segment Mallory's phosphotungstic acid haematoxylin makes it possible to distinguish the fibrinoid of the media containing the necrosed muscle cells and the subendothelial, homogeneous fibrinoid above the smoothened-out internal elastic membrane

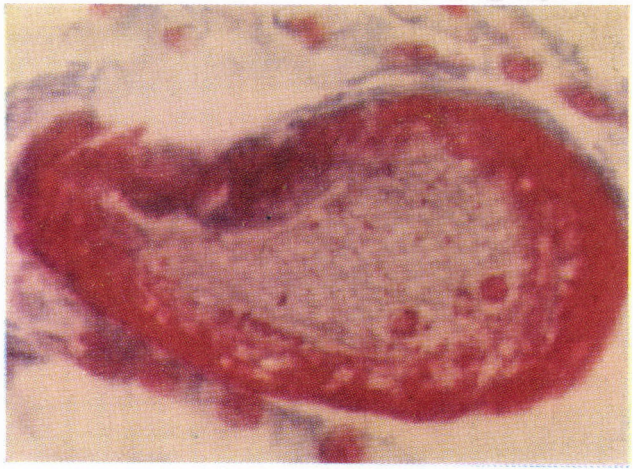

Fig. 5. Circular fibrinoid necrosis extending to most of the vascular wall 5 days after treatment. Azan

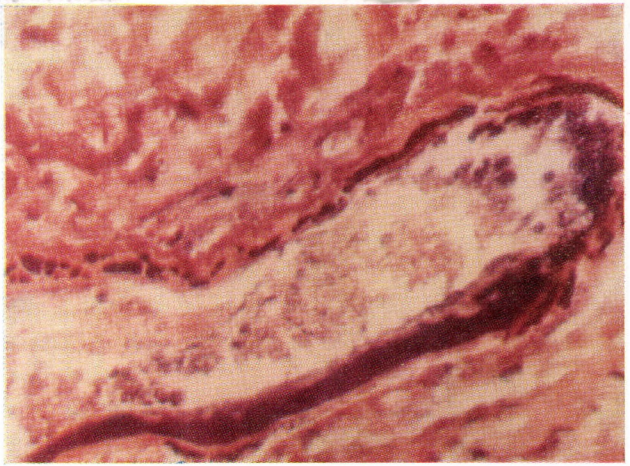

Fig. 6. Fibrinoid necrosis in vascular wall, with isolated and partly confluent cell necroses distinguishable by phosphotungstic acid haematoxylin (upper part). The lower part stains homogeneously. Seven day specimen. Mallory's phosphotungstic acid haematoxylin

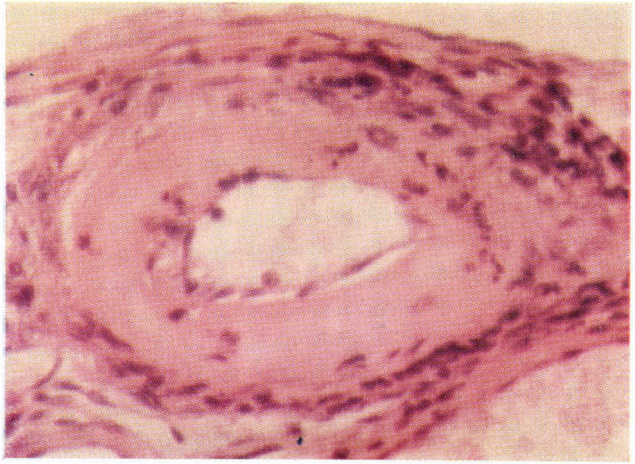

Fig. 7. Fibrinoid necrosis in small blood vessel, with plasma imbibition, broadening of the wall in which the necrotic muscle cells cannot be differentiated either by Mallory's phosphotungsticacid haematoxylin or by polarization optical methods.

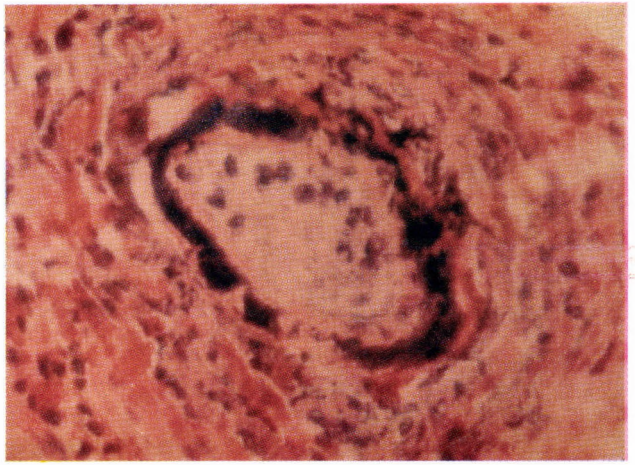

Fig. 8. Severely damaged small blood vessel 21 days after painting with acid, with perivascular granulation. Mallory-positive fibrinoid in part of the perivascular granulation tissue

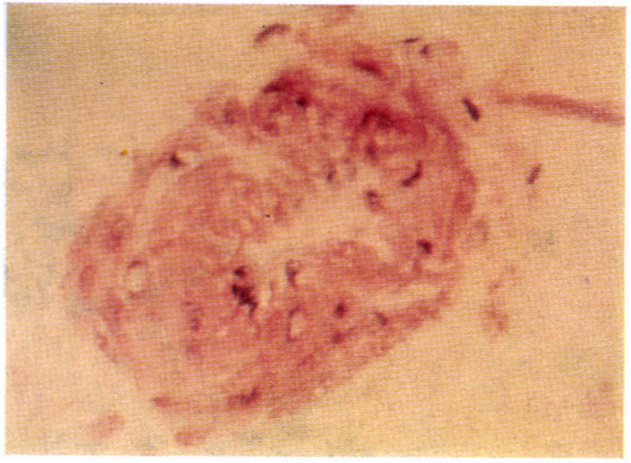

Fig. 1. Vacuolization of endothelium and muscle cells, two days after weak acid treatment. Haematoxylin-eosin

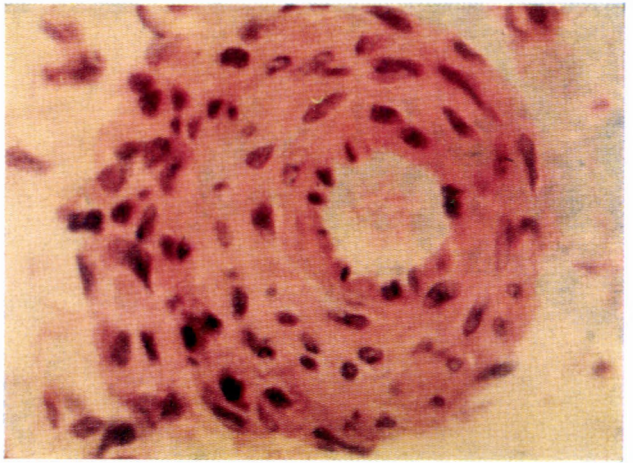

Fig. 2. Thirty days after painting with acid. Slightly damaged small blood vessel, with hypertrophy of the vascular wall showing no fibrinoid necrosis. Haematoxylin-eosin

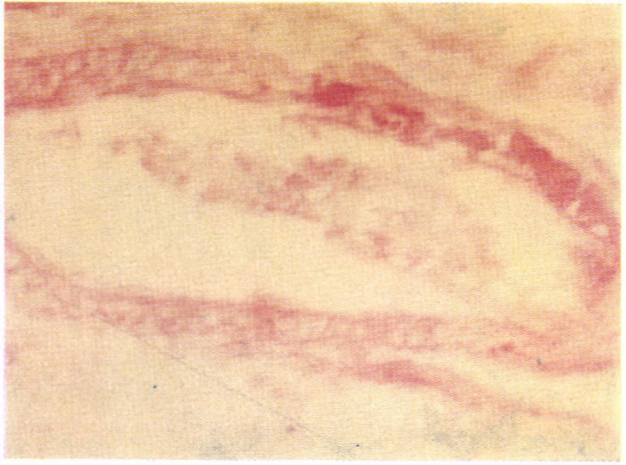

Fig. 3. Muscle cell necroses in the vascular wall, two days after treatment with strong acid, showing PAS- positivity

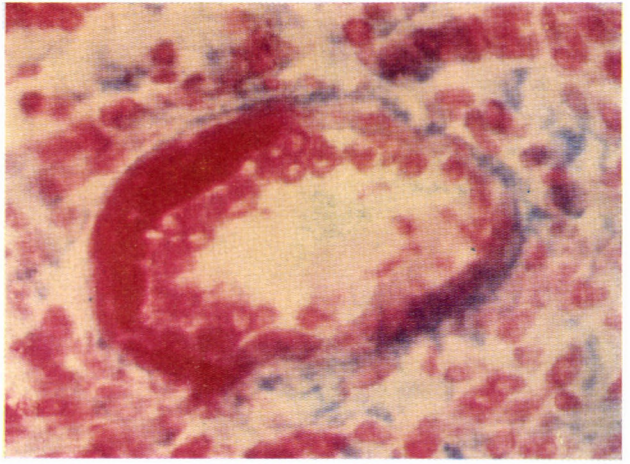

Fig. 4. Segmental confluence of muscle cell necroses in the entire breadth of the media, 7 days after treatment. Azan

Acta Morphologica Academiae Scientiarum Hungaricae 14, 1966

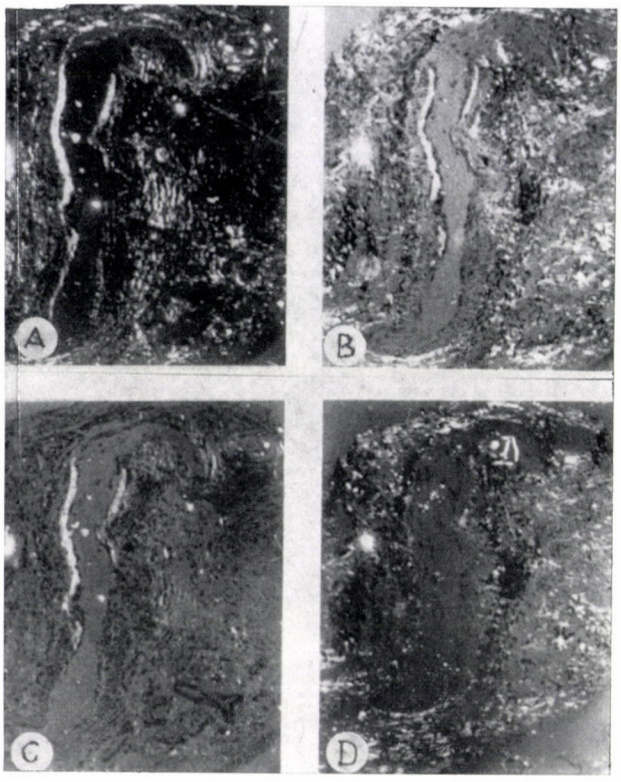

Fig. 11. Subendothelial fibrinoid in the uterine artery shown in Figs. 9 and 10, in polarised light. After covering with Canada balsam (A) the subendothelial fibrinoid shows positive birefringence, identical in direction with that of collagen fibres. The phenol reaction (B) which reverses the positive birefringence of collagen fibres and the aniline reaction (C) which abolishes the birefringence of the fibres, does not affect the positive birefrigence (48 mμ) of the subendothelial fibrinoid. Trypsin digestion dissolved the subendothelial fibrinoid. Phenol reaction after 60 minutes digestion with trypsin (D)

After an average of 30 days, the fibrinoid necrosis of the small blood vessels showed hyalin transformation: the characteristic Azan-red colour disappeared and finally every lesioned vascular wall stained blue. In the vascular wall showing hyaline transformation neither necrosed muscle cells, nor fibrin could be demonstrated by phosphotungstic acid haematoxylin.

Thrombus formation did not occur in our cases.

Discussion

Malkoff [7], Ssolowjew [19], Jaffé [3] and others applied different mechanical means to lesion the vascular wall, but all those authors restricted their studies to large blood vessels.

In similar experiments we have shown [20, 21] that painting with acid was a method by which fibrinoid necrosis could be induced in small blood vessels of the muscular type.

In the pathogenesis of fibrinoid necrosis a decisive significance has been attributed to plasma imbibition by SCHÜRMANN and MCMAHON [16], VAZQUEZ and DIXON [22], KELLAWAY *et al.* [6], SOUSTEK [17, 18] and others, whereas MONTGOMERY and MUIRHEAD and [9, 10], MUIRHEAD and GROLLMANN [12], MUIRHEAD *et al.* [13, 14], GARDNER [1, 2] MASSON and KAWAKITA [8], ZOLLINGER [23] and others have claimed this for a primary damage to the muscle cells of the vascular wall.

Electron microscopic and fluorescence studies equivocally prove that in lesions of the small blood vessels an early influx of plasma components into the vascular wall take place. MOVAT and FERNANDO [11], for instance demonstrated a precipitate in the wall of small blood vessels 3 minutes after inducing allergic inflammation. It is, however, undecided whether plasma imbibition would be responsible for the necrosis of muscle cells and whether it alone would suffice to cause necrosis, and whether the disturbance of permeability and the necrosis of muscle cells could be traced back to a common cause.

In our experiments the first visible sign of the vascular damage was a necrosis of the muscle cells of the media; a plasma imbibition of such a rate which together with the necroses of muscle cells gave rise to the typical fibrinoid, developed only later.

As it has been pointed out by JOBST [5], the identical appearance of the fibrinoid arising in different pathological processes, its staining like fibrin, thus a homogeneous red with Azan its eosinophilia and PAS-positivity as well as the birefringence of certain of its forms, may be traced back to various factors. As indicated by its staining with phosphotungstic acid haematoxylin and its polarisation optical properties, the fibrinoid of the media that arises in the wall of the small blood vessels in response to painting with acid is likewise of variable composition. At first, the necrosed muscle cells strongly binding phosphotungstic acid haematoxylin and showing increased birefringence can still be differentiated in the imbibed vascular wall. Later, even this staining method only reveals a homogeneous vascular wall. The simultaneous cessation of the polarisation optical activity of the media indicates a disintegration of muscle cells, a desorientation of their birefringent elements. When the lesion is more severe, in the necrotic vascular wall and its environment a network corresponding to fibrin can be differentiated on the basis of its birefringence and topochemical reactions (ROMHÁNYI [15], JOBST [5].)

The subendothelial fibrinoid which develops in the larger uterine arteries unlike the fibrinoid of media is on the basis of its birefringence a precipitation of plasma proteins, composed mainly of fibrin under the endothelial layer.

At the beginning, when the subendothelial fibrinoid develops in these blood vessels the changes of the media don't contain fibrin. Thus, the internal elastic membrane seems to block the way of the plasma imbibition and prevent for a time the penetration of fibrinogen into the media.

The above described changes observed in small blood vessels running in intact environment suggest the possibility that a reflex vascular spasm may have played a role in the process.

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Acknowledgement: We are indebted to Mr. S. Kiss for tho photographs.

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ÜBER DIE ENTWICKLUNG DER MIT SÄUREPINSELUNG HERBEIGEFÜHRTEN FIBRINOIDEN GEFÄSSWANDNEKROSE

I. HÜTTNER, H. JELLINEK, T. KERÉNYI und KLÁRA SZEMENYEI

Bei Ratten wurde durch Säurepinselung Fibrinoidnekrose der Kleingefäße der Uterusmuskulatur herbeigeführt. Die erste, lichtmikroskopisch wahrnehmbare Veränderung im Laufe der Entwicklung der Fibrinoidnekrose war die Nekrose der Muskelzellen. Jenes Stadium der Plasmaimbibation, das gemeinsam mit der Muskelzellnekrose zur Entwicklung des typischen Mediafibrinoids führte und im Falle einer schweren Schädigung auch die Präzipitation des Fibrins resultierte, trat nur später in Erscheinung. In den, über eine widerstandsfähigere Membrana elastica interna verfügenden größeren Gebärmuttergefäßen wurde außerdem in manchen Fällen auch die Entwicklung eines — größtenteils aus Fibrinpräzipitation stammendes — endothelialen Fibrinoids beobachtet.

ВОЗНИКНОВЕНИЕ ФИБРИНОИДНОГО НЕКРОЗА СОСУДИСТОЙ СТЕНКИ, ВЫЗВАННОГО СМАЗЫВАНИЕМ КИСЛОТОЙ

И. ХЮТТНЕР, Х. ЙЕЛЛИНЕК, Т. КЕРЕНЬИ, К. СЕМЕНЬЕИ

Авторы вызывали смазыванием кислотой фибриноидный некроз малых сосудов мышц матки крыс. В ходе возникновения фибриноидного некроза первым признаком, наблюдаемым под микроскопом, был некроз мышечных клеток. Та степень впитывания плазмы, которое вместе с некрозом мышечных клеток привело к возникновению типичного фибриноида средней оболочки и в случае тяжелого поражения приводило также и к выпадению фибрина, наблюдалась только позже. В более крупных артериях матки, обладающих более устойчивой внутренней эластической перепонкой, несколько раз наблюдалось возникновение субэндотелиального фибриноида, преимущественно происходящего за счет выпадения фибрина.

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FIBRINOID NECROSIS OF THE VASCULAR WALL IN EXPERIMENTAL MALIGNANT HYPERTENSION

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Malignant hypertension was induced in albino rats by bilateral kidney compression by means of rubber capsules and the development and the various forms of appearance of fibrinoid necrosis in the small blood vessels have been studied.

1. In the development of the fibrinoid of the media of small muscular blood vessels, the progressive necrosis of muscle cells was of fundamental importance.

2. The elastic elements of the vascular wall significantly modified the form of appearence of fibrinoid necrosis. In the small blood vessels possessing a resistant internal elastic membrane, or in the case of fibrosis of the media, above the elastica interna a subendothelial fibrinoid may develop which differs in composition from the fibrinoid of the media.

Fibrinoid necrosis of the small blood vessels in experimental malignant hypertension has been reported by several authors [10, 11, 12, 13, 9, 17, 2a, 1.] GORÁCZ [4] studied the vascular wall lesions in malignant hypertension induced in albino rats by bilateral compression of the kidneys by means of rubber capsule. In the present paper we shall report on a study of the development and the various forms vessel wall's fibrinoid.

In 60 albino rats, weighing 150 to 200 g, rubber capsules were placed on both kidneys, as described by LŐRINCZ and GORÁCZ [7, 8]. After the second postoperative day blood pressure increased from the normal 80 to 115 mm Hg systolic to 150 to 190 mm Hg. The animals were sacrificied at regular intervals from the 2nd till the 30th day and the heart, intestines, mesentery and pancreas were subjected to study, as in previous experiments the changes of the small blood vessels were most marked in these organs. The sections were fixed in formalin and Carnoy's fluid, embedded in paraffin, and stained with haematoxylin-eosin, Azan, Mallory's phosphotungstic acid haematoxylin, Endes' trichrome and resorcin Fuchsin, the PAS and Feulgen reactions, as well as by polarisation optical and fluorescence methods.

In 2 to 4-days, swelling and vacuolisation of the endothelial and smooth muscle elements of the small blood vessels, swelling and sometimes pycnosis of the nuclei of muscle cells, then disseminated necrosis of the muscle cells were visible. The necrosed muscle cells stained an intensive red with Azan (Fig. 1), were PAS-positive and bound phosphotungstic acid haematoxylin more firmly than did the normal cells. The necrosed areas showed an increased positive birefringence in polarised light (Fig. 2), and a greenish-yellow fluorescence with acridine orange (Fig. 3). Their Feulgen-positivity indicated an DNA content (Fig. 4). On the 3rd to 6th days the necroses showed segmental, then circular confluence, extending to the entire media (Fig. 5). The coalescing necroses of

the muscle cells and the widening of the media indicated an increasing influx of plasma. On the 7th to 9th days the vascular wall was eosinophilic, staining a homogeneous red with Azan, PAS-positive, exhibiting a picture typical of

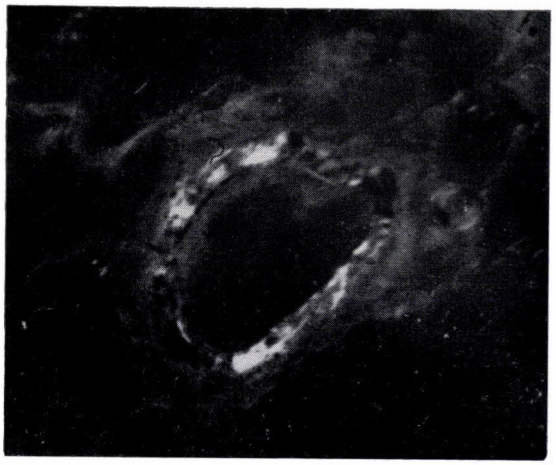

Fig. 2. Muscle cell necroses showing increased birefringence in polarised light in the wall of a small blood vessel 3 days after operation. Under Canada balsam

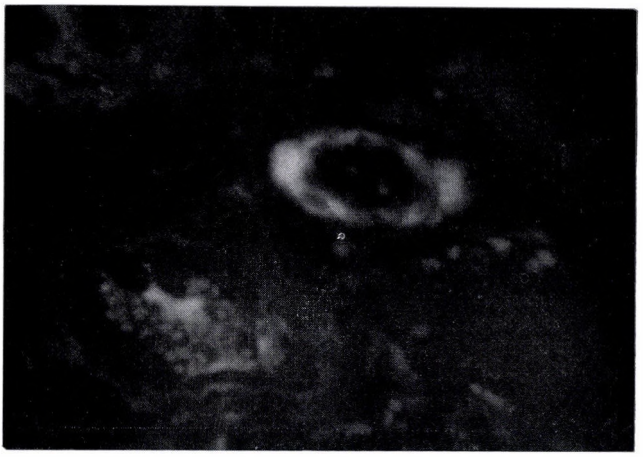

Fig. 3. Intensive greenish-yellow fluorescence corresponding to muscle cell necroses in the wall of a small artery 5 days after operation. Acridine orange 1 : 10000, pH 6

fibrinoid. Initially, the necrosed muscle cells could still be differentiated by their increased binding of phosphotungstic acid haematoxylin and birefringence.

Parallel with the changes of the media, perivascular granulation occurred. The cells of the granulation tissue invaded the necrosed media, to occupy finally the place of the damaged media by infiltrating it circularly down to the

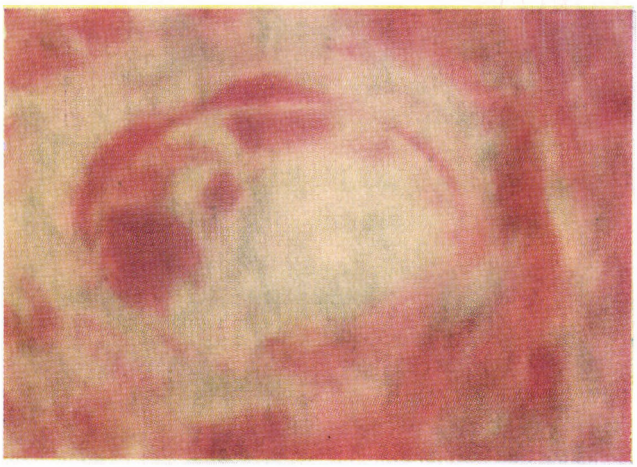

Fig. 10. Necrosing muscle cells and subendothelial fibrinoid in small intestinal subserose arteriole 9 days after operation. Mallory's phophotungstic acid haematoxylin

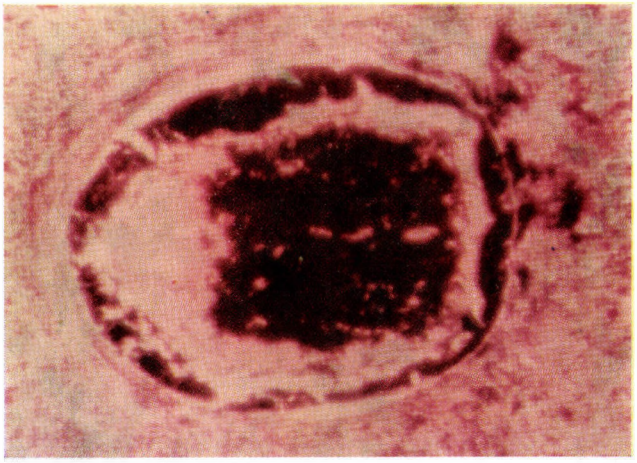

Fig. 12. Mesenterial artery with resistant elastic membrane. Circular subendothelial fibrinoid above smoothened-out internal elastic membrane. At sites the process involves the media and perivascular tissue as well. 21 days after operation. Endes' trichome

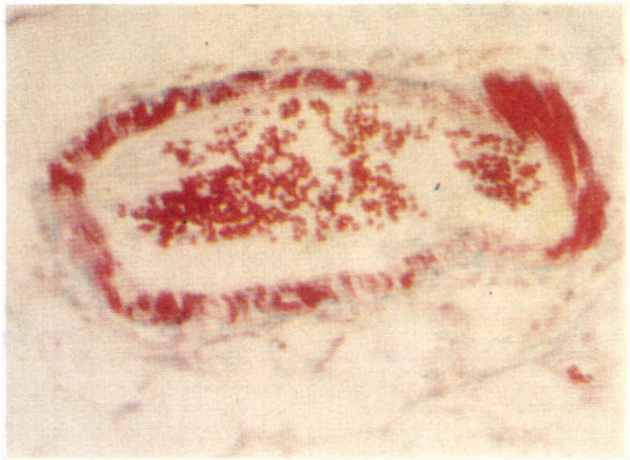

Fig. 1. Isolated and segmentally confluent muscle cell necroses in a mesenterial small blood vessel 3 days after operation. Azan

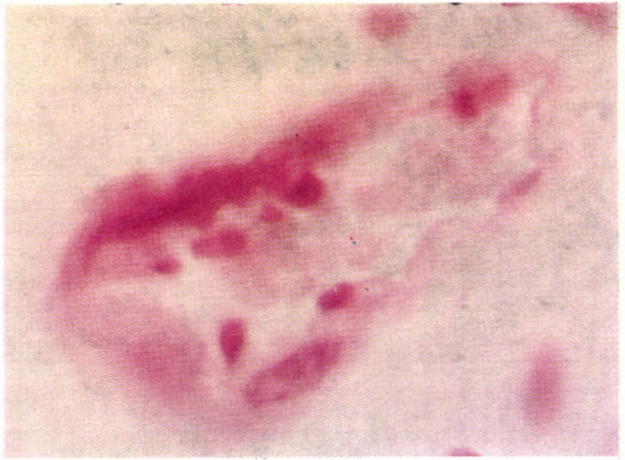

Fig. 4. Early confluent muscle cell necroses with persisting nuclear material, 3 days after operation, in the wall of a mesenterial arteriole. Feulgen reaction

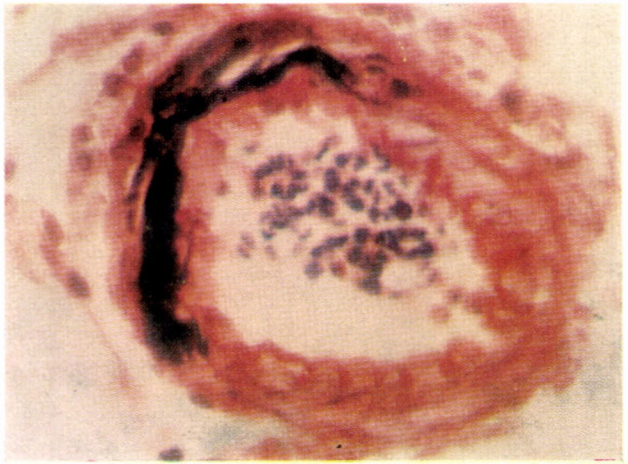

Fig. 5. Segmental confluence of muscle cell necroses in the entire media, 5 days after operation. Mallory's phosphotungstic acid haematoxylin

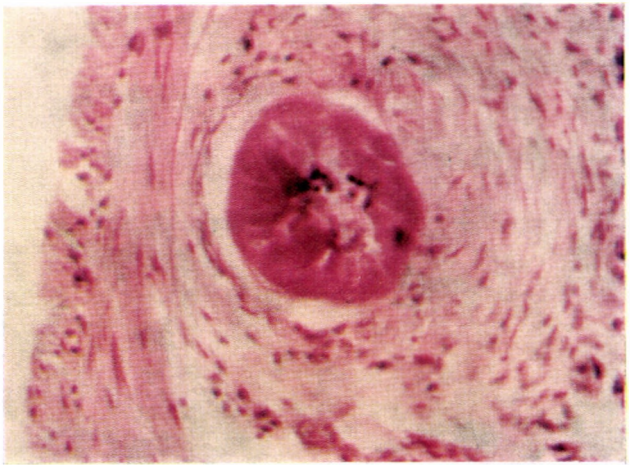

Fig. 6. Small intestinal subserose arteriole 21 days after operation. The media is replaced by granulation tissue, the lumen is almost obliterated by the subendothelial fibrinoid. PAS-

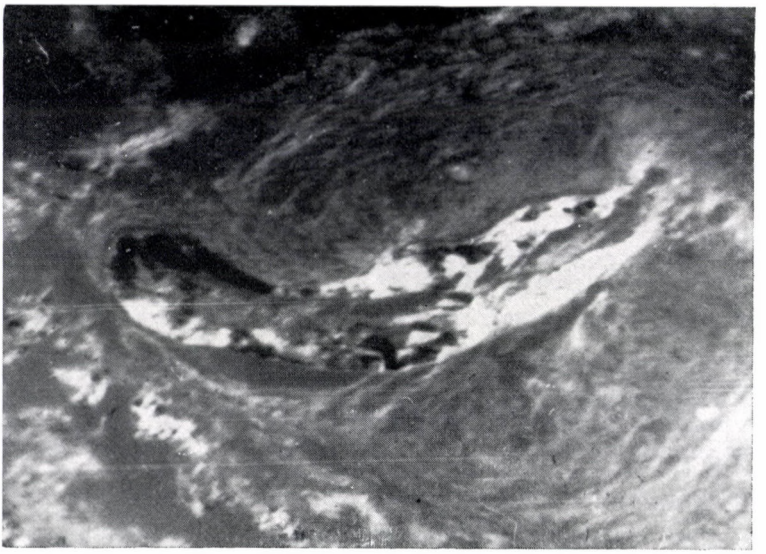

Fig. 7. A lesion similar to that shown in Fig. 6, in polarised light. Birefringent bundles and granules oriented in different directions are visible in the subendothelial fibrinoid. Under Canada balsam

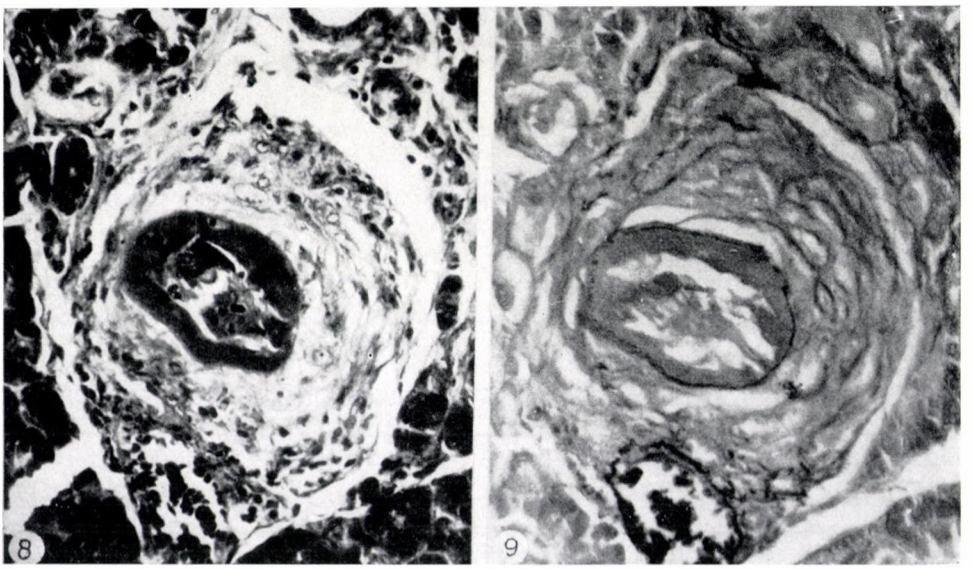

Fig. 8. Pancreatic small blood vessel 17 days after operation, with granulation tissue extending to the lamina elastica interna and with subendothelial fibrinoid. Haematoxylin-eosin
Fig. 9. The same small artery, stained with resorcin-fuchsin

lamina elastica interna, blocking thus the influx of plasma and leading to the appearance of a homogeneous acellular substance staining like fibrinoid and increasing in amount subendothelially. The broad subendothelial fibrinoid narrowed the lumen, occluding it almost completely in some instances (Fig. 6). It stained a dark bluish-black with phosphotungstic acid haematoxylin and contained an increasing quantity of filamentous granular elements oriented in different directions and showing an average birefringence of + 35 to 48 mμ similar to that of fibrin (Fig. 7).

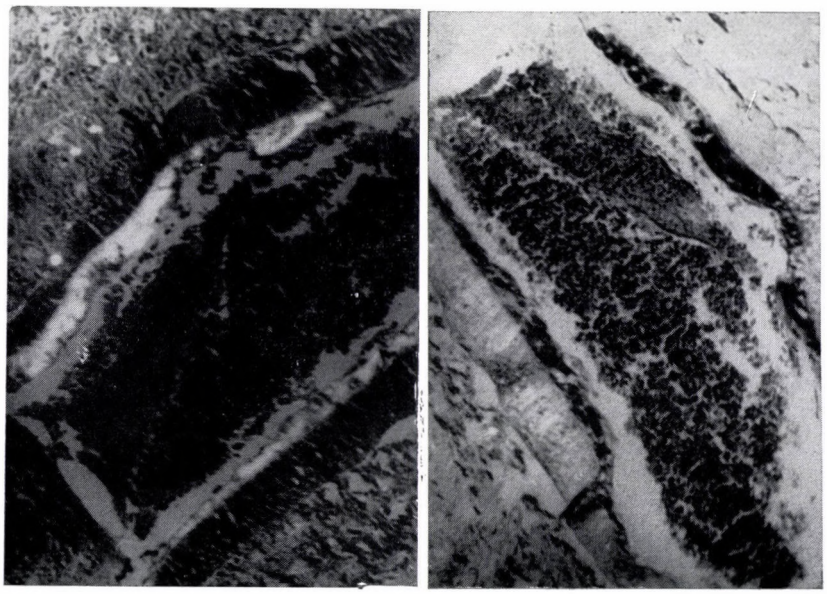

Fig. 11. Subendothelial fibrinoid in polarised light, with opposite compensation. Birefringent bundles and granules oriented in different directions 12 days after operation. Under Canada balsam

In the blood vessels in which the lamina elastica interna was resistant the subendothelial fibrinoid developed simultaneously with the changes in the media. The staining and polarisation optical properties of the subendothelial fibrinoid were similar to the changes observed in small blood vessels surrounded by granulation (Fig. 11). In these blood vessels of the muscular type possessing a marked elastic membrane the influx of plasma took place in stages; after the development of the subendothelial fibrinoid the signs of plasma imbibition appeared also in the media, and together with the muscle necroses a typical picture of medial fibrinoid appeared. Subsequently the process extended radially from the media to the surrounding granulation tissue (Figs. 12, 13). In the plasma invading the perivascular granulation tissue radially, fibrin could be demonstrated (Fig. 14).

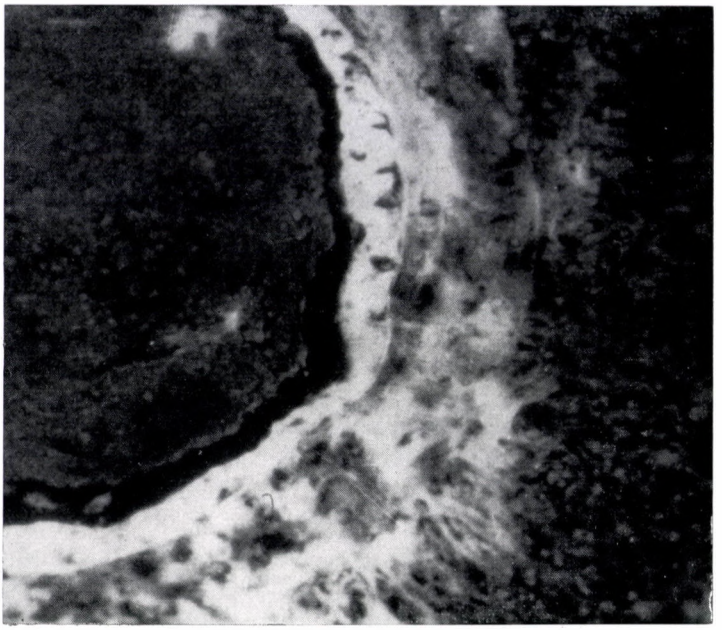

Fig. 13. Mesenterial artery 21 days after operation. Plasma invasion blocked by the internal elastic membrane, then the external elastic membrane. At the lower margin, plasma has penetrated across the external elastic membrane. Thiazine red-eucrysin 1 : 10 000, fluorescence microscopy

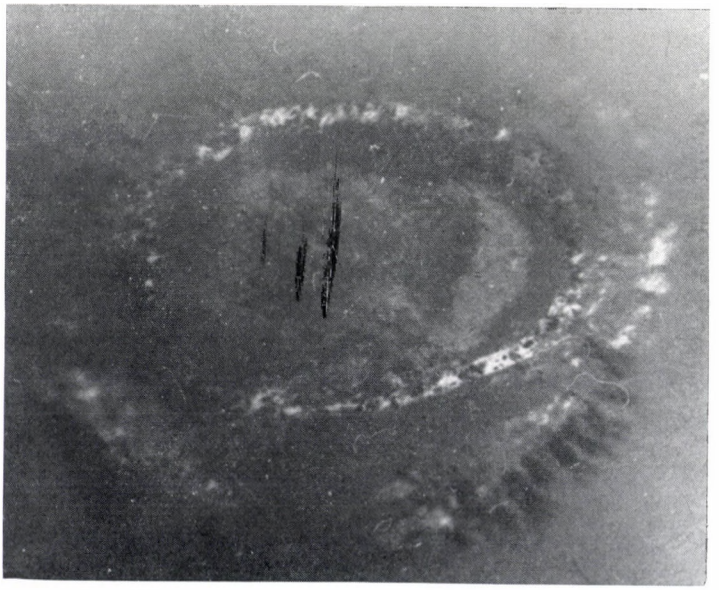

Fig. 14. In the plasma imbibing the necrosed vascular wall and penetrating subendothelially and radially into the environment, fibrin is demonstrable. 21 days after operation. Under Canada balsam. Polarisation optical photograph

After 30 days the entire fibrinoid of the media and parts of the perivascular fibrinoid stained usually blue with Azan, showing hyaline transformation. The subendothelial fibrinoid retained its staining properties and birefringence.

Discussion

Opinions diverge as to the development of vascular fibrinoid necrosis in malignant hypertension. Some authors think that the primary change is a necrosis of muscle cells [10, 11, 12, 13, 9, 17, 2a, 1], whereas others claim this for plasma imbibition. [15, 2, 16, 14].

According to data in the literature, in experimental hypertension a pathological permeability of the blood vessels develops in a few hours following the onset of hypertension [3, 4]. However, plasma imbibition alone cannot be held responsible for the development of muscle necroses. In the experiments of GORÁCZ fibrinoid necrosis failed to occur in those animals, in which the rubber capsule had been removed from the kidneys 2 days, although plasma imbibition was demonstrated by fluorescence on the first postoperative day already [4]. Moreover, the simultaneous presence of plasma imbibition and muscle cell necroses is not always a proof of the entrance of plasma components into the vascular wall being a primary event. OHTA *et al.* [14] reported that in the early stage of experimental necrosing arteriitis, gamma globulins entered the vascular wall and were linked to muscle cells. This suggests the possibility that an accumulation of plasma proteins in the media is due, at least in part, to a damage to muscle cells. As regards the pathogenesis of the fibrinoid in the vascular wall, no view can be put forward, because the forms of fibrinoid appearing in the different layers of the vascular wall differ from one another in composition and mode of development.

On the basis of our investigations it may be stated that the commonest form of fibrinoid necrosis of the small blood vessels, the fibrinoid of the media, originates from the necrosed muscle cells and the plasma components entering the vascular wall. The muscle cell necroses revealed by Mallory's phosphotungstic acid haematoxylin and examination in polarised light can be differentiated for a while even after the onset of excessive plasma influx and the development of typical fibrinoid necrosis.

Subendothelial fibrinoid can always be traced back to as interference with the entrance of plasma into the media. This fibrinoid may develop even in the smallest muscular blood vessels if the damaged media is replaced by a granulation tissue rich in fibres. However, we find it most often under the layer of endothelial cells in muscular arteries possessing a resistant internal elastic membrane. The subendothelial fibrinoid stains in the same way as the fibrinoid of the media but differs from the latter in composition, as revealed by its polarisation optical activity. Its native birefringence is always significantly

higher (positive 35 to 48 mμ) than the increment of birefringence (maximum 12 to 18 mμ), observable in the early stage of muscle cell necrosis leading to the development of fibrinoid of the media in small blood vessels. At the same time, the toluidineblue potassium-ferrocyanide precipitation anisotropy of the subendothelial fibrinoid plotted against pH is similar to that of fibrin and significantly different from that of the fibrinoid of the media [6]. Accordingly the subendothelial fibrinoid would correspond to plasma materials precipitated onto the internal elastic membrana and its birefringence is caused by fibrin. The suggestion made by MONTGOMERY and MUIRHEAD [9] that the subendothelial fibrinoid would originate from necrosed cellular elements of the media is discredited by the fact that the two forms of fibrinoid behave differently under polarised light.

On the basis of its morphology, staining properties and birefringence, the fibrinoid substance spreading radially around the vessels if these are seriously damaged likewise contains fibrin. However, in the case of such a grave damage the permeability disturbance is so severe that fibrin is precipitated in every layer of the vascular wall, therefore the various forms of fibrinoid cannot be differentiated on the basis of their birefringence.

Although these experimentally induced vascular changes cannot be parallelled without restrictions with the vascular changes of human hypertension, yet in many respects their patterns are identical, with the various changes of the small blood vessels described in human pathology as occurring in malignant hypertension and other conditions.

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UNTERSUCHUNG DER FIBRINOIDEN GEFÄSSWANDNEKROSE IN EXPERIMENTELLER MALIGNER HYPERTONIE

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Bei Albinoratten wurde mit der Methode nach LŐRINCZ und GORÁCZ (Komprimierung der Nieren mit einer Gummihülle) maligne Hypertonie herbeigeführt und anschließend die Entwicklung bzw. die Erscheinungsform der Fibrinoidnekrose der Kleingefäße untersucht. Es wurde festgestellt, daß

1. im Zustandekommen des Media-Fibrinoids der kleinen muskulären Gefäße der ausgeprägten Nekrose der Muskelzellen eine grundlegende Bedeutung zukommt,

2. die elastischen Elemente der Gefäßwand die Erscheinungsfrom der Fibrinoidnekrose in bedeutendem Maße modifizieren. In den, über eine widerstandsfähige Membrana elastica interna verfügenden Kleingefäßen oder im Falle von Media-Fibrose kann sich oberhalb der Elastica interna ein „subendotheliales Fibrinoid" entwickeln, das sich bezüglich der Zusammensetzung vom „Media-Fibrinoid" unterscheidet.

ИССЛЕДОВАНИЕ ФИБРИНОИДНОГО НЕКРОЗА СОСУДИСТОЙ СТЕНКИ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ЗЛОКАЧЕСТВЕННОЙ ГИПЕРТОНИИ

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Авторы вызывали на белых крысах двусторонним сдавливанием почек резиновой манжеткой по Леринцу—Горацу злокачественную гипертонию. В связи с этим они исследовали возникновение и формы проявления фибриноидного некроза малых сосудов. Они установили, что

1. в деле возникновения фибриноида средней оболочки малых мускулярных сосудов основное значение имеет прогрессирующий некроз мышечных клеток;

2. эластические элементы сосудистой стенки значительно видоизменяют картину проявления фибриноидного некроза. В малых сосудах, обладающих устойчивой эластической перепонкой или в случае фиброза средней сосудистой оболочки, над внутренней эластической перепонкой может возникать «субэндотелиальный фибриноид», который по своему составу отличается от «фибриноида средней оболочки».

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FIBRINOID NECROSIS OF THE VASCULAR WALL INDUCED BY NORADRENALINE

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The fibrinoid necrosis of the vascular wall induced by noradrenaline infusion in the small blood vessels of the dog's heart has been studied. The first change visible by light microscopy is a necrosis of the muscle cells of the media, which together with the rapidly progressing imbibition with plasma results in the formation of a typical fibrinoid. The process was the same as that observed in response to painting with acid and in malignant hypertension. The form of appearance was influenced exclusively by the intensity of the intervention and by the structure of the vascular wall.

Changes of the heart and small blood vessels in phaeochromocytoma have been described by SZAKÁCS and CANNON [9], TALBOT *et al.* [10], KLINE [4] and others. Similar changes have been reported to follow the administration of high doses of noradrenaline for the treatment of shock by SZAKÁCS and CANNON [9], MOND and MACK [5]. The noradrenaline-induced cardiac and vascular changes have been reproduced experimentally by SZAKÁCS and CANNON [9], PUGH *et al.* [7], as well as SAMSON [8].

Since the vascular changes described by the above authors resemble those found by us in fibrinoid vascular wall necroses induced by various methods [2, 3], a study has now been made of the noradrenaline-induced process leading to fibrinoid necrosis.

Under chloralose anaesthesia 36 dogs of either sex, weighing 6 to 12 Kg, were given in doses of 300, 450 and 700 μg/Kg body weight respectively of noradrenaline in 200 ml physiological NaCl solution in a drip infusion administered in 45 minutes. Before, during and after the infusion we determined the heart rate and measured the blood pressure directly in the femoral artery. The ECG was recorded before, at 5-minute intervals during and once daily after the infusion. During the infusion blood pressure rose from the initial 160 to 180 mm Hg to 260 to 280 mm Hg. The heart rate varied within wide limits, from 60 to 280. The ECG mostly showed repolarisation disturbance and extrasystolic arrhythmia. The clinical aspects of the experiments have described by POGÁTSA and GÁBOR [6].

The animals were killed 48 hours, as well as 2 and 8 weeks after the experiment. The organs were fixed in formalin and Carnoy, embedded in paraffin, stained with haematoxylin-eosin, Azan, Mallory's phosphotungstic acid haematoxylin, Endes'trichome and Weigert's dye. In addition, the PAS and Feulgen reactions, polarisation optical and fluorescence microscopic methods were carried out.

Vascular changes were most marked in the heart; slight vascular changes occurred in the kidneys, intestines and pancreas.

In response to noradrenaline treatment, changes developed simultaneously in the heart muscle and its small blood vessels. Depending on the dose of the

drug, necroses of the fibres appeared scattered or in smaller groups in the muscle of both ventricles.

In the heart of animals killed at 48 hours after infusion, the intramural small arteries exhibited changes, in the right ventricle in the first place, depending in severity on the dose of noradrenaline.

The first changes visible under the light microscopie were swelling, vacuolisation, then necrosis of the muscle cells of the media, found mostly at the junction of media and adventitia. The necrosed muscle cells stained a flame-red with Azan (Fig. 1.), were PAS positive (Fig. 2), bound phosphotungstic acid haematoxylin more firmly than the intact muscle cells, on staining acridine orange 1 : 10 000 pH 6, they were easy to recognize by their intense greenish-yellow fluorescence, and in polarised light their birefringence was stronger than that of intact muscle. After a small dose of noradrenaline only a few muscle cells showed necrosis and few blood vessels were affected. Although the individual variations were considerable, higher noradrenaline doses usually caused changes in more blood vessels, in which the muscle cell necrosis extended to single segments of the vascular wall, or involved a whole circular section of the media (Figs. 3, 4). At the same time the vascular wall increased in diameter, stained homogeneously with haematoxylin-eosin, Azan and PAS, showing the characteristic pattern of fibrinoid necrosis (Fig. 5). For a while the plasma imbibed in it could be separated from the necrosed muscle cells by Mallory's phosphotungstic acid haematoxylin (Fig. 6). In the initial stage of muscle cell necrosis there was an increase of birefringence, as we have described it in connection with fibrinoid necrosis (7, 8); this increase soon ceased, indicating a total disintegration of muscle cells.

In the dogs treated with 700 μg/Kg body weight of noradrenaline, beside the above changes the necrosis extended to the adventitia and the perivascular areas (Fig. 7), where filaments of fibrin were demonstrable by polarisation. For their demonstration we did not use Weigert's or Endes' trichrome dyes as they stained the necrosed muscle cells, too. The vascular changes localised to the media or extending to the environment were often followed by perivascular cellular infiltration (Fig. 8).

In the heart of the animals sacrificed 2 weeks after treatment the necrosis was less extensive than after 48 hours; at sites initial fibrosis, and around the damaged blood vessels cellular infiltration and an accumulation of fibres were visible. In the dogs killed 4 to 8 weeks after treatment there was no necrosis in the heart muscle, only an occasional fibrosis, and fibrous, hyaline transformation in some blood vessels.

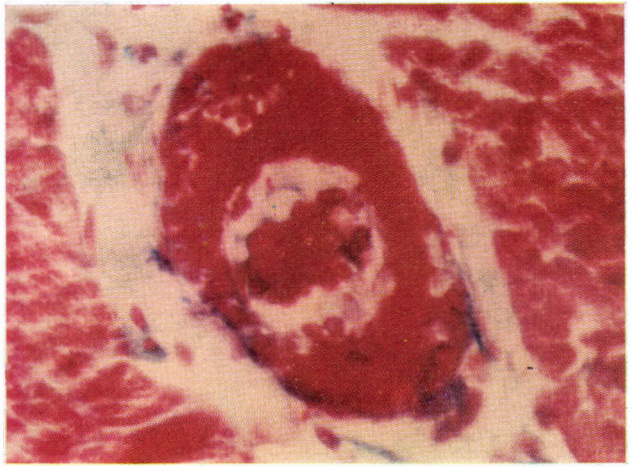

Fig. 5. Typical, homogeneous fibrinoid necrosis in vascular wall, 48 hours after noradrenaline infusion. (Azan)

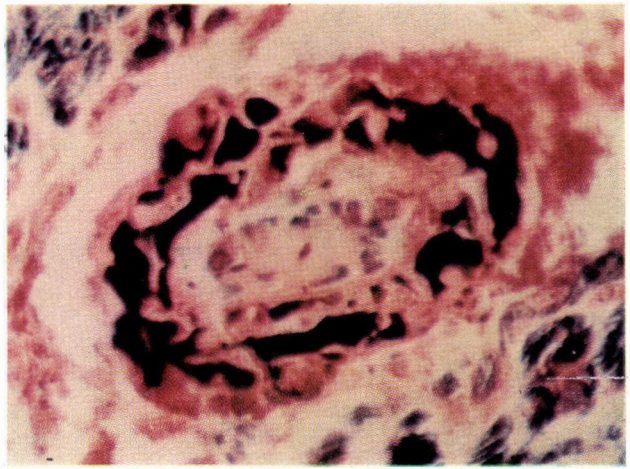

Fig. 6. In the fibrinoid necrosis staining a homogeneous red with Azan, phosphotungstic acid haematoxylin still reveals necrosed muscle cells. 48 hours after noradrenaline infusion.

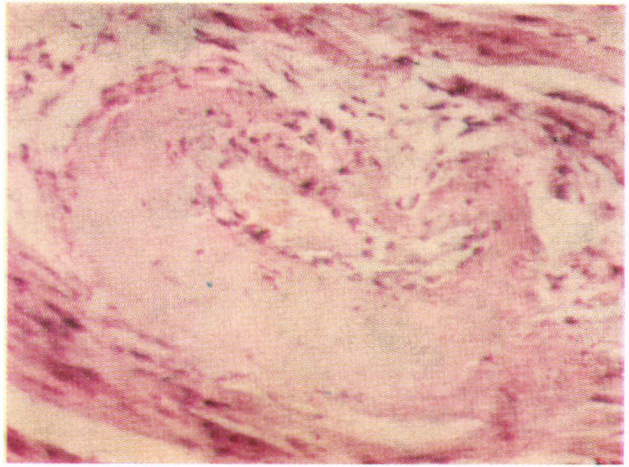

Fig. 7. Grave vascular wall lesion with excessive plasma imbibition. 48 hours after the infusion of 700 g/Kg body weight of noradrenaline. (Haematoxylin-eosin)

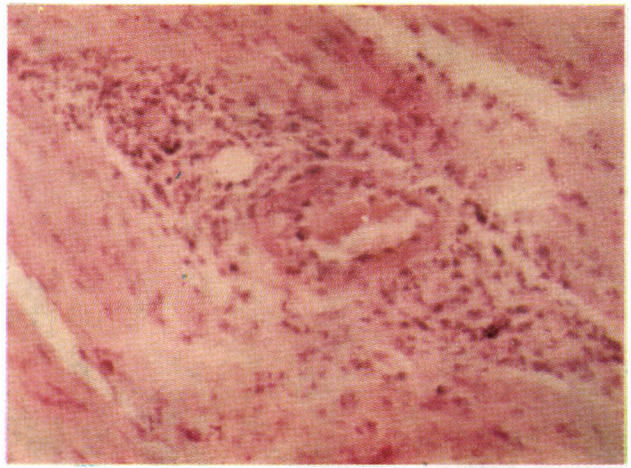

Fig. 8. Perivascular cellular reaction in the environment of a slightly lesioned blood vessel. Dog killed 48 hours after noradrenaline infusion. (Haematoxylin-eosin)

Acta Morphologica Academiae Scientiarum Hungaricae 14, 1966

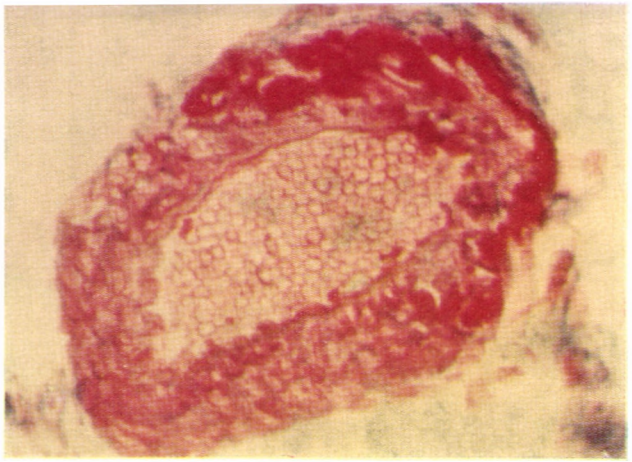

Fig. 1. Necrosed muscle cells in the outer part of the media of a myocardial arteriole, 48 hours after the infusion of noradrenaline (Azan)

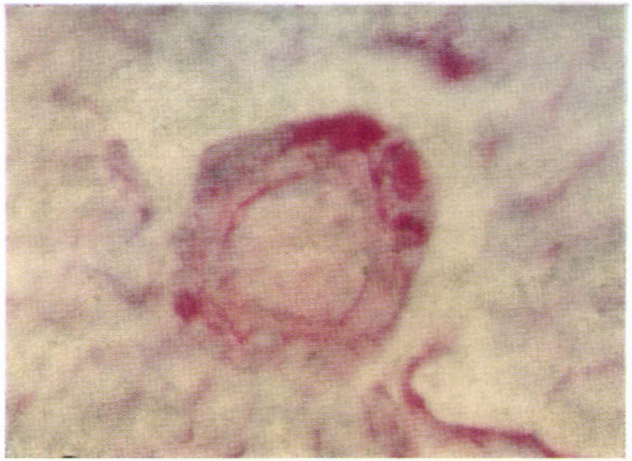

Fig. 2. Isolated muscle cell necroses 48 hours after noradrenaline infusion. PAS reaction

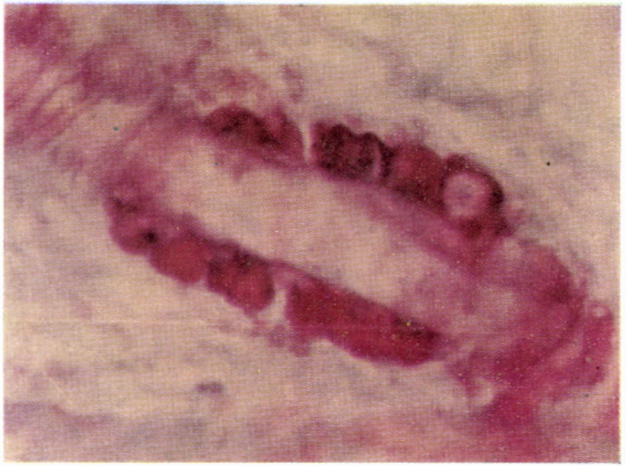

Fig. 3. A group of necrotic muscle cells in the vascular wall, 48 hours after noradrenaline infusion (PAS reaction)

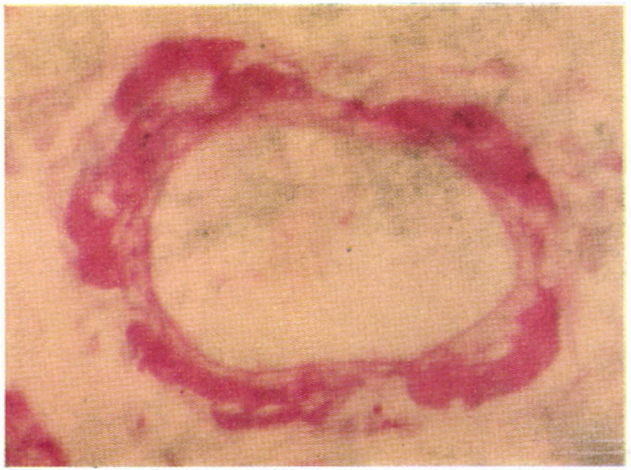

Fig. 4. Necrotic and homogeneous parts of media, 48 hours after noradrenaline infusion (PAS reaction)

Discussion

Fibrinoid necrosis of the small blood vessels of the heart was seen to occur exclusively in the cases exhibiting a marked rise of blood pressure. Vascular changes were more frequent in the right than in the left ventricle. Similar observations have been made by GORÁCZ and KONYÁR [1] in white rats with malignant experimental hypertension. It is thought therefore that a rise of blood pressure must have an important role in the pathogenesis of the fibrinoid necrosis developing in response to noradrenaline. However, tachycardia, hypoxia, and the direct toxic action of noradrenaline may also be responsible for the muscle cell necrosis and plasma imbibition.

Like in the cases of fibrinoid necrosis of the vascular wall induced by painting with acid and by nephrogenic malignant hypertension [2, 3], in the present cases, too, a muscle cell necrosis in the vascular wall was the first change leading to fibrinoid necrosis. The intensive invasion with plasma, which finally led to the homogeneous vascular wall fibrinoid, ensued only later. In the early stage the necrosed muscle cells could still be distinguished by phosphotungstic acid haematoxylin even in the fibrinoid which appeared to be homogeneous by other methods of staining. Thus, the typical fibrinoid necrosis of the media is due to the combined effect of necrosed muscle cells, the plasma elements entering the vascular wall, and the rapid development of plasma imbibition. In the blood vessels, seriously damaged by painting with acid, in nephrogenic malignant hypertension, and in the present experiments alike, the inflowing plasma imbibed the environment of the blood vessel and fibrin was precipitated in it.

The differences from the fibrinoid vascular wall necroses in experimental nephrogenic malignant hypertension [3], i.e. the early disappearance of the birefringence of the necrosed muscle cells, the rapid imbibition with plasma indicative of a serious impairment of permeability, and the absence of subendothelial fibrinoid over the internal elastic membrane of the small blood vessels, beside the different features of this membrane, might be explained by a more acute, faster development of the process. We found subendothelial fibrinoid also in the larger uterine arteries running near the site of painting with acid [2]. Although painting with acid, just like the infusion of noradrenaline, represents an acute effect, the appearance of subendothelial fibrinoid may be explained by an increased resistance of the lamina elastica of the large uterine vessels.

Thus, fibrinoid necrosis is an acute form of damage to the small blood vessels of the muscular type, that develops in essentially the same way in response to the widest variety of acute interventions. Composition, site of appearance, and extent, of the fibrinoid are determined by the intensity of the intervention and by the structure of the vascular wall.

Acknowledgement: We are indebted to Mr. S. Kiss for the photographs.

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ÜBER DIE ENTWICKLUNG DER MIT NORADRENALIN HERBEIGEFÜHRTEN FIBRINOIDEN GEFÄSSWANDNEKROSE

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In den Kleingefäßen von Hundeherzen wurde die Entwicklung der nach Noradrenalin Infusion entstehender fibrinoiden Gefäßwandnekrose untersucht. Das erste, mit Lichtmikroskop wahrnehmbare Zeichen der zu Fibrinoidnekrose führenden Gefäßwandschädigung war die Nekrose der Muskelzellen der Media, die mit der gleichzeitig beobachteten, rasch progredierenden Plasmaimbibation zusammen die Entwicklung des typischen Fibrinoids resultierte. Anhand des Entwicklungsprozesses konnte festgestellt werden, daß die erwähnten Veränderungen mit den, in den vorangehenden Experimenten beobachteten, durch Säurepinselung und maligner Hypertonie herbeigeführten fibrinoiden Gefäßveränderungen übereinstimmen. Die Erscheinungsform beeinflußten lediglich die Intensität der Einwirkung und die Struktur der Gefäßwand.

ВОЗНИКНОВЕНИЕ ФИБРИНОИДНОГО НЕКРОЗА СОСУДИСТОЙ СТЕНКИ, ВЫЗВАННОГО НОРАДРЕНАЛИНОМ

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Авторы исследовали в малых сосудах сердца собак возникновение фибриноидного некроза сосудистой стенки, возникающего вслед за вливанием норадреналина. Первым признаком приводящего к фибриноидному некрозу поражения сосудистой стенки, видимым под микроскопом, было отмирание мышечных клеток средней сосудистой оболочки, которое вместе с быстро прогрессирующим пропитыванием плазмой привело к типичному фибриноидному изменению. Возникновение этого изменения соответствовало возникновению фибриноидных сосудистых изменений при смазывании кислотами и при злокачественной гипертонии. Форма проявления зависела от силы воздействия и от структуры сосудистой стенки.

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ÜBER DIE WIRKUNG DER BLEIPHOSPHATBEHANDLUNG AUF DIE NIERENEPITHELZELLEN VON ALBINORATTEN

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(Eingegangen am 14. Oktober, 1965)

Die auf Wirkung chronischer Bleiphosphatbehandlung in den Nieren von Albinoratten früh entstehenden zytologischen Veränderungen wurden untersucht. Die wichtigsten dieser Veränderungen waren Einschlußkörper in den Zellkernen des Nierenepithels. Diese Einschlußkörper waren im allgemeinen rund, eosinophil bzw. pyroninophil, von konzentrischer Struktur, ihre Größe übertraf die der Nukleolen, sie wiesen schwache PAS-Positivität und Feulgen-Negativität auf. Der Kern einzelner tubulärer Zellen war vergrößert, von unregelmäßiger Form und chromatinreich.

Außer dem gestörten Nukleinsäurestoffwechsel war auch das Plasma der tubulären Epithelzellen geschädigt. Die Schädigung führte zur Karyolyse; die Substanz der aufgelösten Zellen gelangte in das Lumen der Tubuli. In einigen Fällen traten die Einschlußkörperchen vom Kern in toto aus und gelangten vorerst in das Plasma, sodann in das Lumen der Tubuli.

Anstelle der massenhaften Zerstörung der Epithelzellen entstanden Hohlräume, deren Wand lediglich die Basalmembran der Tubuli auskleidete. Falls zwischen einigen dieser Hohlräume infolge des Membranzerfalls eine Kommunikation entstand, kam es zur Zystenbildung in der Nierenrinde.

Einleitung

Auf Wirkung von chronischer Bleivergiftung entstehen Nierenadenome. Im Zusammenhang mit diesen können wir den in den Nierenepithelzellen entstandenen Einschlußkörpern eine Bedeutung beimessen. Blackman [5] gab 1936 bekannt, daß Bleisalzzufuhr bei Menschen und Versuchstieren in den Epithelzellen der Nierentubuli eosinophile, Desoxyribonukleinsäure nicht enthaltende Kerneinschlüsse verursacht. Bei den Versuchstieren (Ratten, Mäusen, Meerschweinchen) erschienen diese Kerneinschlüsse bereits nach 9wöchiger Bleikarbonat-Behandlung. Über ähnliche Beobachtungen berichteten später auch Finner und Calvery [10], Fairhall und Miller [9], sowie Wachstein [21].

Zollinger der in seiner 1953 erschienenen grundlegenden Arbeit sowohl die tumorregende Wirkung des Bleiphosphats analysierte, als auch über die erwähnten intranukleären Gebilde eingehend berichtete, betrachtete diese Körperchen — im Einklang mit Tönz [19] der das Material von Zollinger 1957 nachprüfte — als pathologisch vergrößerte Nukleolen. Da diese Zelleinschlüsse auch hinsichtlich der Nierenadenome von Bedeutung sind, hielten wir ihre Untersuchung für wichtig.

Mit der Histologie der Kerneinschlüsse haben sich WOLMAN [22], BRACKEN und Mitarb. [6] sowie LANDING und NAKAI [14] eingehend befaßt. Aus ihren Mitteilungen geht hervor, daß die Einschlüsse Ribonukleoproteide, Lipoproteine und große Mengen einer SH-Gruppe enthaltenden Substanz — annehmbar Zystein — enthalten.

Die von PORTE und BATZENSCHLAGER [17], BEAVER [4] sowie KILHAM und Mitarb. [13] durchgeführten elektronenmikroskopischen Untersuchungen ergaben, daß die Kerneinschlüsse über eine Konzentrizität und von den Nucleolen abweichende Struktur verfügen.

Laut KILHAM und Mitarb. [13] können die Einschlüsse vom Kern austreten, in das Plasma, ja sogar in das Lumen der Tubuli gelangen. Dieses Verhalten der Einschlüsse geht mit Zellzerfall und -zerstörung einher.

GUEFT und MOLNÁR [11] fanden anläßlich der elektronenmikroskopischen Untersuchung der Leber von mit Bleiazetat behandelten Albinoratten, daß die charakteristische Schichtung der Einschlüsse auch in den Leberzellen in Erscheinung tritt; außerdem konnten sie auch die Schädigung der Mitochondrien nachweisen.

MÜLLER und RAMIN [15] stellten 1963 fest, daß die erwähnten intranukleären Gebilde teils im Zellkern entstehen, teils infolge Plasmaabschnürung in die Kernsubstanz gelangen.

DALLENBACH [8] konnte 1964 in den intranukleären Einschlüssen mit radioaktivem Bleiisotop Blei nachweisen, worauf er zur Feststellung gelangte, daß sich um das im Laufe der Bleivergiftung im Kern abgelagerten Blei Ribonukleinsäure anhäuft, was zur Störung des Ribonukleinsäurestoffwechsels führt. Die von MÜLLER und STÖCKER [16] mit ^{3}H-Zytidin und ^{3}H-1-Phenylalanin durchgeführten autoradiographischen Untersuchungen ergaben, daß die Kerneinschlüsse im Eiweiß — bzw. Ribonukleinsäurestoffwechsel nicht aktiv teilnehmen.

Eigene Untersuchungen

Versuchsmaterial und Methodik

Die Untersuchungen wurden an 100(80 Versuchstiere und 20 Kontrolltiere), durchschnittlich 160 g wiegenden, mit Standard-Diät gefütterten weiblichen Albinoratten durchgeführt. Den Versuchstieren wurden mit der von ZOLLINGER [23] empfohlenen Methode wöchentlich einmal unter die Rückenhaut 20 mg Bleiphosphat (in 1 ml physiologischer NaCl-Lösung suspendiert)injiziert. Da am Ende des 10. Behandlungsmonats bei der Mehrzahl der Tiere Gewichtsabnahme festzustellen war, wurde die Bleiphosphatbehandlung (20 mg) weiterhin nur 2 wöchentlich vorgenommen. Vom 12. Behandlungsmonat an nahmen die Tiere wieder zu, worauf die ursprüngliche Behandlungsweise zur Anwendung kam. Während der 18monatigen Behandlungsperiode erhielten die Tiere insgesamt 65 Bleiphosphat-Injektionen. Diejenigen Tiere, die bis zum Ende des Experiments am Leben blieben, erhielten eine Gesamtmenge von 1300 mg Bleiphosphat. Das letzte Versuchstier ging am Ende des 19. Monats ein. Die ersten beiden Ratten wurden am Ende des 3. Monats getötet, die übrigen Tiere gingen vom 180. Tag des Experiments spontan ein, bzw. wurden im Interesse der besseren Fixierungsmöglichkeit der Organe vor dem Tode dekapitiert. Die Organe der Tiere wurden im allgemeinen in Formalin, einige Nierenteilchen außerdem in Carnoy-Lösung fixiert. Die auf diese Weise angefertigten

Schnitte wurden demnach mit Hämatoxylin-Eosin gefärbt, sodann im Falle eines positiven histologischen Befundes mit Methylgrün-Pyronin, Feulgen, PAS, Best-Karmin, Mallory, Novelli, Elastica-van Gieson, Toluidinblau oder Ölrot gefärbt, sowie nach Gomori mit Silber impregniert.

Ergebnisse

In den Organen der an chronischer Bleivergiftung leidenden Tieren waren zahlreiche Veränderungen zu beobachten. Hier sei lediglich über die zytologischen Nierenveränderungen berichtet, die übrigen Befunde werden an einer anderen Stelle bekanntgegeben [3]. Unter den zytologischen Abweichungen fielen in erster Linie die intranukleären Gebilde auf (Abb. 1), die zuweilen bereits in den Nieren der am Ende des 3. Monats getöteten Tiere nachzuweisen waren (Abb. 2). Die Einschlußkörper kamen vornehmlich in den Zellkernen der proximalen und distalen gewundenen Kanälchen vor, sie waren aber auch in den Zellkernen der *Henle*schen Schleifen zu erkennen. Die Kerne der erwähnten Zellen waren vergrößert, das Chromatin lag teils der Kernmembran angelagert, teils umgab es die intranukleären Einschlüsse (Abb. 3—4).

Außer dem Einschlußkörper trat in zahlreichen Fällen auch ein Nukleolus in Erscheinung; dieser war manchmal von normaler Größe und Färbung, manchmal dagegen vergrößert. Diese Erscheinung konnte zuweilen auch in kerneinschlußfreien Zellen beobachtet werden. In diesem Zusammenhang sei auf die Meinung mehrerer hingewiesen, nach der die Kerneinschlüsse pathologisch vergrößerten Nukleolen entsprechen, die nicht mehr imstande sind, ihren Ribonukleinsäuregehalt in das Plasma zu entleeren.

Während die Färbungseigenschaften der Kerneinschlüsse denen der Nukleolen ähnlich sind — beide sind nämlich im allgemeinen acidophil — lassen sich bezüglich der Färbungintensität gewisse Abweichungen erkennen. Die Kerneinschlüsse färben sich oft wesentlich blasser als die Nukleolen, außerdem hängt die Färbung der Einschlüsse auch von ihrer Größe ab: kleinere Einschlüsse färben sich stärker, größere dagegen blasser, zuweilen finden sich jedoch auch große Einschlüsse, die eine intensive Färbung aufweisen. Die größeren Einschlüsse haben manchmal eine konzentrische Struktur (Abb. 5). Vereinzelt ließen sich mehrere Einschlüsse enthaltende Zellkerne beobachten.

Bezüglich der chemischen Struktur der Einschlüsse konnte mit Hämatoxylin-Eosin- und Methylgrün-Pyronin Färbung ihr Ribonukleinsäuregehalt nachgewiesen werden. Die PAS-Positivität der Einschlüsse war mit dem des Zellplasmas identisch, Best-Karmin- und Fettfärbung fielen jedoch negativ aus. Mit dem *Novelli*schen Verfahren färbten sich die Kerneinschlüsse, den Mitochondrien ähnlich, lebhaft rot. Toluidinblau führte eine blaßblaue Färbung des Plasmas und der Kerneinschlüsse herbei, Metachromasie war nicht nachzuweisen. Mit dem *Mallory*schen Verfahren ließen sich die Einschlüsse rot färben, mit van *Gieson* dagegen blaßgelb. Nach *Wachstein* [21] sind die auf Wirkung

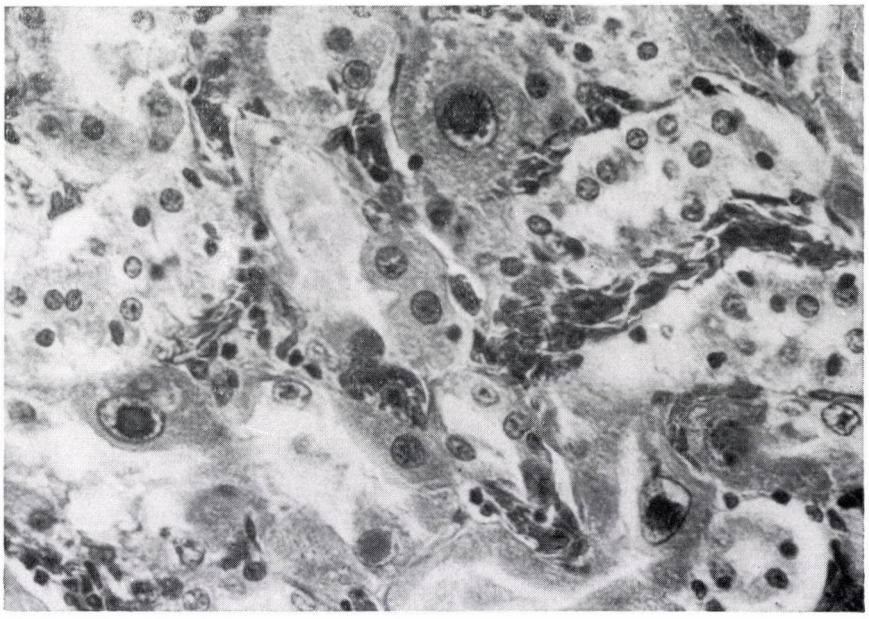

Abb. 1. Intranukleäre Einschlüsse in den tubulären Epithelzellen (Hämatoxylin-Eosin-Färbung 400 ×)

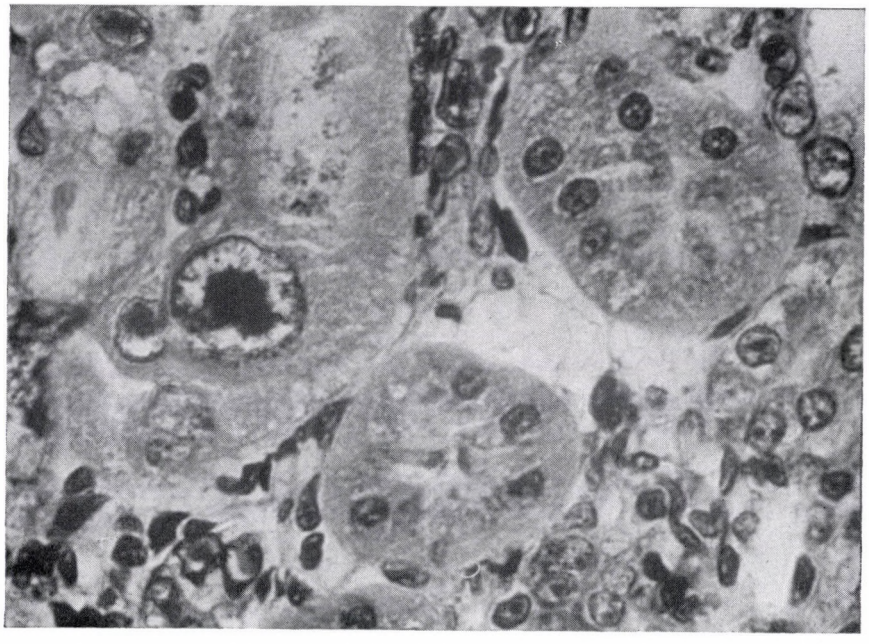

Abb. 2. Intranukleärer Einschlußkörper in der Frühphase des Experiments; an der anderen Seite zwei Nierenkanälchen mit intakten Epithelzellen (Hämatoxylin-Eosin-Färbung, 800 ×)

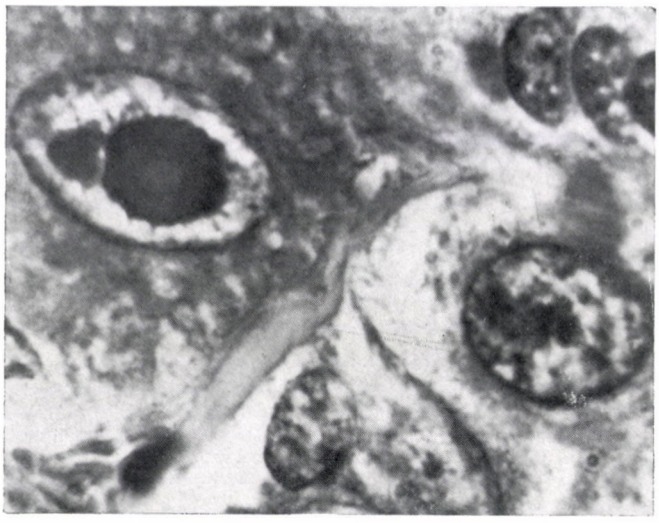

Abb. 3. Intranukleäre Einschlußkörper und Chromatinscholle enthaltende Nierenepithelzelle und Epithelzellen ohne Einschlüsse (Hämatoxylin-Eosin-Färbung, 1200 ×)

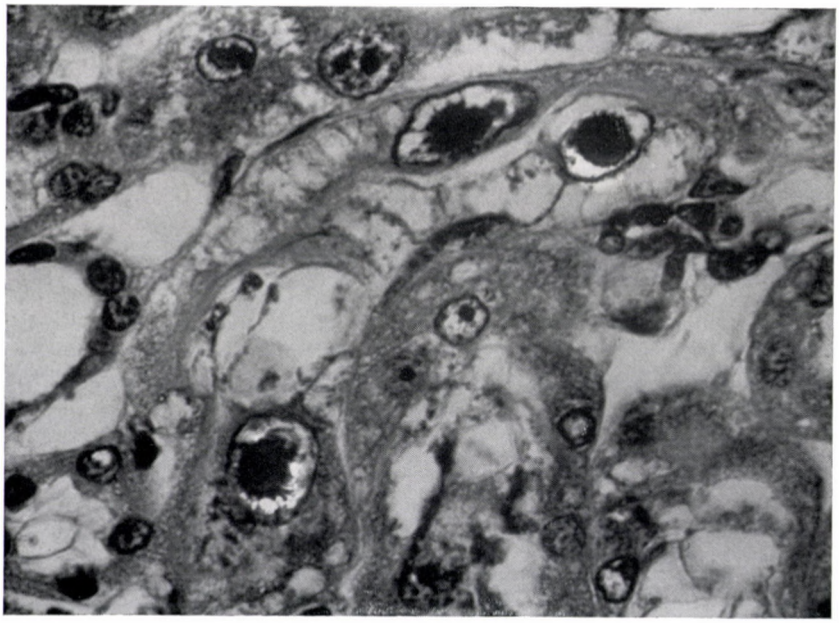

Abb. 4. In der Niere mehrere charakteristische Zelleinschlüsse (Hämatoxylin-Eosin-Färbung 800 ×)

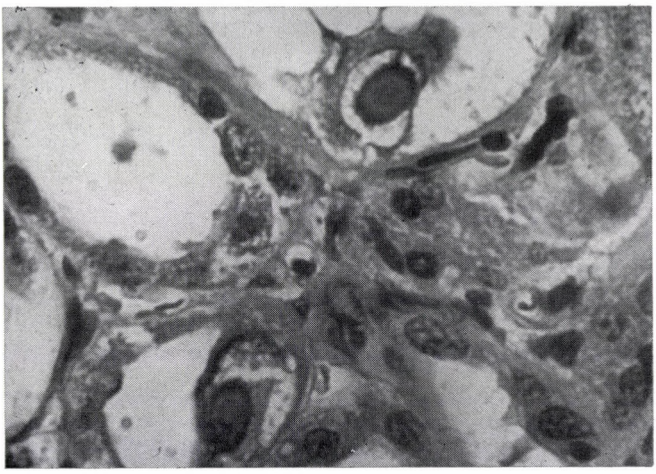

Abb. 5. In den tubulären Epithelzellen Einschlüsse von konzentrischer Struktur (Hämatoxylin-Eosin-Färbung, 400 ×)

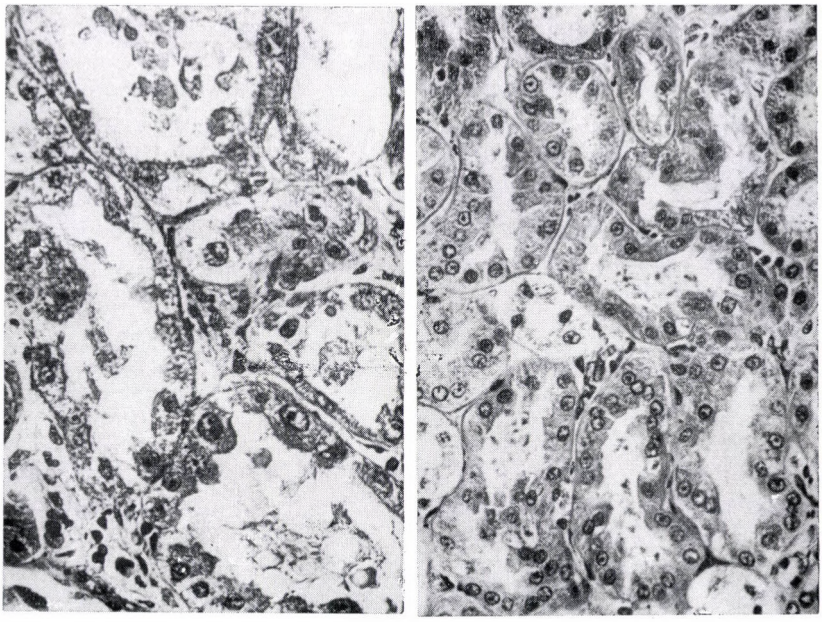

Abb. 7. Links, auf Wirkung von Bleiphosphatbehandlung degenerierte Nierentubuli; rechts, Kontrollpräparat. (Hämatoxylin-Eosin-Färbung, 100 ×)

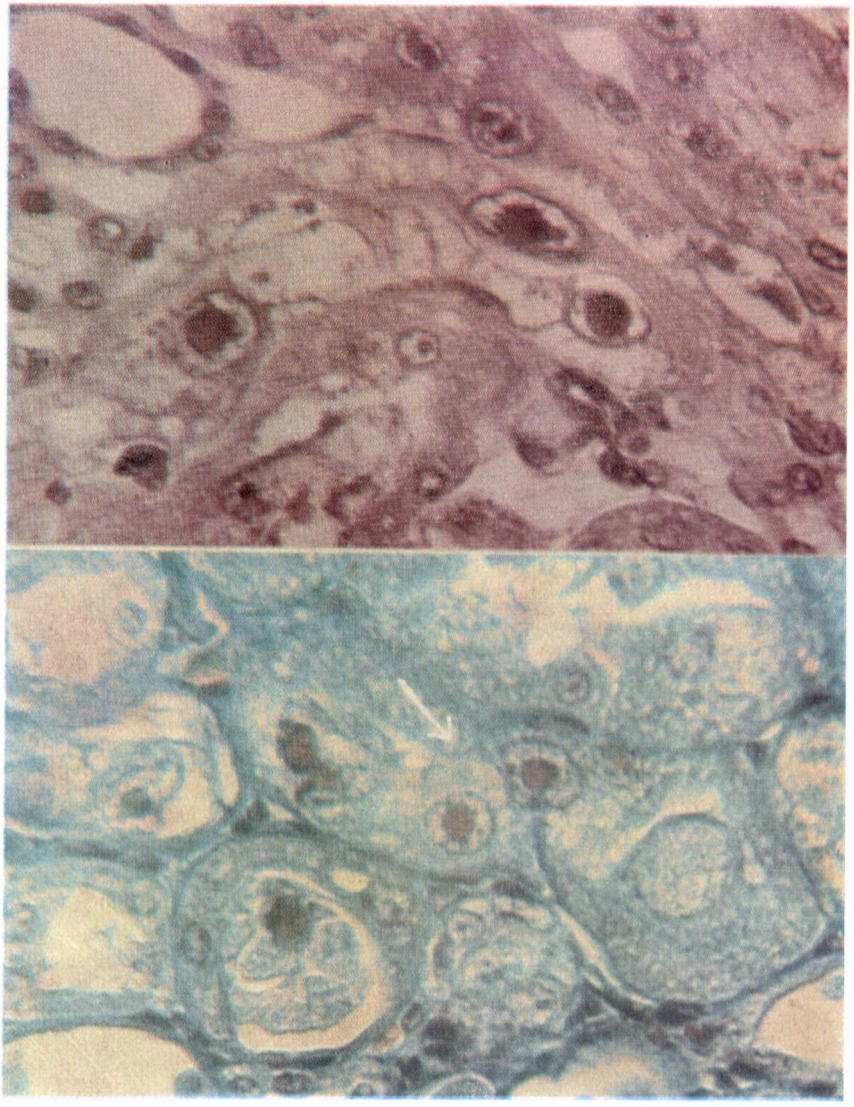

Abb. 6. Oben säurefeste Zelleneinschlüsse mit Ziehl-Neelsen-Färbung, unten Zelleinschlüsse mit Färbung nach Mallory (800 ×)

von Blei und Wismut entstandenen Kerneinschlüsse säurefest. Diese Beobachtung können wir bezüglich der Bleisalze bestätigen (Abb. 6). Auf Grund der *Feulgen*-Reaktion enthalten die Einschlußkörper keine Desoxyribonukleinsäure. Sowohl die Feulgen-Reaktion, als auch die Methylgrün-Pyronin Färbung wiesen darauf hin, daß die Chromatinsubstanz der Epithelzellkerne der Nierentubuli aufgelockert war.

In unserem Material ließen sich auch andere Nukleinsäurestoffwechselstörungen beobachten. In einigen Tubuli waren zerstreut vergrößerte Kerne von unregelmäßiger Form vorzufinden, deren Chromatingehalt das Mehrfache der Norm ausmachte. Einige dieser Zellen enthielten auch Kerneinschlüsse.

Zufolge dieser Befunde ergab sich die Frage, wie sich das weitere Schicksal der intranukleäre Gebilde enthaltenden Zellen gestaltet. Nach KENDREY [12] können die bei Thioazetamidvergiftung in der Leber zustandegekommenen großen Nukleolen vom Kern in toto austreten, ja sogar das Plasma verlassen und in die Gefäßlumina geraten. Auch wir beobachteten in einigen Fällen, daß die Kerneinschlüsse die Kernmembran durchdringen und in das Plasma gelangen; vereinzelt waren auch extrazellulär, im Lumen der Tubuli Gebilde zu beobachten, die aller Wahrscheinlichkeit nach Kerneinschlüsse waren.

Obwohl der angeführte Prozeß auch in unserem Material zu beobachten war, verschwinden die durch Bleivergiftung herbeigeführten intranukleären Gebilde — unseres Erachtens — üblicherweise nicht auf diesem Weg. Im allgemeinen kommt es vorerst zur Auflösung der Zellen, worauf der Kerneinschluß die Kernsubstanz um sich herum aufspreizt, was einen Plasma-bzw. Zellzerfall zur Folge hat.

In manchen Fällen beginnt der Plasmazerfall bereits vor der Karyolyse. Dieser Prozeß verläuft folgendermaßen: Die Plasmafärbung nimmt ab, die Plasmastruktur wird schaumig, zuweilen kommt es zur Vakuolenbildung; einige Plasmateilchen können in das Lumen der Tubuli gelangen, weshalb stellenweise fast bloßgelegte, große Einschlüsse enthaltende Kerne erscheinen. Infolge der Anhäufung des Ribonukleinsäuregehalts der Zellen im Kerneinschluß verliert das Plasma eine bedeutende Menge seines Ribonukleinsäuregehalts, was zur Schädigung bzw. zum Zerfall der Plasmastruktur führt.

Das durch den angeführten Mechanismus herbeigeführte Bild des Zelluntergangs war in unserem Material recht oft zu beobachten. Die Substanz der zerstörten Zellen gelangt in Form einer, zumeist azidophilen, vorerst körnigen, sodann homogenen Masse in die Tubuluslichtungen.

Anstelle der zahlreichen zerfallenen tubulären Epithelzellen bleiben Hohlräume zurück, deren Wand lediglich die Basalmembran der Tubuli auskleidet (Abb. 7). Infolge des Membranzerfalls kommunizieren diese Hohlräume allmählich miteinander, worauf stets größer werdende Höhlen entstehen. Auf diese Weise treten in der Niere vorerst mikroskopische, sodann

makroskopische Zysten in Erscheinung. Über unsere Untersuchungen bezüglich der Zystenentwicklung, der Tubulusepithelregeneration und der Tumorerzeugung wollen wir in einer anderen Mitteilung berichten.

Besprechung

Die primäre Bleiwirkung betrifft aller Wahrscheinlichkeit nach den Nukleinsäurestoffwechsel. Wie darauf die vornehmlich in der Pflanzenwelt durchgeführten Untersuchungen hinweisen, übt das Blei auf die Mitose eine Kolchicin-ähnliche Wirkung aus. Nach AHLSTRÖM [1] ist das Blei ein in der frühen Prophase wirkendes Mitosegift, das seine Wirkung durch eine Blockierung der SH-Gruppen ausübt. VENNER und ZIMMER [20] nahmen an, daß die Schwermetalle unmittelbar die Desoxyribonukleinsäure des Zellkerns schädigen. BÜCHNER und Mitarb. [7] vertraten die Ansicht, daß die primäre Schädigung der Karzinogene das Protoplasma betrifft. Die elektronenmikroskopischen Untersuchungen von TOTOVIĆ [18] ergaben, daß während die Schädigungen des Zytoplasmas bereits nach 1-2wöchiger Behandlung in Erscheinung treten, die charakteristischen Kerneinschlüsse sich nur in der 5.-6. Versuchswoche zeigen.

Anhand unserer Ergebnisse konnte festgestellt werden, daß auf Wirkung chronischer Bleiphosphatbehandlung in den Nierenepithelzellen der Albinoratten intranukleäre Einschlüsse entstehen, die laut ihren Färbungseigenschaften hauptsächlich Ribonukleinsäure enthalten; im Zustandekommen dieser Einschlüsse ist annehmbar auch der Umstand von Bedeutung, daß die Entleerung der nukleären Ribonukleinsäure in das Plasma gestört wird. Wir schließen uns der Meinung von ANGEVINE und Mitarb. [2] an, nach der die in chronischer Bleivergiftung entstehenden intranukleären Gebilde eine spezifische Antwort des Zellkerns bedeuten. Infolge des gestörten Nukleinsäurestoffwechsels kommt es in der Mehrzahl der Fälle zum Zellzerfall; die Vergiftung überleben annehmbar nur die in der Initialphase des Experiments beobachteten, bizarr geformten, monströsen Kerne, die im Laufe der Progression des Prozesses unter Umständen den Ausgangspunkt der Zellproliferation bilden können.

Die von einigen Verfassern beobachteten Zeichen, nach denen einzelne intranukleäre Einschlüsse infolge Plasmaabschnürung zustandekommen würden, waren in unserem Material nicht zu beobachten.

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EFFECT OF CHRONIC LEAD PHOSPHATE TREATMENT ON THE EPITHELIAL CELLS IN THE KIDNEY OF WHITE RATS

J. BALÓ, B. SZENDE and A. BAJTAI

Cytologic changes in the kidney of white rats due to chronic treatment with lead phosphate have been examined. Most characteristic among the early changes was the appearance of inclusion bodies in the nuclei of renal epithelial cells. These bodies were, as a rule, round, larger than the nucleoli, and many of them showed a concentric structure. They were eosinophile, weakly PAS-positive, and Feulgen-negative. The nucleus of some tubular cells was supernormal in size, irregular in shape and rich in chromatin.

Disorder in the metabolism of nucleic acids was accompanied by cytoplasmic degeneration in the tubular epithelial cells. The last phase of cellular damage was cytolysis. The substance of the dissolved cells emptied into the lumen of the tubules. It was observed in certain instances that the whole body of the inclusion emerged from the nucleus, passed into the cytoplasm and thence to the tubules.

Large-scale destruction of epithelial cells leads to the formation of cavities whose wall consists of the basal membrane of the tubules only. Several such cavities may intercommunicate and give thus rise to smaller or larger cysts in the renal cortex.

ДЕЙСТВИЕ ХРОНИЧЕСКОЙ ДАЧИ ФОСФОРНОКИСЛОГО СВИНЦА НА ЭПИТЕЛИАЛЬНЫЕ КЛЕТКИ ПОЧЕК У КРЫС-АЛЬБИНОСОВ

Й. БАЛО, Б. СЕНДЕ и А. БАЙТАИ

Авторы изучали цитологические изменения, возникающие под влиянием хронической дачи фосфорнокислого свинца в почках белых крыс, наблюдающиеся уже в ранней стадии опыта. Из этих изменений наиболее характерные внутриядерные включения, которые наблюдаются в ядрах клеток почечного эпителия. Эти включения имеют обычно круглую форму, они больше ядрышек, часто имеют концентрическую структуру, они эозинофильные или пиронинофильные, дают положительную реакцию на ПАШ и отрицательную реакцию Фельгена. Ядро некоторых тубулярных клеток больше привычного, неправильной формы и богато хроматином.

Наряду с расстройством обмена нуклеиновой кислоты и плазма клеток канальцевого эпителия претерпевает дегенеративные изменения. Поражение клеток приводит в конечном итоге к их растворению. Вещество растворившихся клеток опорожняется в просвет канальцев. Можно наблюдать, что ядерные включения целиком выходят из ядра в плазму и затем попадают в просвет канальца.

Вслед за гибелью большого количества эпителиальных клеток возникают такие полости, стенки которых образуются базальной перепонкой канальцев. Несколько таких полостей могут сообщаться между собой и таким образом в корковом веществе почек возникают большие или меньшие кисты.

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INNERVATION OF LYMPH VESSELS

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Innervation of lymph vessels and lymph capillaries in the mesentery of dogs and cats has been studied with the Gros-Schultze silver staining technique. The adventitia of larger (valvular) lymph vessels is supplied by a very fine nervous plexus which, being derived from large calibered myelinated fibres, can be identified as of sensory function. Smaller unmyelinated fibres of undoubtedly efferent autonomic function supply the muscular coat with a circular terminal plexus of particular density in the wall of the sinusoid enlargements corresponding to the region of the valves. The true lymph capillaries are lacking in any efferent nerve supply, but they are regularly in close attachment to specific kinds of sensory nerve endings.

The innervation of lymph vessels has recently again come into the focus of interest, mainly with respect to the possible influence on lymph flow of the nervous system. New information has been added to the classical findings of Tonkoff (1899) concerning the innervation of lymph nodes, and of DOGIEL (1897) and KYTMANOV (1901) on that of lymph vessels by the recent observations of GELLÉRT et al. (1955), KUBIK and SZABÓ (1955), RUSZNYÁK *et al.* (1957), WOLODIKO (1961), MELNIKOVA (1964) and BORISOV (1964). Most of these data concern larger lymph trunks, which appear to have nervous plexus arranged in several layers. Little is known of the innervation of lymph capillaries and the immediate postcapillary vessels.

LAWRENTJEW (1925) was the first to call attention to the close connexion between Pacinian bodies and lymphatics of the mesentery. Although an occasional close contact between Pacinian corpuscles and arteries as well as lymph vessels has recently been mentioned by KUBIK and SZABÓ (1955) and been observed in the course of the present investigations, the findings do not warrant any direct functional connection as there are many Pacinian bodies and other kinds of encapsulated endings at such parts of the mesentery which is lacking in both kinds of vessels.

Having studied for some years the functional anatomy of mesenterial lymphatics (VAJDA and TÖMBÖL, 1964; VAJDA and TOMCSIK, 1965), it seemed worth while to extend these investigations to their nerve supply, an account of which is presented below.

Materials and methods

Pieces of the mesentery of cats and dogs — preferably of lean animals — were excised after fixation in neutral formol, impregnated with silver according to Gros-Schultze and mounted *in toto*.

Observations

Larger lymph vessels. Nerve fibres that are seen to establish direct relations to lymph vessels having some muscular coating and valves, are either large myelinated fibres, or finer unmyelinated ones. The large fibres are running simply and unattached to any specific structure, whereas the unmyelinated ones reach the large lymph vessels by way of their supplying arterioles, the vasa vasorum lymphaticorum (Figs. 1 and 2). The myelinated fibres are losing their myelin sheath on reaching the lymph vessel and encircle it with spiral branches. The smaller branches appear to be embedded into a common Schwann sheath and are forming a loose plexus on the outer surface of the vessel (Fig. 3). The finer meshes of this plexus cannot be distinguished from the so called vegetative ground plexus (Fig. 4). The unmyelinated fibre strands having reached the lymph vessels distribute chiefly in the region of the valves and immediately centred. They penetrate into the layer between the muscular cells (Figs. 5 and 6), where their course is chiefly circular or rather parallel with the attachment of the valve. The number of terminal fibres and the density of the meshes decreases in central direction of the intervalvular segments. It is interesting to note (Figs. 7 and 8) that nerve fibres running longitudinally to the vessel have a straight, and those running transversally a rather tortuous, course. This might be in connection with the widening of the vessels in transversal direction during increased lymph production or stasis.

Lymph capillaries. Nerve elements can be observed also along the lymphatic capillaries especially in the postcapillary region, indicated by invaginations of the narrower distal portion into the wider central one. These invaginations have been described by VAJDA and TOMCSIK (1965) as characteristic for the transition zone between true lymph capillaries and vessels with true valves. Incomplete valves having but a single cup occur in this region, too. Nerve fibres can be observed in the neighbourhood of both invaginations and single cup valves (Fig. 9), where the first smooth muscle cells are to be found. There is no plexiform innervation around the true lymph capillaries (Fig. 10 and 11). Lacking in muscle cells or other contractile elements like perivascular cells, and having no true basement membrane, the lack of any efferent innervation could be expected. Strangely it is just this part of the mesenterial lymphatics which has particularly intricate relations with nerve endings.

Specific nerve terminals. Two kinds of obviously sensory endings can be distinguished, free and encapsulated ones. Unmyelinated fibres are giving rise to free terminals, whereas myelinated ones are ending in encapsulat-

ed terminations. All of these endings are strictly related to lymph capillaries whether they are in direct contact with the capillary wall (Figs. 12 and 16) or in their close neighbourhood (Figs. 13, 14 and 15).) Whether encapsulated or

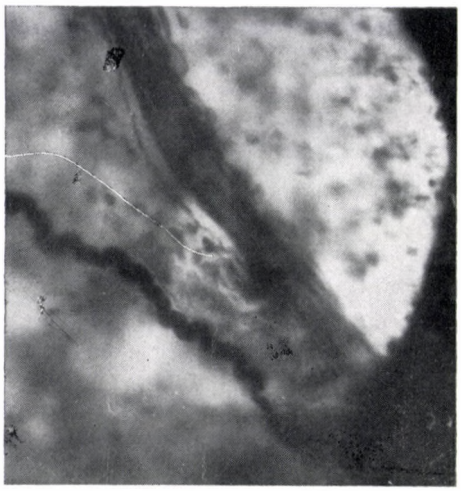

Fig. 1. Myelinated fibre on lymph vessel and unmyelinated fibres accompanying the vasa vasorum lymphaticorum

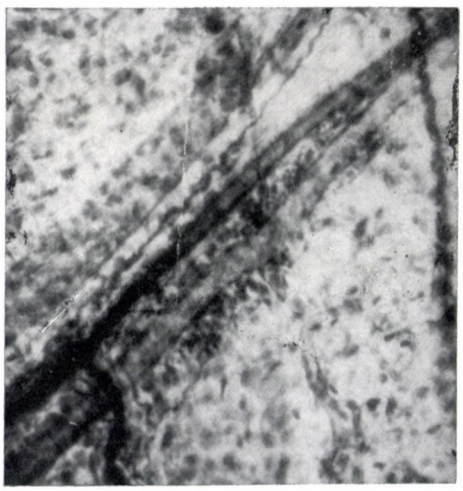

Fig. 2. Unmyelinated fibres accompanying the vasa vasorum lymphaticorum

free, the terminal part of the nerve fibre forms one or sometimes two loose tangels. Inside this the nerve fibre generally breaks up into three to four terminal branches, which after a more or less parallel course are wound up into ball-shaped or ovoid formations. At the endturns, loop formations are

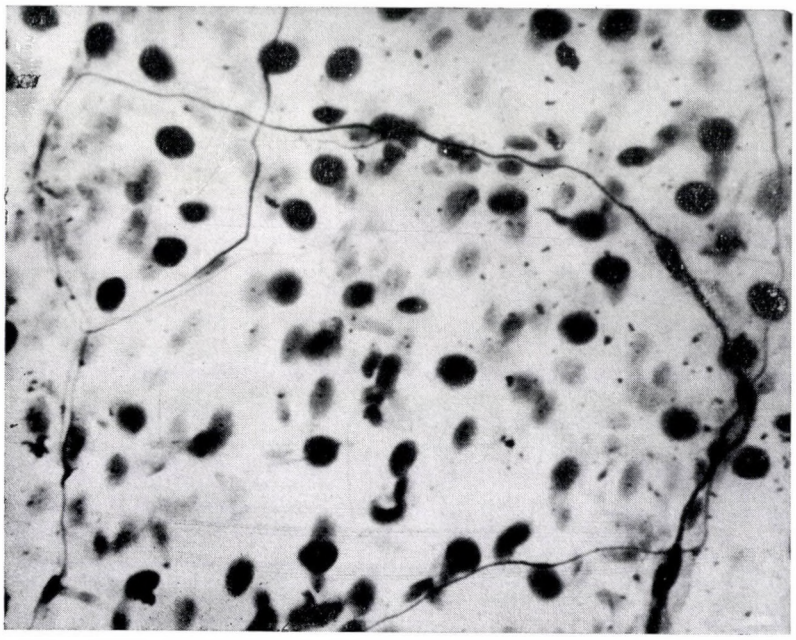

Fig. 3. Adventitial plexus on lymph vessel

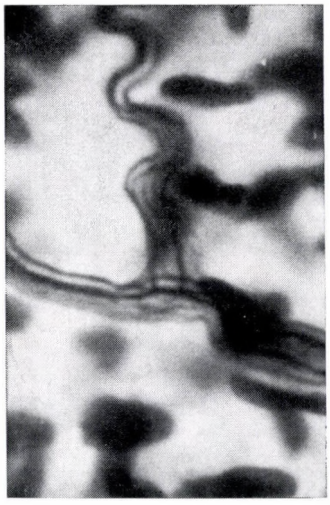

Fig. 4. Several thin nerve fibres embedded into a single Schwann cell strand

frequent. The encapsulated endings do not differ significantly with respect to the terminal course of the nerve fibre, but have less branches and are surrounded by a single layer of connective tissue cells.

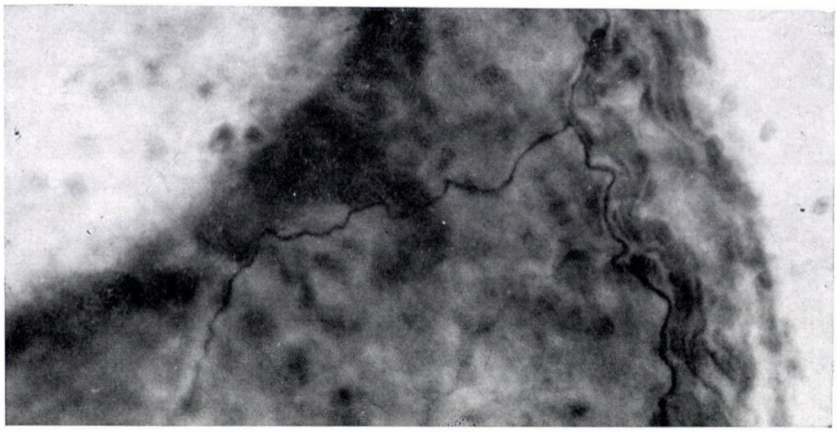

Fig. 5. Fibre plexus near the valve

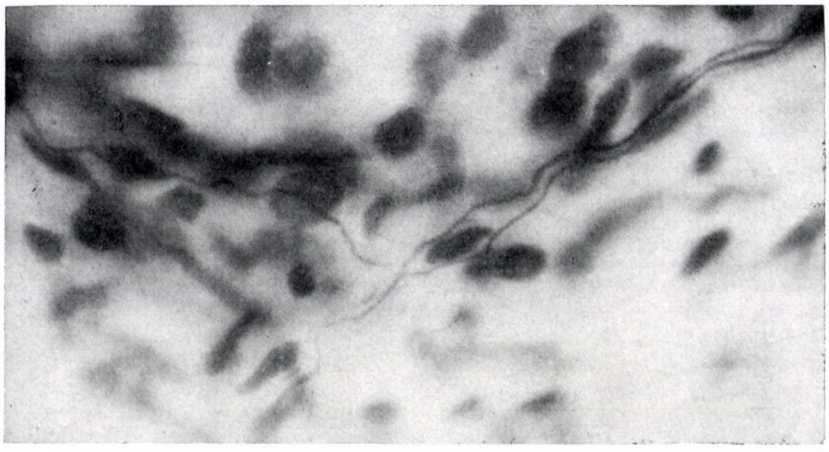

Fig. 6. Circular fibres at the valve

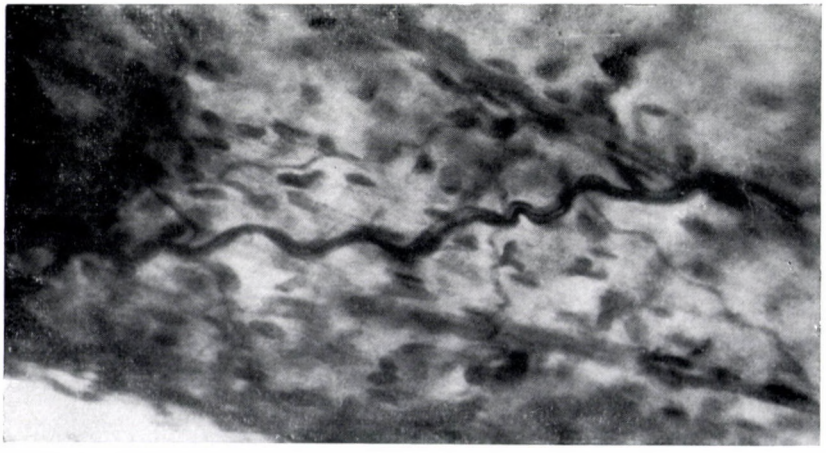

Fig. 7. Plexus of the portion adjacent to the valve

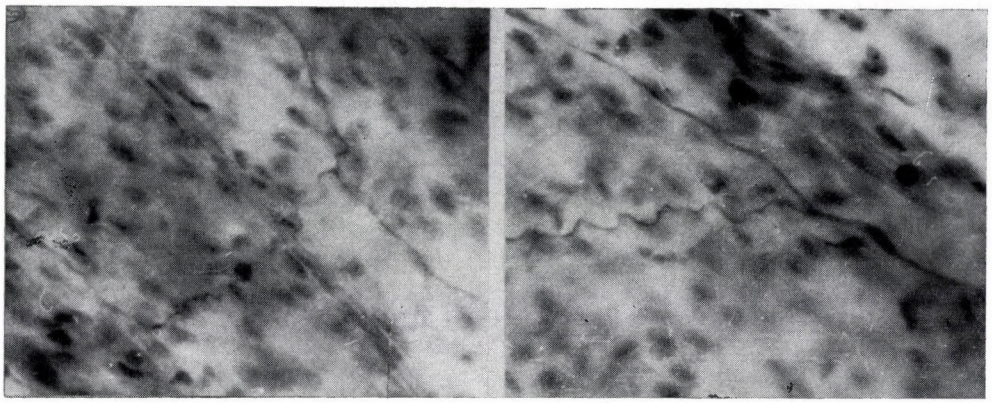

Fig. 8. Twisted fibres running transversally to the lymph vessel

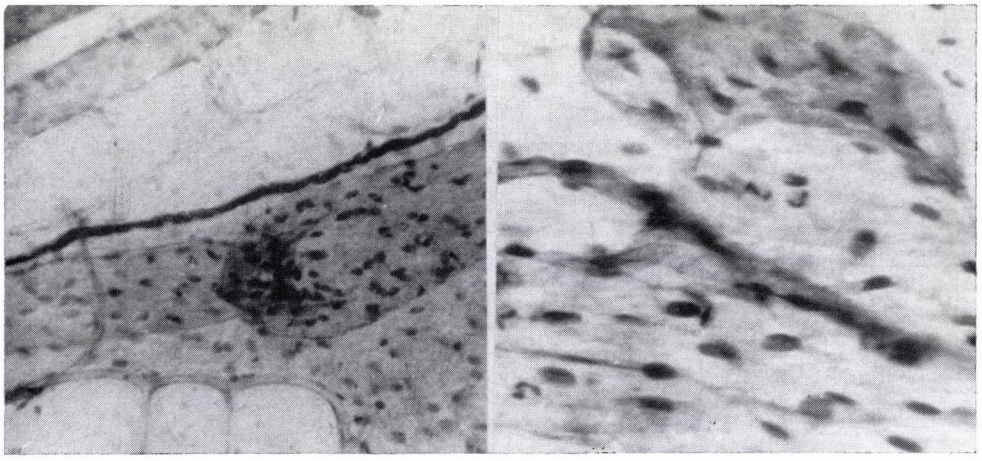

Fig. 9. Myelinated and unmyelinated fibres near invagination and interflow

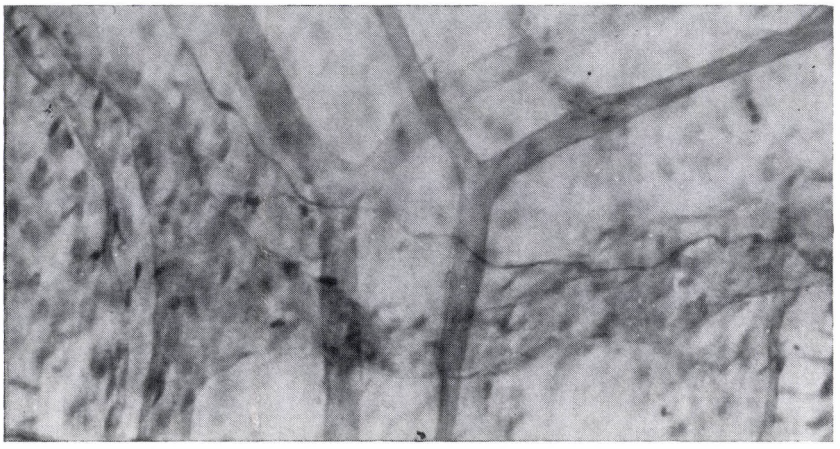

Fig. 10. Unmyelinated fibre following lymph capillary

tract to the reticular layer of the median eminence, and it accumulates in the stratum palissadicum (especially in its β zone), in the form of large globules and coarse granules. Appearance of large globules in the peripheral part of the stratum palissadicum, and the absence of neurosecretion from the stratum reticulare allow the inference that the median eminence of water-loaded birds becomes a depot without outlet towards the portal vessels. Seen under the microscope, the median eminence of pigeons looks much the same whether they have been loaded with water or treated with ACTH (PÉCZELY [25]).

It is probable that in birds water loading reduces the activity of both the neurohypophysis and the median eminence. This would mean that the aldehyde fuchsin-positive neurosecretion which finds access to the portal veins of the adenohypophysis via the median eminence acts as CRF. This would then confirm the results of earlier experiments in which ACTH treatment was applied (PÉCZELY [25]).

A comparison of the respective effects of dehydration and hydration allows to conclude to a correlation between the aldehyde fuchsin-positive neurosecretory substance and the hypothalamic secretion of CRF in the domestic pigeon. The existence of a normofunctional stimulatory mechanism has to be postulated which would act via the adenohypophyseal portal vessels of the median eminence, as proved by the sensitivity to ACTH of the neurosecretion contained in the median eminence as also by its accumulation under the effect of hydration. On the other hand, the observed effect of dehydration admits of the conclusion that — in the aphysiological condition provoked by intense osmotic stress — the large amount of Gomori-positive neurosecretion discharged by the pars nervosa, too, may induce a release of ACTH. This theory needs further confirmation.

The fact that the interrenal tissue of the adrenal gland of birds does not react uniformly to exogenous stimuli makes it probable that it consists of two layers: a peripheral part which secretes aldosterone, and a deeper one which produces glucocorticoids (KONDICS [13 to 16]). NaCl treatment of water deprivation evokes a uniform response from the entire neurosecretory system, whereas the reaction of the interrenal tissue is not uniform. NaCl treatment is followed by atrophy of the peripheral part and by a moderate hypertrophy of the deeper layer. Water deprivation induces hypertrophy in both layers. Hypothalamic CRF and adenohypophyseal ACTH are responsible only for the hypertrophy of the deeper interrenal cells, whereas the activity of the peripheral part must be regulated by some other factor. Further investigations will have to shed light on this factor.

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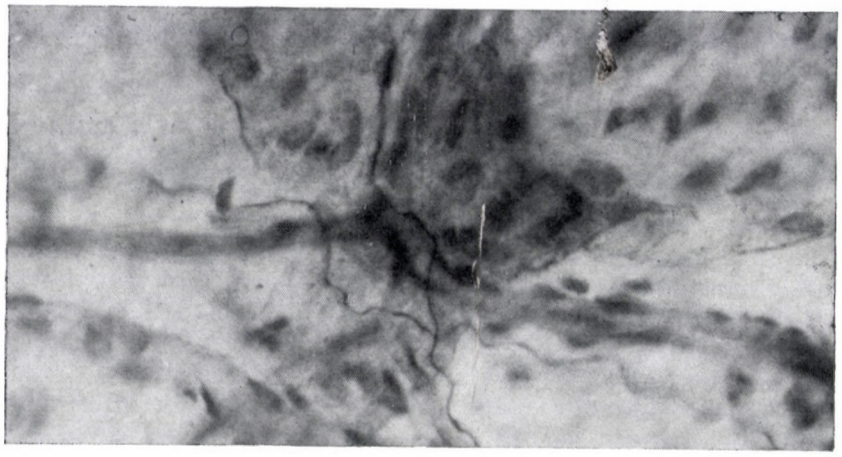

Fig. 11. Unmyelinated fibre following lymph capillary

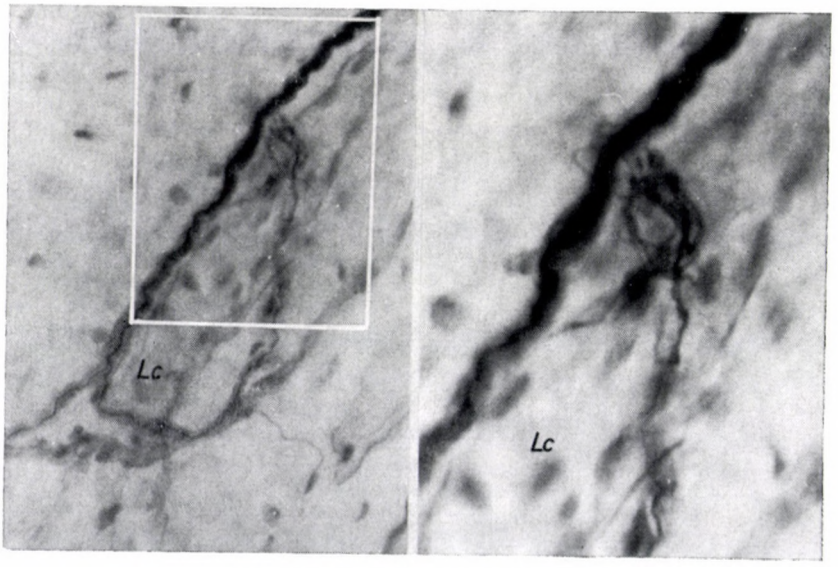

Fig. 12. Ending on lymph capillary

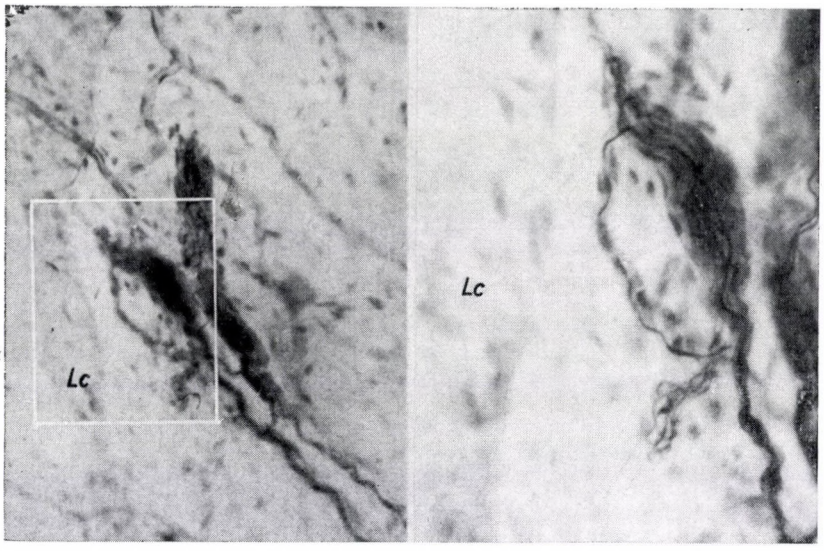

Fig. 13. Partly encapsulated ending near lymph capillary

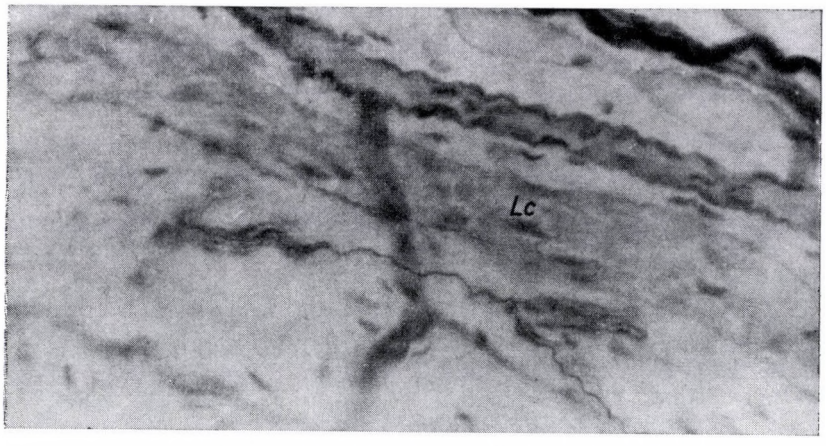

Fig. 14. Endings formed by paired, unmyelinated fibres

Discussion

The innervation of the larger- (valvular)-lymph vessels is shown somewhat diagrammatically in Fig. 17. Large myelinated fibres contribute only to the outer adventitial plexus of vessels (Fig. 17) [2]. The terminal branches of this plexus may be arranged inside a single Schwann cell in the same manner as in the so-called vegetative ground plexus, i.e. several preterminal nerve fibre are embedded into the same Schwann cell cytoplasmic stand. As practically all large myelinated fibres in the vegetative nerves can safely be considered

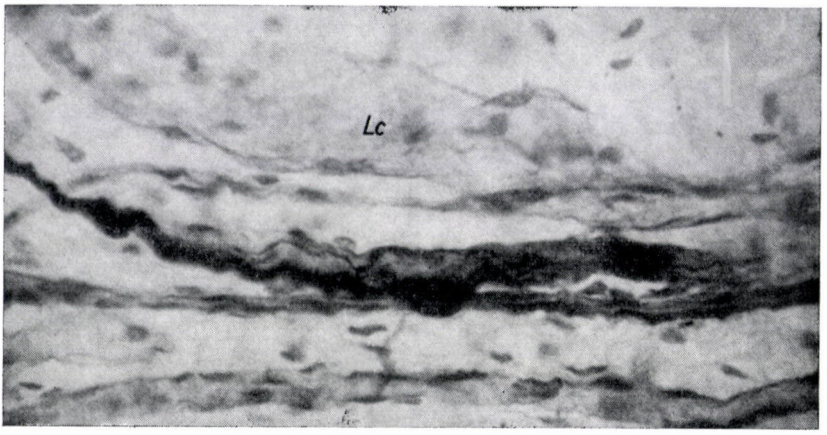

Fig. 15. Encapsulated ending of a myelinated fibre

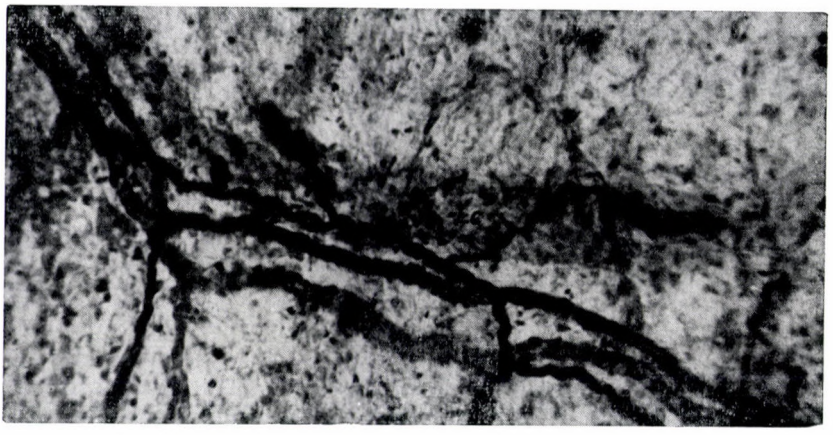

Fig. 16. Endings of paired encapsulated myelinated fibres

to originate from the spinal ganglia and having entered the vegetative nerves through the white communicating rami, the large myelinated fibres of the mesentery obviously belong to primary sensory neurons. The adventitial plexus of the lymph vessels — however fine — is clearly derived from such large myelinated fibres, so that there can be no doubt of their sensory nature. The smaller unmyelinated fibres that reach the lymph vessel together with the vasa vasorum and that penetrate into the muscle layer of the lymphatic, are obviously afferent and of vegetative nature. Their strong concentration at the sites of the sinusoid enlargements corresponding to the attachment of the valves and their decrease in more central regions of the intervalvar segment where there are less muscle cells, are also suggestive of the motor function of this plexus. Direct observation of the movement of lymphatic under the

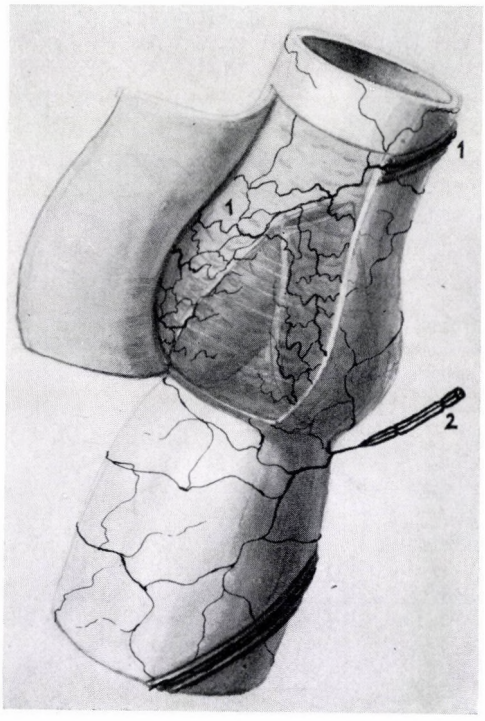

Fig. 17. Semidiagrammatic illustration of the innervation of a large lymph vessel
1) Plexus composed of unmyelinated fibres
2) Adventitial plexus

dissecting microscope also shows the predominantly contractile activity of the wall of the sinusoid enlargements. Under normal conditions the frequency of contractions at these sites is about 2—4/min. If on ligating the two vessels neighbouring the lymphatic under observation the supply of fluid increases, the frequency of contractions increases to 10—12/min. One might speculate, whether the unusually dense sensory plexus in the adventitia of the lymph vessel, or the numerous sensory endings in the close neighbourhood of the lymph capillaries, could serve as receptor of a specific reflex for the adjustment of lymph transport to requirements.

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ÜBER DIE INNERVATION DER LYMPHGEFÄSSE

J. VAJDA

Die mesenterialen Lymphgefäße und -kapillaren von Hunden und Katzen wurden mit dem Silberimpregnationsverfahren nach Gros-Schultze untersucht. Die Adventitia der größeren (Klappen enthaltenden) Lymphgefäße versorgt ein sehr feines Nervengeflächt, welches — hinsichtlich dessen, daß es aus einem großen, markhaltigen Nervenfaser entspringt — wahrscheinlich sensorisch funktioniert. Die Muskulatur der Gefäße innervieren die feinen, marklosen Nervenfasern, die zweifellos eine autonome efferente Funktion erfüllen. Diese letzterwähnten Nerven bilden ein zirkuläres Geflecht, welches bei den Sinusoiderweiterungen — die der Lokalisation der Klappen entsprechen — besonders dicht ist. Bei den echten Lymphkapillaren fehlt die efferente Innervation, diese sind jedoch in der Regel mit irgendeiner spezialer sensorischer Nervenendigung in enger Verbindung.

ИННЕРВАЦИЯ ЛИМФАТИЧЕСКИХ СОСУДОВ

Я. ВАЙДА

Автор исследовал лимфатические сосуды и лимфатические капилляры брыжейки скобак и кошек при помощи метода Гроса—Шульце с пропитыванием серебром. Адвентиция более крупных (обладающих клапаном) лимфатических сосудов осуществляется очень тонким нервным сплетением, которое, ввиду того, что оно происходит от крупных нервных волокон с миелиновым влагалищем — вероятно является сензорным. Меньшие, безмиелиновые нервные волокна, безусловно выполняющие автономную эфферентную функцию, иннервируют мышечный покров сосудов. Последние нервы образуют круговое сплетение. Сплетение особенно густое у синусоидных расширений, соответствующих расположению клапанов. В истинных лимфатических капиллярах отсутствует эфферентная иннервация,но они обычно связаны с окончанием какого-нибудь специального чувствительного нерва.

Dr. János VAJDA: Budapest IX., Tűzoltó u. 58., Hungary

RECENSIO

J. RAEKALLIO:

Die Altersbestimmung mechanisch bedingter Hautwunden mit enzym-histochemischen Methoden

1965. Verlag Max Schmidt-Römhild, Lübeck

The monograph, covering 120 pages and accompanied by 28 black and white photomicrographs and 2 tables, discusses the examination of cutaneous lesions by enzymatic-histochemical methods, and offers a detailed survey of the possibilities of distinguishing vital from postmortal injuries.

To fix the time of injuries and to ascertain their vital or postmortal origin is a prominent task of medico-legal activity, and current methods are often too rough for providing reliable findings in that respect.

RAEKALLIO has elaborated an enzymatic-histochemical procedure which allows to approach the problem early and thus with better prospects of success. Observations made in animals are compared with results obtained on human material with a view to establishing the criteria to be applied in medico-legal work.

The first part of the work deals with the phases of wound healing, the current methods of differentiating vital from postmortal injuries, and the techniques of examination — the pertaining literary data are briefly reviewed. Before going into details, a comprehensive review is presented of the enzyme-histochemical principles, in order to initiate morphologists who are not conversant with such methods.

The second part of the book contains a summary of the author's investigations into the behaviour of non-specific alkaline phosphatase, adenosine triphosphatase, beta glycuronidase, glucose-i-phosphatase, cytochrome oxidase, and succinodehydrogenase. Before evaluating and analysing the results, a detailed methodological information is offered. This chapter is highly instructive, as it discusses the usual methodological errors, and shows the way of avoiding them (e. g. selection of suitable substrates, pH optimum concentration of the reaction mixture, etc.).

It is suggested that the injury is postmortal or has been inflicted within an hour if both positive and negative enzymatic reactions are absent, whereas the presence of both reactions proves the intravital nature of the injury. The method is moreover suitable for the analysis of chronological factors. Compared with the controls, the activity of ATPase and non-specific esterase shows, for instance, a considerable decrease within an hour, but increases markedly after 4 and 6 hours. The monograph contains no information as to whether differences of activity are determined by quantitative histochemical measurements or a comparison of sections, but the reliability of the results has been verified by numerous control experiments.

The description of each enzymatic reaction contains numerous literary references, and in each chapter a detailed bibliography is given.

The concluding part of the work gives a summary of the results and their condensation in the form of tables, to prove that the procedure makes it possible to distinguish between vital and postmortal injuries even in the cases of one-hour survival, i. e. much earlier than with other routine methods.

The monograph is a good example of up-todate morphological analysis which may offer promising possibilites for the advance of medico-legal research.

E. SOMOGYI

Printed in Hungary

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INDEX

Morphologia Normalis et Experimentalis

ACTA HISTOCHEMICA

ZEITSCHRIFT FÜR HISTOLOGISCHE TOPOCHEMIE

Die Acta histochemica hat sich in den 10 Jahren ihres Bestehens zu einer bedeutenden Fachzeitschrift im Weltmaßstab entwickelt. Das wird nicht nur durch die Mitarbeit von bekannten Wissenschaftlern aus vielen Ländern, die dieses Fachblatt sehr zu schätzen wissen, immer wieder bestätigt, sondern auch dadurch, daß rund 90 Prozent der Abonnenten der Acta histochemica im Ausland zu finden sind. Die Zeitschrift bringt Beiträge in deutscher, englischer, französischer, russischer, spanischer und italienischer Sprache, dabei handelt es sich sowohl um Originalarbeiten als um zusammenfassende Darstellungen. (Farbige Tafelabbildungen werden ohne Druckkostenzuschuß veröffentlicht.) Eine besonders wertvolle Bereicherung stellt die regelmäßig beigegebene Bibliographia histochemica dar, die eine sonst nirgends erreichte Vollständigkeit aufweist.

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VEB GUSTAV FISCHER VERLAG JENA

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(Seit 1890)

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J. Botár

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(FROM F. DE CASTRO'S FOREWORD)

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Acta Morphologica Academiae Scientiarum Hungaricae Tomus 14 (3—4), pp. 211—226 (1966)

Institut für Morphologie der Bulgarischen Akademie der Wissenschaften
(Direktor: A. I. HADJIOLOFF), Sophia

ZUR BIOLOGIE DES SEXUALGEWEBES

II. IN-VITRO-ZÜCHTUNG DES SEXUALGEWEBES VON HÜHNEREMBRYONEN

J. JORDANOV, M. ANASTASSOVA-KRISTEVA, A. I. HADJIOLOFF
und A. BOYADJIEVA-MICHAILOVA

(Eingegangen am 23. September, 1965)

Das Verhalten des weiblichen Sexualgewebes (Geschlechts- und Satellitzellen, Keimepithel) von Hühnerembryonen wurde in Gewebekultur untersucht. Es wurde eine Methodik angewandt, die das Fibroblastenwachstum inhibiert, das epithelartige Wachstum der Eierstockgewebe fördert und eine gute vitale Beobachtung unter dem Phasenkontrastmikroskop gestattet. Die Untersuchungen bestätigten das epithelartige Wachstum der Satellitzellen und des Keimepithels. Diese Elemente des Eierstocks zeigen in Kulturen manche typischen Eigenschaften, die sie vom banalen Epithel unterscheiden und dazu veranlassen, sie der Gruppe der sogenannten Coelodermalgewebe (CHLOPIN) zuzuordnen. Das Schicksal der echten Geschlechtszellen in der Kultur ist je nach den Bedingungen, unter die sie bei der Explantation geraten, verschieden. Es wird einwandfrei festgestellt, daß die Geschlechtszellen in vitro durch mitotische Teilung proliferieren können. Dies ist der Fall, wenn die Geschlechtszellen in den Frühstadien der Differentiation sind (Ovogonien), und wenn sie die Möglichkeit haben, unter einer Schutzmembran zu wachsen, die von den Satellitzellen gebildet wird. Fehlt eine dieser Bedingungen, dann degenerieren die Geschlechtszellen, evtl. nach kürzerem oder längerem Überleben. Die Satellit- und die Geschlechtszellen zeigen auch manche gemeinsamen Eigenschaften in ihrem Verhalten in Gewebekultur: Proliferation in Form von Strängen und Membranen, Wachstum auch bei beschränktem Sauerstoffzutritt, Bildung von Symplasten. Diese Tatsachen sprechen zugunsten ihrer morphofunktionellen, wahrscheinlich auch genetischen Einheit und rechtfertigen die Auffassung, daß sie zwei Elemente eines einheitlichen Grundgewebes, des Sexualgewebes sind.

Die ersten Untersuchungen über das Wachstum der Gonadengewebe in vitro datieren aus der Zeit der Einführung der Gewebezüchtungstechnik. Die ersten Versuche in dieser Richtung führte 1913 MACCABRUNI (zit. OLIVO, 1934) aus. In jener Zeit sind Untersuchungen auch von anderen Autoren veröffentlicht worden,* doch gelten heute noch die 1938 von MURATORI ausgesprochenen Worte, daß man zu keinen definitiven Resultaten gelangt sei.

Je nach dem in vitro beobachteten Wachstum der Gonadengewebe lassen sich die von den verschiedenen Autoren dargelegten Angaben in sechs Gruppen einteilen (Tabelle I). Wenn man von der ersten Gruppe absieht, in der nur eine Bindegewebeproliferation, eventuell von Makrophagenmigration begleitet, festgestellt wird, beschreiben alle anderen Autoren auch ein epithelartiges Wachstum in Form von Strängen und Membranen. Die einzelnen Forscher weisen auf

* Die vorliegende Arbeit betrifft das histotypische Wachstum der Gonaden und hat nicht unmittelbar mit den Untersuchungen des sogenannten organotypischen Wachstums in Gewebekulturen zu tun, die mit den recht präzisen Untersuchungen von MARTINOVITCH (1938) ihren Anfang nahmen.

das Vorherrschen des einen der beiden Wachstumstype hin, meist des bindegewebigen Wachstumstyps. Was den anderen Wachstumstyp betrifft, so hält die größere Zahl der Autoren ihn für das Wachstum eines banalen, unspezifischen Epithels, wenn sie auch auf manche morphologisch-physiologische Eigentümlichkeiten desselben hinweisen. Am besten sind letztere in den Arbeiten von MICHAILOW (1937) und KOLESNIKOWA (1940) beschrieben. Diese Autoren fanden, daß die epithelartige Formationen der Gonaden in vitro von den Satellitzellen (Sertolischen und Follikularzellen) herrühren und ordnen diese, entsprechend den Angaben und der Auffassung ihres Lehrers CHLOPIN, den Coelodermalgeweben zu, zu denen auch die Mesothelien gehören.

Am wenigsten geklärt und bis heute umstritten ist die Frage des Verhaltens der echten Geschlechtszellen (Gonoblasten und Gonozyten) in vitro. Während manche Autoren, wie aus Tabelle I ersichtlich, eine Degeneration dieser Elemente feststellen, beschreibt eine kleinere Gruppe von Autoren eine aktive Migration und sogar progressive Erscheinungen der Geschlechtszellen, wie z. B. mitotische Aktivität, die Bildung vielkerniger Symplasten oder eine gewisse Differenzierung. Nur zwei Forscher, MURATORI und SMITH (1958) beobachteten eine Proliferation der Geschlechtszellen in vitro. Die Versuche des erstgenannten sind jedoch von recht kurzer Dauer, während die Beschreibung des zweiten Autors nicht systematisch genug und nicht überzeugend ist. Sie erwähnen nicht, daß epithelartige Membranen auch von den Satellitzellen sowie vom Keimepithel herrühren können, was bereits mehrfach von anderen Forschern festgestellt worden ist. Einen besonderen Platz nehmen die Untersuchungen von CHAMPY und MORITA (1928) und MENDELSOHN (1937) ein, die das Keimepithel als indifferentes Ausgangsstadium der Geschlechtszellen auffassen und daher die epithelartigen Membranen für eine Derivation der undifferenzierten Geschlechtszellen halten. CHLOPIN (1946) hält eine Proliferation der Geschlechtszellen in vitro für wenig wahrscheinlich.

Wir haben uns daher die Aufgabe gestellt, die Herkunft und das Verhalten der in vitro epithelartig wachsenden Eierstockgewebe vom Huhn zu verfolgen, da, wie aus Tabelle I ersichtlich, die meisten widersprechenden Angaben hierüber vorliegen.

Material and Methodik

Benutzt wurde Material von Eierstöcken 7—21 Tage alter Hühnerembryonen. Meist haben wir Fragmente von der Eierstockrinde 12 bis 16 Tage alter Embryonen gezüchtet; wie aus früheren Untersuchungen (HADJIOLOFF und KRISTEVA, 1962) bekannt, ist die Anzahl der Ovogonien an diesen Tagen bedeutend größer, und die Ovogonien, mit den künftigen Satellitzellen vermischt, bilden runde oder ovale Nester im Cortex des Organs; zudem ist die Differenzierung der Ovogonien, die sich speziell durch den meiotischen Vorgang des Zellkerns ausdrückt, wie schon d'HOLLANDER (zit. MURATORI und BATTAGLIA) festgestellt hat, entweder noch nicht eingetreten, oder sie hat kaum begonnen; dies könnte für die eventuelle Proliferation der Geschlechtszellen in vitro günstig sein. In manchen Fällen wurde das Organ vorher eine halbe Stunde bei 38 °C mit 0,25 bis 0,5% Trypsinlösung (Trypsin

Tabelle I

	Beobachteter Wachstumstyp	Untersuchungsobjekt	Autor
I	Wachstum von Fibroblasten (oder Epithelzellen, die sich in Fibroblasten verwandeln).	Eierstock von menschl. Embryonen	*Maccabruni*, 1913. zit. *Olivo*
		Follikelepithel vom Eierstock einer Frau	*Mjassojedoff*, 1925
		Hoden von Säugetieren	*Esaki*, 1925. zit. *Muratori*
		Eierstock einer Frau	*Olivo*, 1934.
II	Wachstum von Fibroblasten und banalem Epithel; Degeneration der Gonoblasten evtl. nach kurzem Überleben oder gewisser Differenzierung.	Eierstock von 4—8monatigen menschl. Embryonen und einer Frau	*Wolff* und *Zondek* 1925.
		Gonaden von 8—19tägigen Hühnerembryonen.	*Fano* und *Garofolini*, 1928.
		Eierstöcke von neugeborenen Nagetieren und von menschl. Embryonen	*Ulesko-Stroganova*, 1935.
		Eierstöcke von 10—12tägigen Hühnerembryonen	*Galgano*, 1938
		Hoden und Eierstöcke von kleinen Ratten	*Dux*, 1939.
III	Wachstum von Fibroblasten und spezifischen zur Coelodermal Gruppe gehörenden Epithel; Degeneration, evtl. gewisses Überleben der Geschlechtszellen.	Hoden von 1—30 Tage alten Kaninchen	*Michailow*, 1937.
		Eierstöcke von 1 bis 26tägigen und erwachsenen Kaninchen	*Kolesnikowa*, 1940.
IV	Wachstum von Fibroblasten und Epithel; aktive Migration der Geschlechtszellen, evtl. von progressiven Erscheinungen begleitet: Mitosen, Differenzierung, Bildung von Symplasten.	Gonaden von 3½tägigen Hühnerembryonen	*Dantschakoff*, 1932.
		Hoden von Hühnerembryonen, Kücken und Hähnen	*Wermel*, 1933.
		Hoden von erwachsenen weißen Ratten	*Chruščov* und *Diomidova*, 1937.
V	Wachstum von Fibroblasten und Germinativepithel (im Sinne undifferenzierter Geschlechtszellen).	Hoden von jugendlichen Kaninchen, Eierstöcke von erwachsenen Kaninchen, Hoden von Hähnen u. a.	*Champy* und *Morita*, 1928.
		Hoden von erwachsenen Kaninchen	*Mendelsohn*, 1937.
VI	Wachstum von Fibroblasten und Geschlechtszellen in Form von epithelialen Strängen und Membranen.	Eierstöcke von 5—21täg. Hühnerembryonen und Kücken	*Muratori*, 1937.
		Eierstöcke von 11—17täg. Hühnerembryonen	*Battaglia*, 1958.
		Hoden von 16—17täg. Hühnerembryonen und Hähnen	*Smith*, 1958

»Difco«) behandelt, was die Erhaltung kleinster Rindezellenkomplexe, wie auch Zellsuspensionen ermöglichte. Außer den bekannten Züchtungsmethoden in hängenden Tropfen nach Maximow, in Carrelschen Flaschen und auf Deckgläsern in flüssigem Medium auf dem Boden von Petrischalen, haben wir noch folgende angewandt:

I. Verfahren. Eine Modifikation der Maximow-Methode. Das Deckglas, auf dem 4 bis 5 Gewebestückchen in dünner Hühnerplasmaschicht explantiert sind, wird, mit den Explantaten nach unten, direkt auf einen flüssigen Nährboden in der Exkavation des Objektträgers gelegt (Abb. 1. oben). Alle 3—4 Tage wird das Deckglas in einen anderen hohlgeschliffenen Objektträger mit frischer Nährlösung übertragen. Die Explantate werden von der Bodenseite des Objektträgers aus beobachtet. Vor dem Übertragen der Kultur kann man sie bei stärkeren Vergrößerungen untersuchen. Zu diesem Zweck wirft man das Deckglas

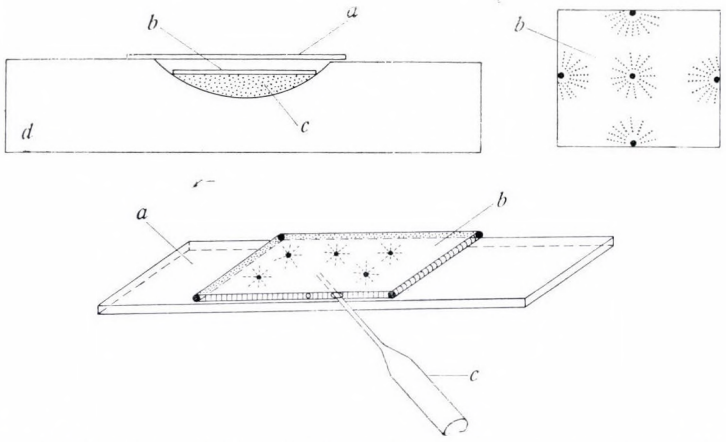


Abb. 1. Oben: — I. Verfahren. Hohlgeschliffener Objektträger (*d*), Deckglas mit den Explantal ten (*b*) und Glimmerplättchen (*a*), Nährboden (*c*). Unten: — II. Verfahren. Kammer au- Objektträger (*a*) und Deckglas (*b*). Pasteurpipette (*c*) zur Einführung des Nährbodens

mit einer Ruckbewegung auf die Glimmerplättchen (Lage wie beim Maximow-Verfahren) wonach man die Kultur von der Glimmerseite aus steril und ohne Gefahr raschen Eintrocknens beobachten kann.

Dieses Verfahren schafft für das Fibroblastenwachstum ungünstige mechanisch-physikalische Bedingungen: dünnes Koagulum, Oberflächenkontakt. Der begrenzte Sauerstoffzutritt an die Explantate hat sich gerade für die epithelartige Proliferation günstig erwiesen.

II. Verfahren. Eine Modifikation der mykologischen Züchtungskammer von GRIGOROW (1955). Die Kammer besteht aus einem Deckglas (24 × 30 mm) und einem Objektträger. Die Gewebestückchen werden vorher auf das Deckglas gelegt, auf dem eine dünne Plasmaschicht ausgestrichen ist. Auf den Objektträger werden 4 (3 mm hohe) Paraffintropfen aufgetragen, auf die man das Deckglas (mit den Explantaten nach unten) auflegt. Der schmale Zwischenraum zwischen den beiden Gläsern wird mit Paraffin ausgefugt. Mit einer Pasteurpipette wird die Nährlösung durch ein in die Paraffinfuge eingestochenes Loch in die Kammer eingeführt (Abb. 1. unten). Das Wechseln der Nährlösung geschieht alle 2—3 Tage. Die ideale Beobachtung im Phasenkontrast, die dieses Verfahren darbietet, ist sehr wertvoll; die Geschlechtszellen, wie auch andere Autoren (MURATORI, BATTAGLIA, SMITH) hervorheben, zeigen bei Phasenkontrast charakteristische Merkmale, die sie von anderen Zellarten unterscheidet.

Als Nährboden verwenden wir: 20% Kalbs- oder Hühnerserum, 25% proteinhaltiges Dotterdialysat ColD und das übrige — proteinfreies Dotterdialysat CelD (die Dialysate nach JORDANOV, 1960, gewonnen) mit keinem oder kleinem (1—2%) Embryonalextraktzusatz. Außerdem wurden der Nährlösung Penicillin (200 E/ml) und Streptomycin (200 γ/ml) zugesetzt.

Viele Kulturen wurden fixiert, gefärbt und als Totalpräparat beobachtet. Zum Fixieren verwendeten wir Carnoy, Serra (mit 4%igem Formol), 10% neutrales Formol u. a. Gefärbt

wurde mit Hämatoxylin nach Böhmer, Carazzi, Meyer, manchmal mit Sudan III kombiniert; angewandt wurden auch die Färbung nach Pappenheim, die Methylgrün-Pyronin-Methode nach Unna-Pappenheim u. a.

Ergebnisse

Unsere Beobachtungen an Eierstockexplantaten, die in Plasmakoagulum nach den klassischen Methoden gemacht wurden, bestätigten die Ergebnisse der Autoren, die sich derselben Methoden bedienten. Dem Fibroblastenwachstum in der Tiefe des Koagulums folgt vom 2—3. Tag nach der Explantation eine Proliferation epithelartiger Stränge und Membranen, und zwar hauptsächlich auf der Oberfläche des Koagulums und des Deckglases (Glimmer). Da die Kultur ein Gemisch von beiden Elementen darstellt, ist die Beobachtung jedes Elements für sich nicht einfach. Mit der Vergrößerung und Verdichtung der Wachstumszone in den nächstfolgenden Tagen wird die Beobachtung dadurch erschwert, daß manche morphologische Unterschiede zwischen den beiden Gewebearten immer mehr verblassen. Bei der Züchtung kleiner Zellkomplexe, die durch Trypsinisieren erhalten worden sind, lassen sich die epithelartig wachsenden Gewebe leichter von den Fibroblasten differenzieren. Immerhin stellt die Kultur auch hier ein Gemisch dar. Noch schwieriger ist es, das Schicksal der Geschlechtszellen — Ovogonien und Ovozyten — nach den klassischen Züchtungsmethoden zu verfolgen.

Etwas andere Resultate und Beobachtungsbedingungen erhält man nach den von uns angewandten Verfahren I und II bei geringem Luftzutritt. Das typische epithelartige Wachstum beginnt in diesem Fall schon am Tag nach der Explantation, während das Fibroblastenwachstum stark inhibiert ist. In den ersten Tagen bilden sich multiple Proliferationen in Form von Strängen und Membranen. Im Ablauf einer Woche erreicht die Kultur ihre maximale Größe von 6—7 mm Durchmesser. Sie ist immer noch dünn genug, um eine gute vitale Beobachtung bei Phasenkontrast zu bieten.

Im großen und ganzen haben wir die typischen Eigenschaften des epithelartigen Gonadenwachstums beobachtet. Die Proliferation beginnt mit Strängen von ziemlich breiten, flachen, länglich-dreieckigen oder rhomboidalen Zellen, die mit ihren Rändern engen Kontakt unterhalten. Die Zellkerne sind ovoid, mit einem oder zwei runden, stabförmigen oder hieroglyphenähnlichen Nukleolen. Die Mitochondrien sind im Zytoplasma verstreut. Die Zellstränge anastomosieren, indem sie kleine leere ovale Felder — »Fenster« — begrenzen. Mit dem fortschreitenden Wachstum verwandeln sich die dem Explantat proximalen Bezirke der Wachstumszone im allgemeinen in eine ganze Membran, während der periphere Rand die beschriebene Strangstruktur beibehält (Abb. 2). Auch in unserem Fall waren der Zellpolymorphismus, die Lockerung der peripheren Zone und in den späteren Tagen auch die häufige Trennung von Zellinseln sowie die Bildung vielkerniger Symplasten charakteristische Er-

scheinungen. In den ersten Tagen des Wachstums ist die Art der Fetteinschlüsse in der Kultur sehr typisch. Die Zellstränge erscheinen unter dem Mikroskop als würden sie von feinen, stärker lichtbrechenden Körnchen besprengt sein, die an dem einen oder an beiden Polenden des Zellkerns liegen. Vereinzelte, sehr große, neben dem Zellkern liegende Fettropfen lassen sich schon in den ersten Tagen der Züchtung entdecken. Sie hängen, wie CHAMPY und MORITA, später auch MICHAILOW bemerken, mit einer Phagozytose von degenerierenden Geschlechtszellen im Explantat zusammen.

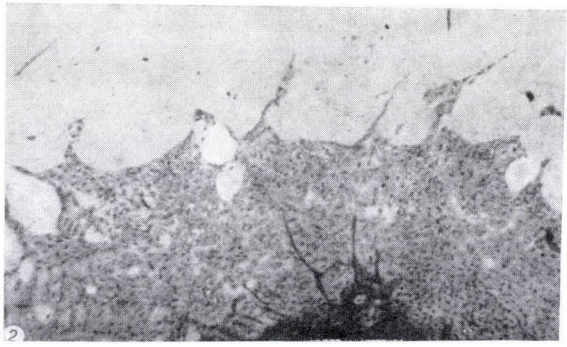


Abb. 2. Sechstägige Kultur von Eierstockcortex eines 17tägigen Hühnerembryos (nach dem I. Verfahren). Carnoy, Pappenheim-Färbung. Vergr. 30×

Die in vitro epithelartig wachsenden Eierstockgewebe zeigen eine Neigung zur Verflüssigung des Plasmakoagulums. Bei den beiden von uns angewandten Verfahren mit dünner Koagulumschicht ist diese Erscheinung selten zu beobachten.

In bezug auf die Frage, *welche Gewebselemente des Eierstocks ein epithelartiges Wachstum in vitro zeigen*, so decken sich unsere Untersuchungsangaben ebenfalls mit den Ergebnissen von MICHAILOW und KOLESNIKOWA. Mit Hilfe unserer Verfahren war es möglich, durch vitale Beobachtung im Phasenkontrast die Anfangsproliferation der Stränge und Membranen zu verfolgen und uns davon zu überzeugen, daß sie auf Kosten des sogenannten Keimepithels und der Satellitzellen geschieht. Besonders deutliche Bilder zeigten die Kulturen von 14tägigen Eierstöcken. In jenen Stellen des explantierten Fragments, in denen das Keimepithel erhalten geblieben ist, sieht man bereits am zweiten Tag nach der Explantation eine Verflachung und Verlängerung seiner Zellen, was von Mitose- und Migrationserscheinungen begleitet ist. Wenn das Keimepithel infolge Ausschneidens des Fragments zerstört ist, kann man schon nach 24 Stunden in den unmittelbar an dem Explantatrand liegenden Nestern von Geschlechts- und Satellitzellen beobachten, wie die kleineren Satellitzellen aus den Nestern stromartig proliferieren und Stränge bilden, die sich am nächsten

Tag zu einer Membran vereinigen. Während dieses Zeitraums zeigen die Geschlechtszellen keine wesentlichen Veränderungen. Noch besser läßt sich dies bei Explantaten beobachten, die durch starke Zerkleinerung oder Trypsinisierung der Fragmente gewonnen worden sind. Man kann in der Kultur dann Komplexe kleinerer, schwächer phasenpositiver Satellitzellen und größerer, stark phasenpositiver Geschlechtszellen sehen. Am nächsten Tag der Züchtung proliferieren die ersteren in Form typischer Stränge, während die zweiten auf ihrem Platz bleiben.

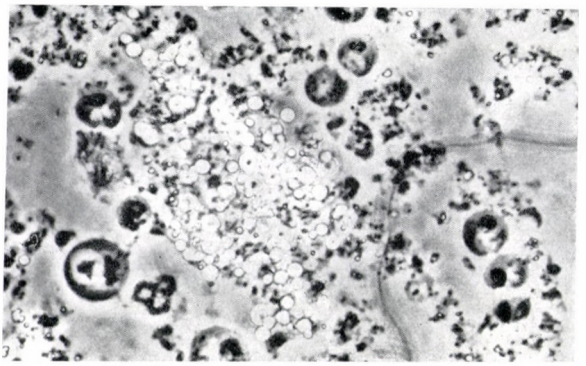


Abb. 3. Kultur vom stark zerkleinerten Eierstockcortex eines 19tägigen Hühnerembryos sofort nach der Explantation. Man sieht vereinzelt isolierte Geschlechtszellen und Komplexe anderer Eierstockzellarten. Phasenkontrast. Vergr. 360×

Besondere Beachtung wurde von uns dem *Verhalten der Geschlechtszellen* in vitro entgegengebracht. Der Vorzug unserer Verfahren besteht darin, daß es möglich ist, die Geschlechtszellen bei der Phasenkontrastmikroskopie sicher zu identifizieren, abgesehen davon, daß es andererseits möglich ist, ein und dieselben Zellen in verschiedenen Zeiträumen nach der Explantation zu verfolgen. Abbildungen 3 und 4 zeigen Aufnahmen von Geschlechtszellen im Phasenkontrast. Außer dem großen blasenartigen Kern, dessen Chromatin je nach dem meiotischen Stadium verschieden strukturiert ist, fallen das starke phasenpositive, fast homogene Ektoplasma der Zelle und das eigentümliche paranukleäre Gebilde, der sog. Corpus Balbiani, auf. Dieser besteht aus einer stark phasenpositiven Mitochondrienanhäufung um ein helleres Feld, die Zentrosphäre, die ein oder zwei Körner, Zentriolen, enthält. Mit Hilfe unseres zweiten Verfahrens war eine gute Beobachtung sowohl isolierter als auch in Gruppen angesammelter Zellen möglich. Es hat sich erwiesen, daß nicht nur das Trypsinisieren, sondern auch das bloße Zerkleinern des Cortex die Trennung einzelner Zellen herbeiführt. Das zeigt, daß die Geschlechtszellen in den Nestern sehr schwach miteinander vereint sind und daß sie durch bloße mechanische Einwirkung gelöst werden können (Abb. 3). Eine andere Eigenschaft der Geschlechtszellen

ist, daß sie ein höheres spezifisches Gewicht haben als die anderen Zellen, so daß sie bei Einführung der Zellsuspension in die Kammer als erste auf das untenliegende Glas fallen. Diese Eigenschaften der Geschlechtszellen könnte man zu ihrer Isolierung von den anderen Zellen ausnutzen.

Bei mehrstündiger Beobachtung isolierter Geschlechtszellen stellten wir die von MURATORI, BATTAGLIA und SMITH beschriebenen Erscheinungen fest: Hervorspringen pseudopodienähnlicher Fortsätze; turbinen- oder wellenartige Bewegungen des Ektoplasmas in Uhrzeigerrichtung (von MURATORI »Zirkusbewegungen« genannt); langsame Formveränderung des Zellkerns. In Über-

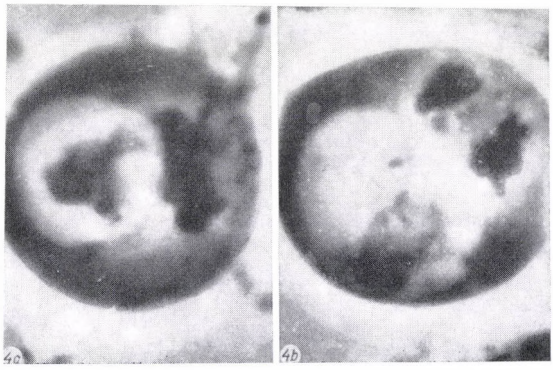


Abb. 4. Geschlechtszelle aus der Kultur in Abb. 5. Phasenkontrast. Vergr. 800× (nachvergrößert)

einstimmung mit MURATORI und BATTAGLIA haben auch wir uns davon überzeugt, daß diese Bewegungen überhaupt nichts mit einer Verschiebung der Zellen selbst, d. h. mit einer echten Amöboidbewegung, gemein haben, wie dies DANTSCHAKOFF annimt. Unsere Untersuchungen haben ergeben, daß alle obenbeschriebenen Erscheinungen in der Kultur auch bei Zimmertemperatur auftreten können. Bei Zimmertemperatur haben wir auch die Bildung vielkerniger Symplasten beobachtet, die andere Autoren beschrieben haben. Bereits 1—2 Stunden nach der Explantation sind zwei oder mehr Kerne sowie ein doppelter oder in Form einer komplizierteren Anhäufung erscheinender Balbianische Körper feststellbar.

Das weitere Schicksal der Geschlechtszellen in der Kultur. Je nach den Bedingungen, unter die die Geschlechtszellen bei der Explantation geraten und je nach ihrem Entwicklungsstadium konnten wir mehrere Arten von Erscheinungen feststellen.

Ein großer Teil der Geschlechtszellen degeneriert und stirbt ab. Das bezieht sich vor allem auf die bei der Zerkleinerung oder Trypsinisierung des Eierstocks im Explantat im einzelnen oder in kleineren Komplexen isolierten Geschlechtszellen. Die obenerwähnten Bewegungen des Ektoplasmas der Zellen

oder der von ihnen gebildeten Symplasten hören auf, im Zytoplasma erscheinen Vakuolen und Lipide, die Zellkerne erfahren Pyknose und Karyorrhexis, und die Zellen desintegrieren nach 24—28 Stunden. In den kleinen Komplexen von Geschlechts- und Satellitzellen erfolgt dieser Zerfall auf dem Hintergrund der von den rasch proliferierenden Satellitzellen gebildeten Stränge und Membranen. Manche der untergehenden Geschlechtszellen werden, wie bereits erwähnt, von den Satellitzellen phagozytiert. In anderen Fällen — und das bezieht sich auf die Geschlechtszellen von der freien Oberfläche der Eierstock-

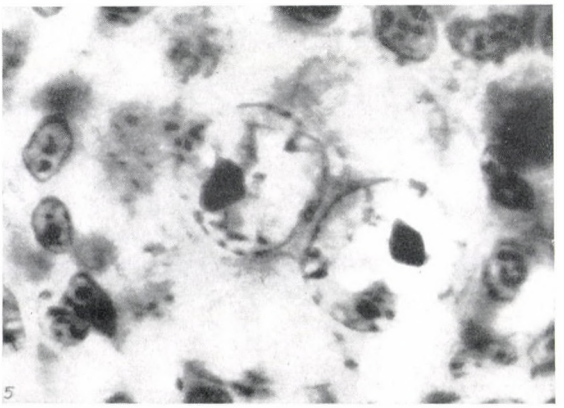


Abb. 5. Sechzehntägige Kultur vom Eierstock eines 17tägigen Hühnerembryos. Am Rande des Mutterstücks haben sich Primordialfollikel differenziert (zweikerniger Ovozyt). Carnoy, Hämatoxylin nach Böhmer. Vergr. 800×

fragmente — werden diese Zellen einzeln oder in Gruppen und Scharen von den wachsenden epithelartigen Membranen mitgeschleppt und können sich in die Wachstumszone begeben, wo sie ihre runde Form mehr oder weniger beibehalten und nur einige Tage lebend bleiben. Im Innern des Fragments beobachtet man ebenfalls den Untergang von Geschlechtszellen infolge beschränkter Ernährung.

Ganz andere Bilder zeigt der der Wachstumszone angrenzende periphere Teil des Fragments. Hier können die Nester von Geschlechts- und Satellitzellen ohne Regressionserscheinungen erhalten bleiben, und die Geschlechtszellen können in den folgenden Tagen spätere meiotische Phasen zeigen, wobei es sogar zur Bildung echter Primordialfollikel kommen kann (Abb. 5). Häufiger ist aber im peripheren Teil des Fragments eine andere Erscheinung zu beobachten. Sie tritt auf, wenn die Satellitzellen die Möglichkeit haben, rasch auf der Oberfläche des Koagulums zu proliferieren und die Geschlechtszellen in unmittelbarem Kontakt mit dem Deckglas bleiben. In diesem Fall beginnen vom dritten und vierten Tage an auch die Geschlechtszellen zu proliferieren. Es bilden sich zwei übereinander liegende Schichten in der Wachstumszone.

Unter dem Mikroskop, vom Deckglas aus gesehen, sieht man in der ersten Brennpunktebene die Geschlechtszellen und in der zweiten die Satellitzellen. Man könnte annehmen, daß die in dieser Weise lagernden Satellitzellen den Geschlechtszellen eine Art Schutz gewährleisten und den Stoffwechsel zwischen ihnen und dem Medium vermitteln.

Die Proliferation der in die Meiosis eingetretenen Geschlechtszellen in Kulturen von älteren Eierstöcken (nach dem 16—17. Bebrütungstag) scheint nur in einer einfachen zweidimensionalen Ausbreitung der Geschlechtszellen

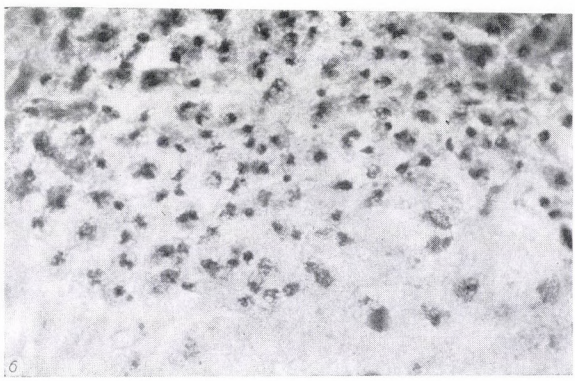
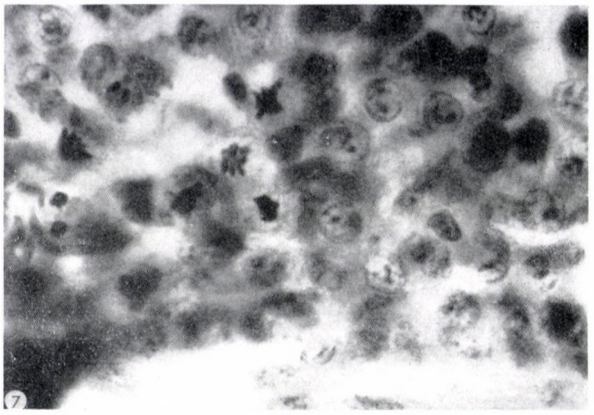


Abb. 6. Dreitägige Kultur vom Eierstock eines 19tägigen Hühnerembryos. Oben: — Membran von degenerierenden Geschlechtszellen in meiotischen Phasen. Unten: Satellitzellmembran in einer anderen Brennpunktebene. Carnoy, Pappenheim-Färbung. Vergr. 360×

unter den Membranen der Satellitzellen zu bestehen. In diesen Fällen sind Mitosen nicht feststellbar, die Zellen leben eine gewisse Zeit weiter (Abb. 6). Eine echte Proliferation durch mitotische Teilungen erfolgt in Kulturen von jüngeren Eierstöcken (bis zum 14—15. Bebrütungstag), bei welcher sich die Geschlechtszellen vor oder im Frühstadium der Meiosis (Typ a- und Typ b-Stadium nach d'Hollander, zit. MURATORI) befinden. Häufig stellt diese Proliferation ein Gemisch von Geschlechts- und Satellitzellen dar, mit Übergangsformen zwischen den einen und den anderen, was an einen Dedifferenzierungsprozeß der Geschlechtszellen denken läßt. Im allgemeinen besteht die Proliferation aber nur aus sich mitotisch teilenden Geschlechtszellen, die unter gewissen Bedingungen (Serum von legenden Hennen in der Nährlösung) zum Teil auch meiotischen Verwandlungen unterworfen werden können. In diesen Fällen bilden sie Gruppen und Stränge, die später zu ganzen Membranen konfluieren (Abb. 7. u. 8). Die Zellen haben eine gerundet polygonale Form, sie sind pflastersteinartig angeordnet und bleiben meist in engem Kontakt miteinander. Im Phasenkontrast erscheinen sie durch das stark phasenpositive Zytoplasma und die halbmondförmige Mitochondrienanhäufung voneinander abgegrenzt. In den

mit Hämatoxylin gefärbten Postfixationspräparaten sind die Zellgrenzen schwach ausgedrückt, das Zytoplasma der Zellen ist in höherem oder minderem Grade basophiler (Pyroninophiler) als das der anderen Zellen, der Kern zeigt ein netzartiges Chromatin, mit einer bis drei nahe am Zentrum gelegenen



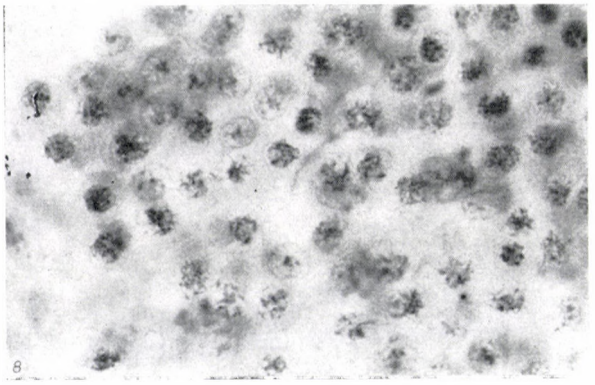


Abb. 7. Neuntägige Kultur vom Eierstock eines 14tägigen Hühnerembryos (nach dem I. Verfahren). Die dunklere Zone oben: Membran von Ovogenien mit einigen Mitosen. Die hellere Zone unten: Membran von Satellitzellen in einer anderen Brennpunktebene. Carnoy, Hämatoxylin nach Carazzi. Vergr. 360× (nachvergrößert)

Abb. 8. Neuntägige Kultur vom Eierstock eines 14tägigen Hühnerembryos (nach dem I. Verfahren). Die Nährlösung enthält Serum von legender Henne. Die Geschlechtszellenmembran besteht aus Ovogonien und Ovozyten in verschiedenen Phasen der frühen Meiose. Serra, Hämatoxylin nach Meyer. Vergr. 800×

größeren Chromatinmassen (Typ »a« und Typ »b« nach d'Hollander (Abb. 7), oder auch verschiedene Bilder der frühen Prophase der Meiose (Abb. 8). Manchmal kommen die Grenzen zwischen den einzelnen Zellen einer Gruppe überhaupt nicht zum Vorschein, die Gruppe als ganzes ist scharf umrissen, und es handelt sich offensichtlich um eine Bildung symplastischer Natur.

In den darauffolgenden Tagen schreitet die Proliferation der Geschlechtszellen durch mitotische Teilungen weiter fort. Um die Mitte der zweiten Woche erreicht die Wachstumszone der Geschlechtszellen ihre Maximalgröße. Der Durchmesser dieser Zone erreicht eine Länge von 2/3 des Durchmessers der Wachstumszone der Satellitzellen. Gegen Ende der zweiten Woche sind die Lipide und die Vakuolen im Zytoplasma vermehrt, die Geschlechtszellen können voneinander dissoziieren, wobei die Verbindung zwischen ihnen durch dünne Fortsätze aufrechterhalten bleibt. Auf diese Weise werden sie den Fibroblasten sehr ähnlich, wie dies mit den Satellitzellen der Fall ist.

Diskussion

In bezug auf das histotypische Wachstum der Gonaden in vitro sind in der Literatur drei Grundfragen ungeklärt: 1. Stellt das beobachtete epithelartige Wachstum der Gonadengewebe etwas Spezifisches dar, wodurch es sich vom Wachstum des banalen Epithels unterscheidet? 2. Welche Gewebselemente der Gonaden zeigen ein derartiges Wachstum? 3. Ist eine Proliferation der echten Geschlechtszellen (Gonoblasten) möglich und in welcher Weise verläuft sie?

Unsere Untersuchungen bestätigen in bezug auf den Eierstock von Hühnerembryonen die Angaben anderer Autoren (Wermel, Ulesko-Stroganowa, Živ, Michailow, Kolesnikowa, Chruščow und Diomidova, Dux usw.), daß die epithelartigen Stränge und Membranen, die man in vitro beobachtet, von den Satellitzellen und dem sogenannten Keimepithel des Eierstocks herrühren. In Übereinstimmung mit Michailow und Kolesnikowa haben auch wir spezifische Eigenschaften dieser Gewebebestandteile des Eierstocks beobachtet, durch die sie sich vom banalen Epithel unterscheiden. Demnach sind sie der Gruppe der Chlopinschen Coelodermalgewebe zuzuordnen, zu der auch die Mesothelien gehören. Dies entspricht der Auffassung vom Ursprung der Satellitzellen und des Keimepithels vom Coelommesothel der Urogenitalfalte. Übrigens verraten diese Gewebe manche Eigenschaften, die sie einerseits dem Wachstum der Epithelien und andererseits den vom Mesenchym herrührenden Geweben annähern. So sind die Zellen bestrebt, in engem Kontakt untereinander zu bleiben, indem sie Stränge und Membranen bilden; sie besitzen auch die Fähigkeit, das Plasmakoagulum zu verflüssigen; andererseits zeigen sie einen erheblichen Polymorphismus, mit Neigung zur Dissoziation und zum Übergang der gebildeten Membranen in lockere Strukturen, eine Bildung von Symplasten, sowie eine gewisse phagozytäre Fähigkeit. Damit ließe sich auch erklären, warum manche der älteren Autoren (Maccabruni, Mjassojedoff, Essaki, Olivo), die wahrscheinlich nicht den epithelartigen vom Fibroblastenwachstumstyp unterschieden haben, nur den letzteren an Gonadekulturen beschreiben. Dasselbe gilt auch für einige neuere Forscher (Muratori, Smith), die

ebenfalls nichts vom epithelartigen Wachstum der Satellitzellen und des Keimepithels erwähnen. Sogar aus manchen von diesen Autoren gezeigten Bildern geht klar hervor, daß dieser Wachstumstyp zu Unrecht als Fibroblastenwachstum qualifiziert worden ist.

Wir stellten noch eine Besonderheit der Satellitzellen und des Keimepithels in vitro fest, nämlich ihre Fähigkeit, auch bei beschränktem Sauerstoffzutritt in Kulturen intensiv zu proliferieren, was sie ebenfalls von den Fibroblasten unterscheidet.

Wir haben die von MURATORI, BATTAGLIA und SMITH beschriebenen eigenartigen Bewegungen des Ektoplasmas der Geschlechtszellen in vitro beobachtet. Bemerkenswert ist, daß wir diese Erscheinungen auch bei Zimmertemperatur feststellen konnten. Unseres Erachtens sind sie eher als Ausdruck einer Reaktion der Geschlechtszellen auf die unmittelbare Einwirkung der ungewöhnlichen Bedingungen des neuen Milieus aufzufassen. Wir stellten auch eine andere Besonderheit der Geschlechtszellen fest, nämlich ihr höheres spezifisches Gewicht im Vergleich mit den übrigen Zellarten des Eierstocks sowie ihr verhältnismäßig späteres Anhaften an das Glas nach der Explantation.

Unsere Angaben sprechen weiterhin dafür, daß das Schicksal der Geschlechtszellen in der Kultur, je nach ihrem Entwicklungsstadium und den Bedingungen, unter die sie bei der Explantation geraten, verschieden ist. Das erklärt die widersprechenden Schlußfolgerungen der verschiedenen Autoren zu dieser Frage. Voraussetzungen für eine echte, durch mitotische Teilungen erfolgende Proliferation der Geschlechtszellen in vitro sind: 1. daß sich diese bei der Explantation nicht im fortgeschrittenen Stadium der Meiosis befinden, d. h. am besten von Eierstöcken bis zum 14—15. Bebrütungstag genommen werden; 2. daß sie in keinem unmittelbaren Kontakt mit der Nährlösung stehen, sondern überdeckt von einer von den proliferierenden Satellitzellen gebildeten Membran wachsen. Fehlt eine dieser Bedingungen, so sind die Geschlechtszellen zu Degeneration und Untergang verurteilt, oder sie können günstigstenfalls nur noch einige Tage überleben. Dies war der Fall bei den Beobachtungen von FANO und GAROFOLINI, ULESKO-STROGANOVA, ŽIV, MICHAILOW, KOLESNIKOWA, BATTAGLIA. Andererseits haben MURATORI und SMITH, die zweifellos eine Proliferation der Geschlechtszellen in Kulturen festgestellt haben, nicht anzugeben vermocht, unter welchen Bedingungen diese Proliferation zustandekommt. Die »Migration« einzelner Geschlechtszellen oder Gruppen von Geschlechtszellen in den ersten Tagen in die Wachstumszone führen wir, ebenso wie andere Autoren (ULESKO-STROGANOWA, MICHAILOW, KOLESNIKOWA, BATTAGLIA) darauf zurück, daß diese Zellen von der wachsenden Schicht der Satellitzellen mitgeschleppt werden. Hierzu trägt u. a. die recht schwache »Verkittung« der einzelnen Geschlechtszellen im Explantat bei. Selbst MURATORI, der sich der Mikrokinematographie bediente, konnte sich nicht einmal davon überzeugen, daß die Pseudopodien und die Zirkusbewegungen des Ekto-

plasmas der Geschlechtszellen mit einer Verschiebung, d. h. mit einer echten Amöboidbewegung der Geschlechtszellen zusammenhängen. Unsere langdauernden Beobachtungen veranlassen uns eine derartige Bewegung kategorisch in Abrede zu stellen. Unseres Erachtens kann von einer Migration der Geschlechtszellen nur dann gesprochen werden, wenn sie unter den obenerwähnten beiden Bedingungen durch Mitose proliferieren. Demnach erscheint die von DANTSCHAKOFF als Argument zugunsten der Theorie von der Wanderung der Geschlechtszellen im Embryo vorgebrachte Bildung von Pseudopodien seitens dieser Zellen nicht stichhaltig genug.

Die von uns festgestellte Abhängigkeit des Wachstums der Geschlechtszellen vom Wachstum der Satellitzellen demonstriert die morphophysiologische Einheit der beiden Gewebselemente auch unter Explantationsbedingungen. Unseres Erachtens könnte man aber, wenn man den Charakter des Wachstums der beiden Elemente in vitro vergleicht, auch an ihre genetische Einheit denken. Dem Verhalten der Satellitzellen und der Geschlechtszellen in vitro sind folgende Eigenschaften gemein: sie wachsen als epithelartige Stränge und Membranen und neigen zu Lockerung in der Peripherie, indem sie unter ungünstigen Ernährungsbedingungen ein fibroblastähnliches Aussehen annehmen können; sie proliferieren in der Gewebekultur auch bei beschränktem Sauerstoffzutritt; sie bilden Symplasten. In manchen Fällen beobachtet man auch Übergangsformen zwischen den beiden Elementen. Letzteres bedarf noch des Beweises durch gründlichere Untersuchungen und Anwendung einer geeigneten Methodik.

Im Gegensatz zu MICHAILOW (S. 198) möchten wir sagen: es scheint, daß das von uns beschriebene Tatsachenmaterial und die daraus gezogenen Schlüsse eher gegen eine »Keimbahn« sprechen. Sie sprechen ebenso auch zugunsten der von HADJIOLOFF (1931/32, 1962) verteidigten Auffassung von der einheitlichen Natur der Satellit- und der Geschlechtszellen und ihrer Zugehörigkeit zu einem Grundgewebe — dem Sexualgewebe.

Die dargelegten Untersuchungen bilden den von uns zu bearbeitenden Teil eines kollektiven Themas mit der Abteilung für Morphologie des Instituts für Experimentelle Medizin der Ungarischen Akademie der Wissenschaften und des Instituts für Histologie der Medizinischen Universität Budapest (Direktor: Prof. Dr. I. Törő).

Den technischen Assistentinen R. Kuseva, A. Christova und A. Stojanova danken wir an dieser Stelle für die uns geleistete wertvolle Hilfe.

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A CONTRIBUTION TO THE BIOLOGY OF THE SEX TISSUE

II. In-vitro cultivation of the sex tissue of chick embryos

J. JORDANOV, M. ANASTASSOVA-KRISTEVA, A. I. HADJIOLOFF AND A. BOYADJIEVA-MICHAILOVA

The behaviour of the female sex tissues (sexual cells, satellite cells, germinal epithelium) of chicken embryos has been studied in tissue cultures. A method — inhibiting the growth of fibroblasts, promoting the epithelioid growth of ovarian tissue and allowing good vital inspection under the phase microscope — has been elaborated. The experiments have demonstrated the epithelioid growth of the satellite cells and the germinal epithelium. These elements of the ovary reveal in tissue cultures certain typical properties which distinguish them from common epithelium and justify their being classified under the head of coelodermal tissues (CHLOPIN). The fate of the true sexual cells in the culture depends on the conditions of explantation. It has been found that sexual cells are capable of mitotic proliferation *in vitro.* It happens at the early stages of differentiation (oögonium) when the sexual cells can grow under a protective membrane composed of satellite cells. The sexual cells degenerate after a shorter or longer survival if these requirements are not satisfied. The behaviour of satellite and sexual cells shows certain similarities in tissue cultures, such as proliferation in the form of strings and membranes, growth under anoxic conditions, formation of symplasms. These observations argue for their morphologico-functional and probably also genetic identity, and justify the concept that they are two elements of a common ground substance, namely the sex tissue.

БИОЛОГИЯ ПОЛОВОЙ ТКАНИ

II. Выращивание половой ткани зародышей цыплят

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Авторы исследовали в культурах тканей поведение женской половой ткани (половые клетки, клетки-сателлиты, зародышевый эпителий) зародышей цыплят методикой, задержающей рост фибробластов, способствующей эпителоидному росту яичниковой ткани и обеспечивающей возможность хорошего прижизненного наблюдения под фазоконтрастным микроскопом. Результаты экспериментов подтверждают эпителоидный рост клеток-сателлитов и зародышевого эпителия. Эти элементы яичников показывают в культурах несколько типичных свойств, которые отличают их от обычного эпителия и позволяют причислить их к группу так наз. целодермальных тканей *(Хлопин)*. В зависимости от того, в какие условия истинные половые клетки попадают при эксплантации, их судьба в культурах различна. Авторы устанавливают, что пролиферация половых клеток in vitro может произойти путем митотического деления. Это наблюдается в ранней фазе дифференциации половых клеток (овогоний), а также, если они имеют возможность расти под защитной оболочкой, образующейся из клеток-сателлитов. При отсутствии одной из указанных предпосылок половые клетки, после более или менее продолжительного срока, перерождаются. Сателлиты и половые клетки показывают в тканевых культурах и некоторые общие особенности поведения: они разрастают в виде тяжей и оболочек, развиваются также при ограничении притока кислорода и образовывают симпласты. Эти факты говорят за то, что в морфофункциональном и, вероятно, также в генетическом отношениях их можно рассматривать как единство, как элементы единой основной ткани: половой ткани.

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EFFECT OF THIRST AND WATER LOAD ON THE HYPOTHALAMIC REGULATION OF THE ADRENAL IN THE PIGEON (COLUMBA LIVIA DOMESTICA L.)

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Domestic pigeons were deprived of water for 4 and 8 days, others were subjected to a water load, and the effect of these treatments on the neurosecretory system and also the reaction of the interrenal tissue of the suprarenal gland have been studied. Hydration and dehydration affected the preoptic division of the supraoptic nucleus in the first line. Neither thirsting nor the water load elicited a significant reaction from the entopeduncular nucleus, nor did the external zone of the median eminence respond to thirsting. Water loading induced accumulation of aldehyde fuchsin-positive matter in the stratum palissadicum. The neurohypophysis filled with secretion on hydration and was drained on dehydration.

It seems that the suprarenal gland is regulated by the hypothalamus via the median eminence and, in aphysiological conditions, also via the neurohypophysis. Water loading stops the drainage of the neurohypophysis, reduces or arrests the portal activity of the median eminence and induces atrophy of the interrenal tissue of the adrenal. Thirsting does not influence the portal function of the median eminence so that the resulting adrenal hypertrophy may be due to the large amount of neurosecretory substance discharged by the pars nervosa. It is, therefore, assumed that the aldehyde fuchsin-positive neurohormone of birds acts as CRF.

The organism of mammals presumably contains an ACTH-releasing substance of hypothalamic origin. This substance is probably lysine vasopressin in the rat (DE WIED [30, 31]) and arginine vasopressin in the dog (KWAAN and BARTELSTONE [18]). Other authors claim that the ACTH-releasing factor is not a neurohormone of the vasopressin type but a structurally similar polypeptide (SAFFRAN et al. [28]; GUILLEMIN et al. [9]). The corticotrophin-releasing factor (CRF) is presumably synthetized in the neurosecretory cells of the hypothalamus and stored in the neurohypophysis. This seems to be borne out by the experiments of RUMSFELD and PORTER [27] in which ACTH release was induced by bovine posterior pituitary extract, a phenomenon attributed by them to the presence of arginine vasopressin.

While the principal object of experiments *in vitro* was to study the role of neurohypophyseal extracts in the release of corticotrophin, surgical manipulations were carried out in order to elucidate the role of the median eminence-adenohypophysis system. MCCANN and HABERLAND [3], after extirpating the median eminence in rats, found that the acutely stimulated adenohypophysis was incapable of releasing the accumulated ACTH. KOVÁCS et al. [17] observed

that extirpation of the hypophyseal stalk reduced the activity of the pituitary-adrenocortical apparatus in the rats. The diabetes insipidus brought about by the intervention subsided a few weeks later. This must have been due to a reduction of adrenocortical activity. It has been claimed by PETERS [24] that the anterior pituitary of the rat inhibits vasopressin secretion, a phenomenon ascribed by him to ACTH.

The results of ENDRŐCZI and LISSÁK [6] point to the role of neural factors in the release of ACTH. They have shown that stimulation of the supraoptic and paraventricular nuclei does not significantly change the level of corticoids in adrenal venous blood.

Hypothalamic CRF has also been studied in amphibians. JØRGENSEN and LARSEN [10] found that lysine vasopressin and — physiologically — arginine vasotocin released ACTH in the toad, *Bufo bufo*. In birds, no CRF has been demonstrated and it is only indirectly that the existence of a hypothalamic ACTH-releasing factor can be inferred to. LEGAIT [19] decreased the activity of the neurosecretory cells in the domestic fowl by chronic ACTH treatment. PÉCZELY [25] induced accumulation of neurosecretion in the stratum palissadicum of the external zone of the median eminence by treating pigeons with ACTH. KAWASHIMA et al. [11] deprived crown sparrows of water and observed neurosecretion from the anterior part of the median eminence, a phenomenon they suggested was interconnected with the hypertrophy of the interrenal substance of the adrenal.

The present experiments were designed to study the effect of hyperosmosis and hyposmosis on the neurosecretory system and the suprarenal gland, in the hope that the results might provide information on the ACTH-releasing effect of the birds' neurohormones.

Material and method

Forty pigeons of both sexes were divided into four groups. Members of Group I served as controls, those of Group II were deprived of water during four, those of Group III during eight days, while members of Group IV were loaded with water through a gastric tube for ten days. The animals were illuminated during 11 hours daily. After decapitation, the brain of the birds was fixed in Bouin's fluid, embedded in paraffin, and 5 μ thick sections were stained with Gomori's paraldehyde-fuchsin combined with a short haematoxylin contrast staining.

The nuclear volume of the neurosecretory cells was determined with the aid of the Fischer-Inke nomogram, using the formula $\frac{\pi}{6}LB^2$ and 2500-fold magnification by projection; 200 nuclei were measured in each division. The frequency distribution of the logarithm of the nuclear volumes was established for all the five divisions in respect of Groups I to IV. We determined the arithmetic mean of the frequency distributions (M) and the standard deviation (σ); in order to analyse the empirical density function of the frequency distributions, we determined the relative frequency of the cases over the range $M \pm \sigma$ so as to be able to compare the empirical density functions with the density function of the normal distribution of the corresponding standard deviation. Student's "t" test was applied for establishing statistical significance of the differences between the arithmetic means in all groups and in respect of all the five divisions. In the present case, $n = 2000$. $p = 0.05$, and the difference

was significant if $t \geq 1.96$. Beyond this, because of possible errors inherent in the methods, only those statistically significant cases were regarded as biologically evaluable in which $(m_1 - m_2) > 0.05$.

Results

Each morphologically and topographically well distinguishable division has been evaluated separately (PÉCZELY [25]). The nomenclature of the divisions was borrowed from ARAI [1] as also from FARNER and OKSCHE [7].

Supraoptic nucleus, preoptic division

Group I. The neurosecretory cells of small and medium size, situated along the wall of the preoptic recess and the ventrolateral surface of the hypothalamus, showed different functional states. The perikaryon usually contained

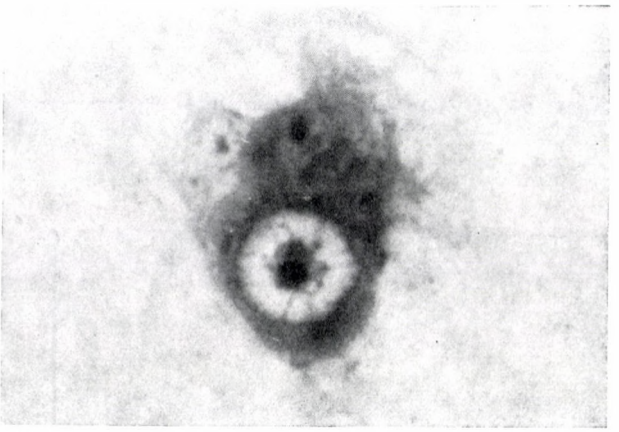


Fig. 1. Preoptic division of the supraoptic nucleus in untreated pigeon. Aldehyde fuchsin, × 2960

granular secretion and Nissl's bodies were observed along the cell membrane. Other cells contained colloidal globules. Occasional transport of secretion could be observed, and there were pearl string structures on the processes (Fig. 1 and Diagram 1).

Group II. The neurosecretory cells of the division were found to have increased, and there was usually a thick zone of Nissl's bodies around the cell membrane. Delicately granular secretion often formed a cape-like structure. Some cells contained colloidal globules or vacuoles. Nuclear volume was increased (Fig. 3 and Diagram 1).

Group III. The neurosecretory cells were considerably enlarged. There were less Nissl's bodies than in Group II. The perikaryon was filled with fine powderlike secretion. Nearly all cells were vacuolated. Only a few colloidal

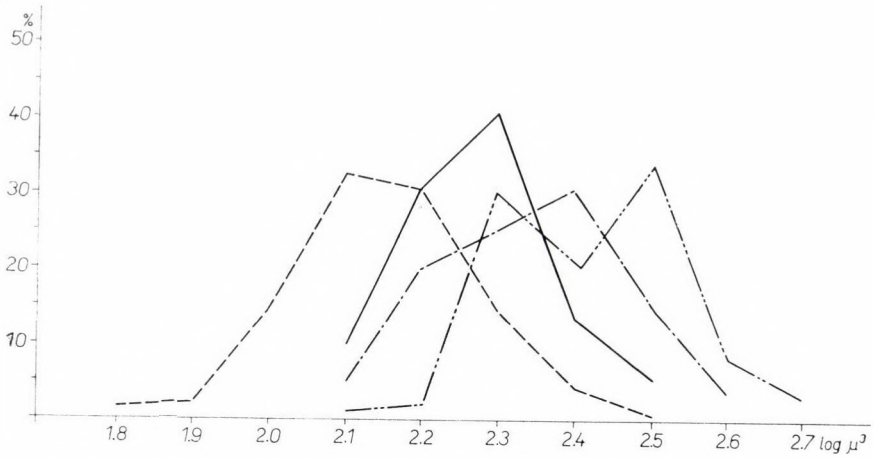


Diagram 1. Preoptic division of supraoptic nucleus
——— = controls; —— · —— = 4-day dehydration; — · · — = 8-day dehydration; · · · · · · = hydration

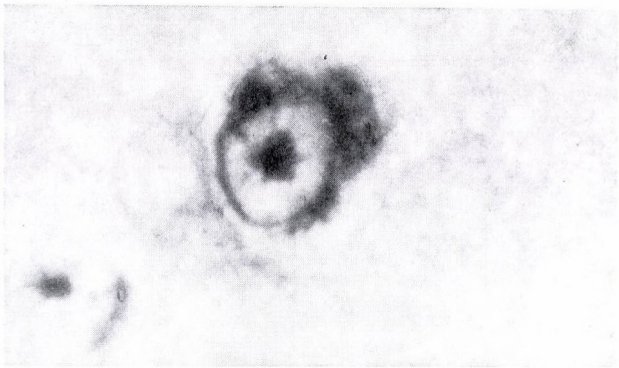


Fig. 2. Preoptic division of the supraoptic nucleus in hydrated pigeon. Aldehyde fuchsin, × 2960

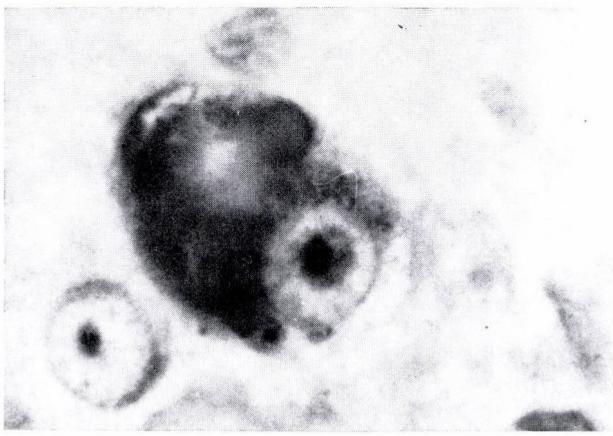


Fig. 3. Preoptic division of the supraoptic nucleus in pigeon after 4 days of thirsting. Aldehyde fuchsin, × 2960

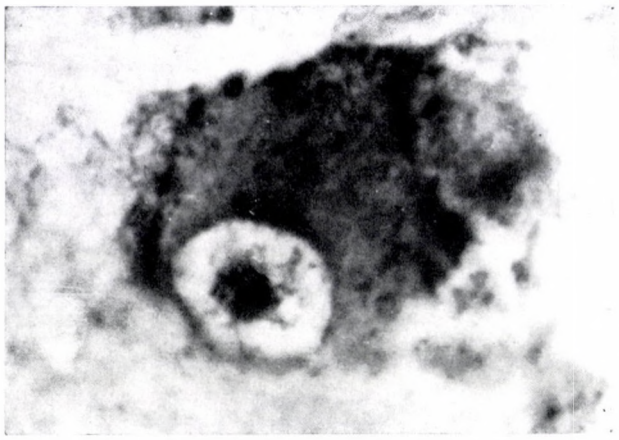


Fig. 4. Preoptic division of the supraoptic nucleus in pigeon after 8 days of thirsting. Aldehyde fuchsin, × 2960

globules were observed. The nuclei were eccentric and conspicuously swollen (Fig. 4 and Diagram 1).

Group IV. The cytoplasmic mass was strikingly small. It contained some coarsely granular secretion next to the nucleus. As a rule, no basophilic marginal zone was seen. Both the nucleus and the nucleolus were small (Fig. 2 and Diagram 1).

Lateral division

Group I. The division consisted of elongated cells containing a considerable amount of granular secretion and occasional colloidal globules. A thin basophilic marginal zone was observed in most cases (Diagram 2).

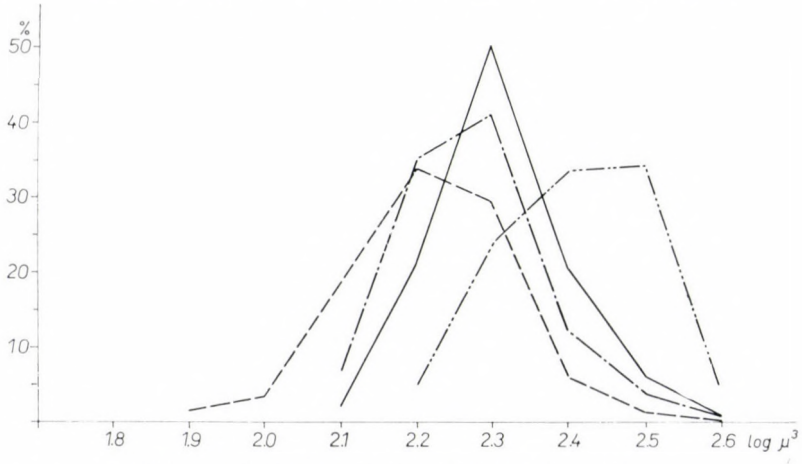


Diagram 2. Lateral division of supraoptic nucleus
——— = controls; — - — = 4-day dehydration; — - - — = 8-day dehydration; - - - - - - = hydration

Group II. The volume of the cytoplasm was not significantly different from that of the controls. Delicately granular secretion was seen around the nucleus. Most of the colloidal globules had disappeared. Nissl's bodies formed a thin marginal zone. Nuclei were slightly eccentric, and they were somewhat smaller than in Group I (Diagram 2).

Group III. The neurosecretory cells considerably increased; there was a broad basophilic marginal zone beneath the membrane. The number of vacuolated cells was less than in the preoptic division. Some cells contained colloidal globules. The nuclei were eccentric, their volume was increased (Diagram 2).

Group IV. The cytoplasmic mass was smaller than in the controls. There were few cells with an extremely narrow basophilic marginal zone. No cell processes were seen. Nuclear volume was smaller than in the controls (Diagram 2).

Medial division

Group I. The cells contained delicately granular secretion. Hardly any colloidal globules were observed. The processes of some neurosecretory cells, together with their secretion, were clearly visible (Diagram 3).

Group II. The neurosecretory cells seemed to be less active than those of the preoptic division. They were somewhat swollen and contained a larger volume of secretion. Marginal granulation was fairly pronounced without a change in nuclear volume (Diagram 3).

Group III. The neurosecretory cells were slightly swollen. There was in most cases a thin basophilic marginal granulation beneath the cell membrane. A cape-like structure of fine granules was seen in nearly all cells. Nuclei were slightly eccentric, their volume was increased (Diagram 3).

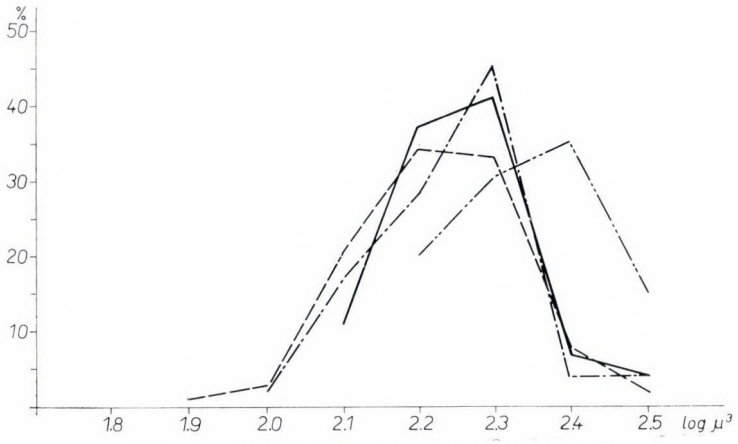


Diagram 3. Medial division of supraoptic nucleus
———— = controls; — - — = 4-day dehydration; — - - — = 8-day dehydration; - - - - - - = hydration

Group IV. Elongated cells containing a conspicuously small mass of cytoplasm were encountered. The nucleus often protruded from the cytoplasm. Occasionally marginal granulation was found. A small amount of coarse, aldehyde fuchsin-positive granules occurred next to the nuclei. Degenerating cells with pyknotic nuclei were also to be seen. Nuclear volume was smaller than in the controls (Diagram 3).

Entopeduncular nucleus

The cells failed to respond to water loading or to 4-day thirsting, but grew in size after 8 days of dehydration, and the amount of basophilic matter increased accordingly in them (Diagram 4).

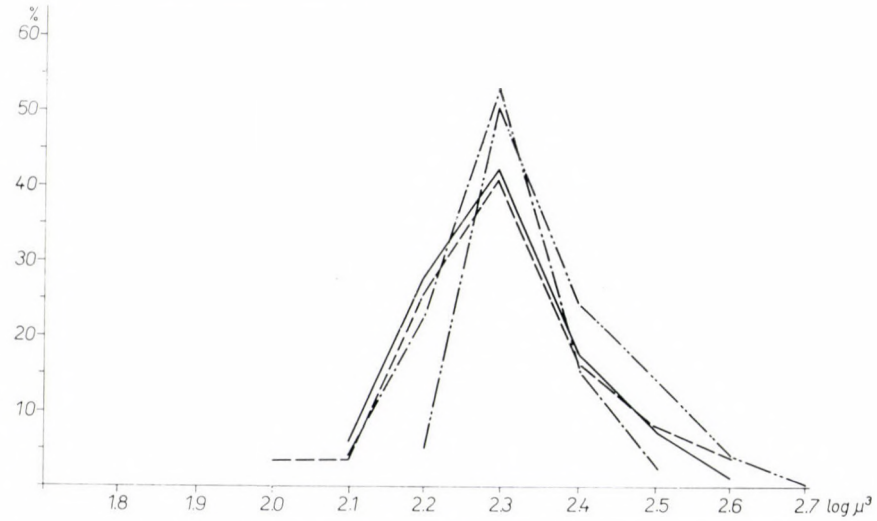


Diagram 4. Entopeduncular nucleus
——— = controls; — - — = 4-day dehydration; — - - — = 8-day dehydration; - - - - - - = hydration

Paraventricular nucleus

The two cell complexes of this nucleus, namely the periventricular and diffuse parts, behaved identically.

Group I. The cytoplasmic volume of the neurosecretory cells was fairly small. The cytoplasm contained some granular secretion and, occasionally, a few colloidal globules. Marginal granulation was not pronounced (Diagram 5).

Group II. The neurosecretory cells were somewhat enlarged, and they contained correspondingly more secretion. The marginal zone of Nissl's bodies was fairly marked in some cells. Nuclear volume was slightly decreased (Diagram 5).

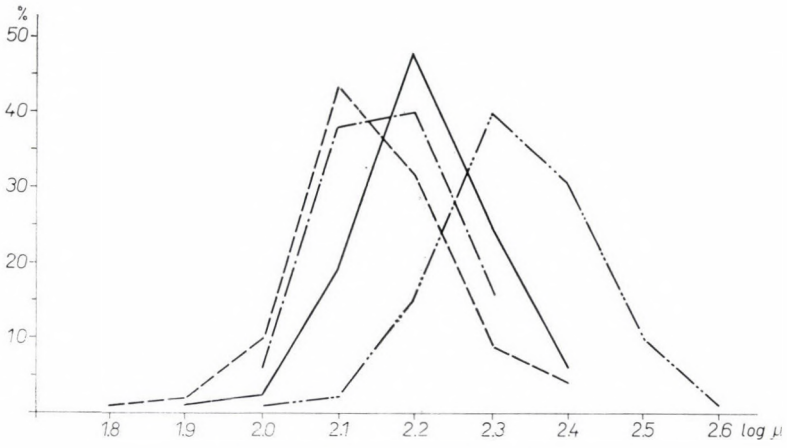


Diagram 5. Paraventricular nucleus
——— = controls; — - — = 4-day dehydration; — - - — = 8-day dehydration; - - - - - - = hydration

Group III. The cells were swollen; they contained a broad basophilic marginal zone; there was a cape-like structure in the perikaryon. Some cells were vacuolated. The nuclei were eccentric, enlarged (Diagram 5).

Group IV. The cytoplasmic mass was so much decreased that, in some cases, it formed but a narrow band around the nucleus. The secretion consisted of coarse granules and, occasionally, of colloidal globules. No cape-like formation was observed. The amount of basophilic matter was much less than in the controls. There were some degenerated cells. Nuclear volume was decreased (Diagram 5).

Statistical-mathematical evaluation

As can be seen from Table II, standard deviation was not significant (between 0.08 and 0.12).

In the preoptic division of the supraoptic nucleus, nuclear volume in Group IV (animals loaded with water) was significantly inferior to that in Group I (controls), whereas nuclear volume in Groups II and III (thirsting for 4 and 8 days respectively) was significantly larger than in the controls ($M_1 - M_2 > 0.05$).

In the lateral division, nuclear volume was significantly less in Group IV than in Group I ($M_1 - M_2 > 0.05$). Group II was not significantly different from the control group, while nuclear volume in Group III was significantly larger than in Group I ($M_1 - M_2 > 0.05$).

In the medial division, nuclear volume in Groups II and IV was not significantly different from the controls, but that in Group III was significantly higher than in Group I ($M_1 - M_2 > 0.05$).

In the paraventricular nucleus, nuclear volume in Group IV was significantly inferior to that in Group I ($M_1 - M_2 > 0.05$). The difference between Group II and the controls was biologically negligible, whereas nuclear volume in Group III was significantly larger.

In the entopeduncular nucleus, neither thirsting nor water loading resulted in significant differences (Tables I, II, III).

Table I

Values of "M"

	Supraoptic nucleus			Paraventricular nucleus	Entopeduncular nucleus
	Preoptic division	Lateral division	Medial division		
I	2.28	2.30	2.26	2.21	2.29
II	2.35	2.28	2.26	2.17	2.30
III	2.42	2.41	2.35	2.33	2.29
IV	2.15	2.23	2.23	2.15	2.30

I = Controls; II = 4-day dehydration; III = 8-day dehydration; IV = Hydration

Table II

Values of "σ"

	Supraoptic nucleus			Paraventricular nucleus	Entopeduncular nucleus
	Preoptic division	Lateral division	Medial division		
I	0.10	0.09	0.10	0.09	0.11
II	0.12	0.09	0.11	0.08	0.08
III	0.11	0.10	0.10	0.10	0.11
IV	0.12	0.11	0.12	0.10	0.12

I = Controls: II = 4-day dehydration; III = 8-day dehydration; IV = Hydration

Table III

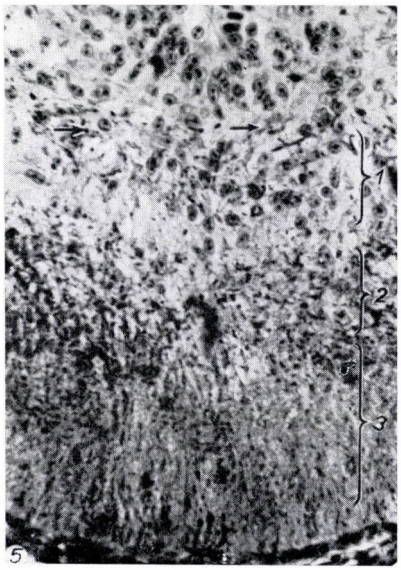
Values of "t"
(significance of differences between controls and the other groups)

	Supraoptic nucleus			Paraventricular nucleus	Entopeduncular nucleus
	Preoptic division	Lateral division	Medial division		
I—II	19.6	*7.1	*Ø	*15.0	*Ø
I—III	42.3	36.8	28.3	40.4	*11.5
I—IV	36.3	—22.1	*8.13	—20.2	*2.8

I = Controls; II = 4-day dehydration; III = 8-day dehydration; IV = Hydration. — = significantly less; * = no evaluable difference; no sign = significantly more.

Median eminence

Group I. The fibrous layer of the internal zone was well visible and showed the stringlike arrangement of Herring's bodies. Fibres, containing globules of secretion, were seen to run to the external zone at some points. The reticular layer of the external zone formed a plexiform and looped system of fibres and contained numerous coarse globules and fine granules of secretion. The internal (α) and external (β) zones of the stratum palissadicum were filled with neurosecretion. Typical, radially running fibres carried the secretion (Fig. 5).



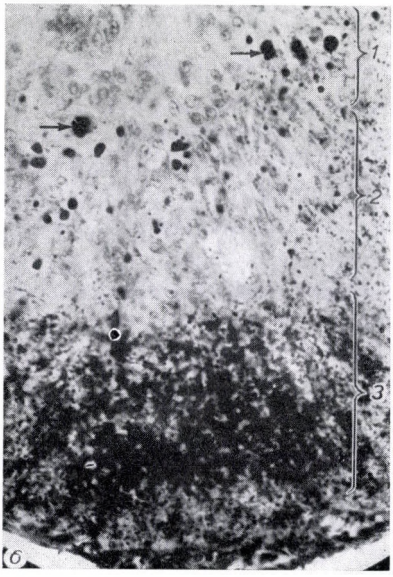


Fig. 5. Median eminence of untreated pigeon. 1: Stratum fibrosum — Herring's bodies. 2: Stratum reticulare. 3: Stratum palissadicum. Aldehyde fuchsin, × 640

Fig. 6. Median eminence of hydrated pigeon. 1 = Stratum fibrosum — Herring's bodies. 2 = Stratum reticulare (draining). 3 = Stratum palissadicum (accumulation of secretion). Aldehyde fuchsin, × 640

Groups II and III. There was hardly any difference between the two dehydrated groups as regards secretion contained in the median eminence. The fibrous layer of the internal zone contained few globules of secretion in transport. They were much smaller than those observed in the controls. No Herring's bodies could be seen. Fibres running towards the external zone carried a fair number of neurosecretory granules different in size. The reticular layer of the external zone contained more globules and grains of neurosecretion than in the controls. The size of the granules of secretion grew smaller towards the stratum palissadicum. In contrast to the members of Group IV (hydrated birds), the entire width (and so also the upper-inner part) of the reticular layer contained

secretion. Like in the controls, the stratum palissadicum was filled with fine powderlike neurosecretion. Bundle-like inflow of coarse globules of secretion from the reticular layer was seen at some points (Fig. 7).

Group IV. The fibrous layer of the internal zone was characterized by the presence of extremely large Herring's bodies, whereas no transport of delicately granular secretion was seen on the fibres. The fibres running towards the reticular layer of the external zone contained a little granular secretion. The amount of secretion contained in this zone (especially in its in ner part) was

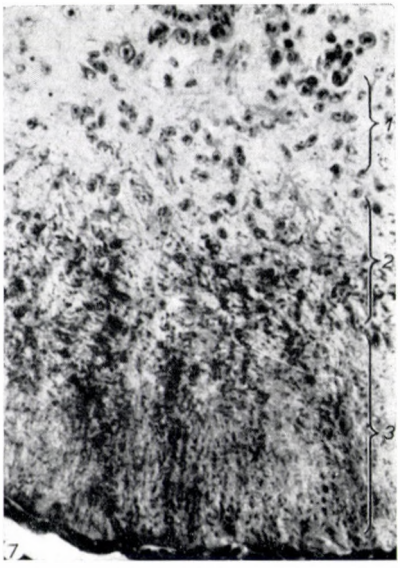


Fig. 7. Median eminence of pigeon after 8 days of thirsting. 1 = Stratum fibrosum (disappearance of Herring's bodies). 2 = Stratum reticulare (as in untreated controls). 3 = Stratum palissadicum (as in untreated controls). Aldehyde fuchsin, × 640

considerably smaller than in the controls. Fibres in contact with the stratum palissadicum contained coarse globules of secretion. The fibres of this layer carried considerably larger and more numerous globules of secretion than in the control group. The globules were found to have accumulated in some cases exclusively at the bottom of zone α and in zone β. The globules of secretion in the stratum palissadicum were much coarser than the corresponding globules of the controls (Fig. 6).

Neurohypophysis

Group I. This organ was rich in secretion in most cases (Fig. 8). Large globules of secretion were observed on the pericapillary fibres.

Group II. A considerable loss of neurosecretory substance was observed. The pars nervosa seemed to be hypertrophic (Fig. 10).

Group III. The posterior lobe was almost completely drained, and it was only at a few points that very fine granules of secretion could be seen at

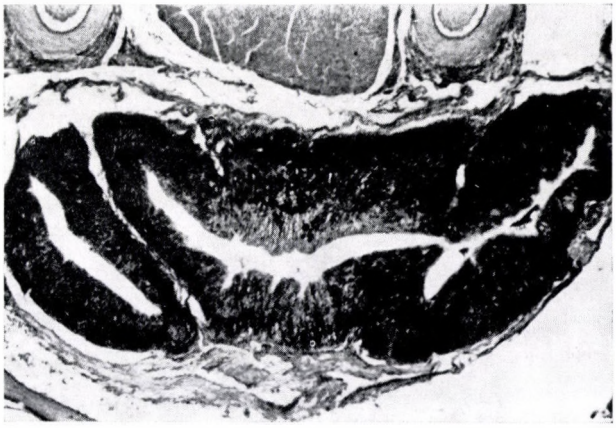


Fig. 8. Neurohypophysis of untreated pigeon. Aldehyde fuchsin, × 103

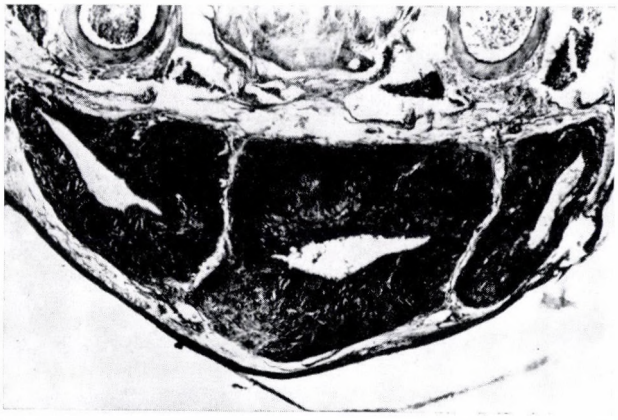


Fig. 9. Neurohypophysis of hydrated pigeon. Aldehyde fuchsin, × 108

the terminal portion of the axons. The neurohypophyseal tissue was markedly hypertrophic (Fig. 11).

Group IV. Water loading caused a narrowing of the neurohypophysis. It contained more secretion than that of the controls; large areas filled with neurosecretion were observed (Fig. 9).

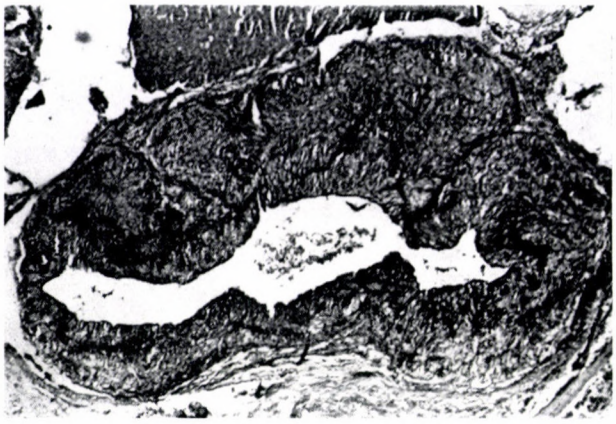


Fig. 10. Neurohypophysis of pigeon after 4 days of thirsting. Aldehyde fuchsin, × 108

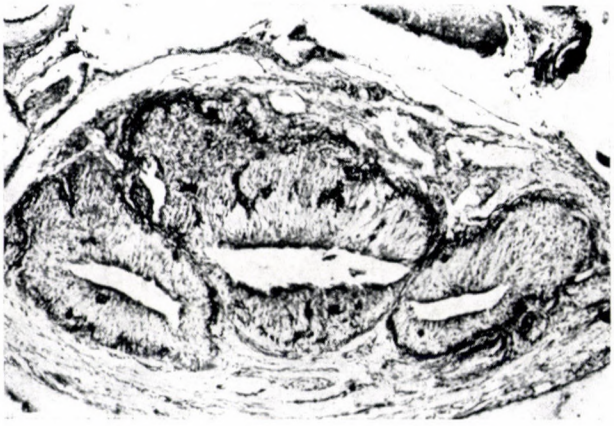


Fig. 11. Neurohypophysis of pigeon after 8 days of thirsting. Aldehyde fuchsin, × 108

Discussion

It is known (KAWASHIMA et al. [11]; KONDICS [14]) that water deprivation induces a hypertrophy of the interrenal tissue in the suprarenal gland of birds. KONDICS has moreover shown that the deeper interrenal cell bundles become atrophic in the first place. He regards them as corresponding to the zona fasciculata in mammals.

Dehydration induced by thirsting or treatment with NaCl causes hypertrophy of the neurosecretory cells in birds; the neurohypophysis is drained, while the amount of secretion contained in the median eminence undergoes no essential change (OKSCHE et al. [21—23]). Osmotic stress affects, thus, only the neurosecretion contained in the posterior lobe, a phenomenon shown by the

experiments of KOBAYASHI et al. [12] in which dehydration promoted acid-phosphatase activity (a reliable sign of neurosecretion) in the pars nervosa and failed to influence it in the median eminence.

In contrast to these findings, KAWASHIMA et al. [11] demonstrated the osmotic sensitivity of the anterior part of the median eminence in the white crowned sparrow, and suggested that the neurosecretion released therefrom acts as CRF which would explain the hypertrophy of the interrenal part of the suprarenal gland. In our present experiments no such phenomenon was observed, and the findings supported the obvious hypothesis that osmotic stress induces a liberation of aldehyde fuchsin-positive matter from the neurohypophysis but has no such influence on the external zone of the median eminence.

As regards the internal zone, the disappearance of Herring's bodies from the supraoptico-hypophyseal tract is a noteworthy phenomenon, one that must have been due to increased transport and drainage of secretion through the pars nervosa. In birds deprived of water for 4 and 8 days, the inner part of the reticular layer contained more secretion than in the control animals. OKSCHE et al. [23] observed the same phenomenon in the quail. Mobilization of neurosecretion was due presumably to that the inner part of the reticular layer, usually poor in secretion, was passively filled simultaneously with the onset of the general transport of secretion. This process did not affect the portal function of the median eminence.

A considerable quantity of neurohormone was released from the pars nervosa under the effect of thirsting. Provided there exists a CRF of the vasopressin-arginine vasotocin type in birds, only the ACTH-releasing action of the substance excreted by the neurohypophysis can be responsible for the hypertrophy of the interrenal tissue of the adrenal gland. By making the neurohormones originating in the pars nervosa to pass from the blood to the pars distalis, JØRGENSEN et al. [10] achieved increased ACTH liberation in frogs. It being known that neurohypophyseal extracts act as CRF in mammals (RUMSFELD and PORTER [27]; PORTER and JONES [26]), it is safe to assume that neurosecretion liberated from the pars nervosa by intensive osmotic stress may induce a release of ACTH *in vivo*. How does the neurohormone of the pars nervosa gain access to the adenohypophysis? Does its concentration suffice for producing the necessary effect after having reached the intravascular department or does it reach the ACTH-producing cells of the anterior pituitary in a direct way? The latter alternative is improbable because — in contradistinction to mammals (DANIEL and PRICHARD [4]; DUVERNOY and KORITKÉ [5]) — no short portal vessels have so far been detected in birds (VITUMS et al. [32]). There seems to be no direct vascular communication between the neurohypophysis and the adenohypophysis in birds; the respective vessels supplying the anterior and the posterior lobe have separate and independent courses.

Hypertrophy of the interrenal tissue caused by thirsting may, on the other hand, be regarded as a stress which does not postulate direct communication between the Gomori-positive neurosecretory system of the hypothalamus on the one side and the adenohypophyseal—interrenal system on the other. This theory seems to be disproved by the atrophy of the interrenal adrenal tissue induced by hydration; both hydration and dehydration mean a stress for mammals.

The reaction of the neurosecretory cells to water deprivation depends on the duration of thirsting. Four-day thirsting elicited a significant increase in the activity of the preoptic division of the supraoptic nucleus, while there was no change in the activity of the medial division and the paraventricular nucleus. It was likewise mainly the preoptic division of the supraoptic nucleus which became hypertrophic after eight days of thirsting, but there was, in addition, a marked increase in the activity of the lateral and medial divisions and the paraventricular nucleus. These findings confirm the statement (Oksche et al. [21, 23]; Kawashima et al. [11]) that it is in the first line the medial area of the supraoptic nucleus (i.e. the preoptic and the medial division) which reacts to osmotic stimulation.

While in birds water loading induces a hypertrophy of the interrenal tissue of the adrenal (that of the deeper cell bundles in particular) (Kondics [16]) in mammals, it is the zona fasciculata of the adrenal cortex which becomes hypertrophic (Nagareda and Gaunt [20]; Baisset et al. [2].) Hypertrophy induced in mammals is presumably due to the stress effect of water loading which mobilizes ACTH and reduces the activity of the neurosecretory apparatus. Water loading means, therefore, a stress for the mammal, while this stress is insignificant in birds or, else, hydration means no stress for them. Atrophy of the interrenal tissue and reduced neurosecretory activity in pigeons loaded with water substantiate the assumption that neurosecretory substance acts as CRF.

The hypothalamic neurosecretory cells react fairly uniformly to water loading. Their microscopic inspection shows decrease in, and reduced transport of, secretion. The preoptic division is particularly sensitive to exogenous influences.

The entopeduncular nucleus does not react significantly either to hydration or to dehydration. By being independent of exogenous influences, this group of neurosecretory cells is sharply different from other such groups. The present findings are in this respect in harmony with earlier ones (Péczely [25]; Farner and Oksche [7]; Uemura and Kobayashi [29]).

Secretion accumulates in the neurohypophysis of the pigeon loaded with water, so that neurosecretory draining is stopped or reduced to a minimum. The flow of secretion becomes slower, and large Herring's bodies are deposited along the supraoptico-hypophyseal tract. Secretion ceases to flow from the

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DIE WIRKUNG VON WASSERENTZUG UND WASSERBELASTUNG AUF DIE HYPOTHALAMISCHE STEUERUNG DER NEBENNIEREN DER HAUSTAUBE (COLUMBA LIVIA DOMESTICA)

P. PÉCZELY

Die Wirkung von 4- und von 8tätigem Wasserentzug bzw. von Wasserbelastung auf das neurosekretorische System der Haustaube wurde im Hinblick auf die interrenale Gewebsreaktion der Nebenniere untersucht. Es ließ sich feststellen, daß nach Dehydratation und nach Wasserbelastung in erster Linie der präoptische Abschnitt des Nucleus supraopticus reagiert. In der Antwortreaktion auf Wasserbelastung offenbart sich die funktionelle Verschiedenheit der anderen Abschnitte in geringerem Grade. Der Nucleus entopeduncularis zeigt weder nach Dehydratation, noch auf Wasserbelastung eine signifikante Änderung. Die Außenzone der Eminentia medialis reagiert nicht auf Wasserentzug. Im Stratum palissadicum erzeugt die Wasserbelastung die Akkumulation einer aldehydfuchsinpositiven Substanz. Nach Dehydratation entleert sich die Neurohypophyse, während sie sich nach Wasserbelastung mit Sekret füllt.

Auf Grund dieser Ergebnisse kann angenommen werden, daß die hypothalamische Steuerung der Nebennieren durch Vermittlung der Eminentia medialis und bei aphysiologischen Verhältnissen auch durch die Neurohypophyse erfolgt. Nach Hydratation hört die Entleerung der Neurohypophyse auf, die Funktion der Eminentia medialis portalis vermindert sich oder wird eingestellt und das interrenale Gewebe der Nebennieren atrophiert. Wasserentzug übt keine Wirkung auf die Funktion der Eminentia medialis portalis aus, deshalb ist es wahrscheinlich, daß die Hypertrophie der Nebennieren durch eine aus der Pars nervosa in bedeutender Menge entleerte neurosekretorische Substanz bedingt ist. Dies läßt die Annahme zu, daß das Gömöri-positive neurosekretorische Neurohormon der Vögel über »Corticotrophine-Releasing Factor« Eigenschaft verfügt.

ДЕЙСТВИЕ ЖАЖДЫ И ВОДНОЙ НАГРУЗКИ НА ГИПОТАЛАМИЧЕСКУЮ РЕГУЛЯЦИЮ НАДПОЧЕЧНИКОВ У ДОМАШНЕГО ГОЛУБЯ (COLUMBA LIVIA)

П. ПЕЦЕЛИ

Автор исследовал действие жажды в течение 4 или 8 дней и водной нагрузки на нейросекреторную систему домашнего голубя и связь между этим действием и реакцией межпочечной ткани надпочечников. Он установил, что под влиянием обезвоживания или водной нарузки реагировала в первую очередь преоптическая область nucleus praeopticus. В ответной реакции на водную нагрузку функциональное различие остальных участков проявляется в меньшей степени. Nucleus entopeduncularis не показывает достоверного изменения, ни после обезвоживания, ни после гидратации. Внешняя зона среднего возвышения гипоталамуса не реагирует на жажду. После пробы с водой в палисадном слое накопляется альдегидфуксин-положительное вещество. После жажды нейрогипофиз опорожняется, а после гидратации он наполняется выделением.

Полученные результаты указывают на возможность того, что гипоталамическое регулирование надпочечников осуществляется посредством среднего возвышения, а в нефизиологических условиях также посредством нейрогипофиза. После гидратации опорожнение нейрогипофиза прекращается. Уменьшается или прекращается также портальная функция eminentia mediana гипоталамуса и межпочечная ткань надпочечников атрофируется. Жажда не влияет на портальную функцию eminentia mediana гипоталамуса и, следовательно, ответственным за возникновение гипертрофии надпочечников является, предположительно, нейросекреторное вещество, выделяемое в большом количестве из pars nervosa. На основании сказанного можно предполагать, что Гомори-положительный нейросекреторный нейрогормон птиц обладает свойством фактора Corticotrophin Releasing Factor (CRF).

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STEROIDAL INFLUENCE ON ADRENOMEDULLARY CATECHOL HORMONES OF THE PIGEON

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The endocrine interrelationship of adrenal medulla of pigeon to some steroidal preparations have been studied. Two corticoids, namely cortisone acetate and desoxycorticosterone acetate, and sex steroids — i.e. stilbestrol, stilbestrol plus testosterone propionate, testosterone propionate alone, and estradiol dipropionate have been used to observe alterations in the cytochemically demonstrable catechol hormones in this avian species. The glucocorticoid and the combined sex steroid treatment (stilbestrol with testosterone) produce a differential action on the medullary catechol hormone in the pigeon. Cortisone produces noradrenalin depletion while the combined sex steroid therapy elevates noradrenalin content accompanied by an overall medullary hypertrophy (about + 30%). Stilbestrol treatment alone also shows some chromaffin hypertrophy (about + 15%) associated with noradrenalin increase. Testosterone reveals a decrease in the noradrenalin content. Estradiol and desoxycorticosterone remain completely ineffective in producing perceptible alterations in medullary hormonal concentration. In general, steroidal therapy in pigeons does not bring out any significant change in the total catechol amine concentration as revealed by chromate-dichromate reaction.

The role of corticoid in methylation of medullary catechol hormones, association of noradrenalin release with the pressor homeostasis as well as the part played by the sex steroids in producing artificial avian pheochromocytoma have been pointed out and briefly discussed.

The action of medullary hormone is at least partially controlled by the action of cortical steroids — this has been a subject of research for some time. CLEGHORN et al. [1] reported that after adrenalectomy, dog has been found to be less sensitive to the pressor effect of adrenalin. This has also been confirmed in cases of infused noradrenalin [9]. LEVINE (cited by VOGT [17]) has demonstrated that the vasoconstrictor effect of noradrenalin is only maintained in the presence of cortisone and allied compounds. According to VOGT [17] cortical hormone appears to permit the response of different tissues to the stimulating action of medullary amines. Storage-release mechanisms of adrenalin and noradrenalin are directly controlled by cortical hormones (cortisone and hydrocortisone) was first established by ROFFI [12]. As far as our knowledge goes these observations provide the first direct evidence of the influence of cortical hormones on chromaffin cells. Finding of similar nature with extra-adrenal chromaffin tissue of the rat has also been shown by LEMPINEN [6, 7].

Besides corticoids another steroid capable of producing changes in the medullary hormonal milieu is the estrogenic hormone [8]. This author has

shown that after administration of estrogenic substances or growth hormone, a chromaffin hyperplasia, sometimes pheochromocytoma is resulted. Based on rather scanty data our previous observation [10] also reports that estrogen causes a loss of noradrenalin content in the pigeon adrenal medulla.

Thus, from previous paragraphs it appears that certain glucocorticoids as well as ovarian steroids may effectively bring out changes in the amine-hormonal concentration of the adrenal medulla. In view of this, we thought that it might be of interest to see the action of other steroids (as well as these) on the concentration of avian medullary hormones.

The present report describes the effect of cortisone acetate, desoxycorticosterone acetate, diethylstilbestrol, diethylstilbestrol plus testosterone propionate and estradiol dipropionate on the cytochemically demonstrable catecholamines of the domestic pigeon.

Material and methods

Adult male pigeons average body weight 250—300 gm were divided into six experimental groups. Details of the experimental schedule are given in Table I. After termination of respective experiment both control and treated groups were sacrificed by cervical dislocation and their adrenal glands were taken out immediately and plunged into appropriate fixatives for cytological and cytochemical studies. For total catechol amines, adrenals were treated with a mixture of 5% potassium dichromate and 5% potassium chromate (10 : 1) solutions for 24 hours and subsequently transferred to 10% formalin for 24 hours. To detect noradrenalin the glands were fixed in saturated solution of potassium iodate for 24 hours and in turn these were immersed in 10% formalin for a day. Followed by these treatments sections were prepared

Table I

Experimental schedule

Experiment	Group	Treatment
I	Cortisone (12)*	5 mg of cortisone acetate ("Cortogen" Schering, N. J.) in sterile water injected intramuscularly daily for 15 days
	Control (12)	None
II	DCA (6)	Weekly injection of 5 mg of desoxycorticosterone acetate ("Percorten" Ciba) in oil given for 64 days
	Control (6)	None
III	Stilbestrol (6)	45 mg of diethylstilbestrol pellets ("Stikap" May and Baker) implanted at the neck region. Kept for 45 days
IV	Stilbestrol and Testosterone (6)	From the second day after the stilbestrol implantation a weekly injection of 5 mg of testosterone propionate ("Oreton" Schering N. J.) was given for 45 days
	Control for III and IV (6)	None
V	Testosterone (6)	Weekly injection of 5 mg of testosterone propionate ("Perandren" Ciba) in oil was given. Total period of treatment was 45 days
	Control (6)	None
VI	Estradiol (6)	5 mg of estradiol dipropionate ("Ovocyclin" Ciba) in oil injected weekly for 36 days
	Control (6)	None

* Numericals in parenthesis indicate the number of pigeons used.

after a 30 minute embedding in paraffin at 56 °C (HILLARP and HÖKFELT's [5] technique as modified by FALCK and HILLARP [2]; GHOSH and GHOSH [4]). Hematoxylin and eosin and Heidenhain's azan technique were used as routine cytological procedure.

Results

It has been observed that cortisone treatment provokes a marked loss in the body weight of the pigeon ($P < 0.005$). A loss of appetite and low intake of food is also noticed in the corticoid group. The testoid and the synthetic estrogen (Group IV) elicits an increase in the glandular weight ($P < 0.001$) while body weights of the treated pigeons remain practically unchanged. Aggressiveness, restlessness and occasional mounting become prominent features of this hormonal administration. The food intake of these birds, however, remains normal. High rate of mortality also characterizes this group of treatment. In all other cases of steroidal treatment no significant change in body weight, gland weight and behaviour could be noted.

Control

Histological. The microscopic examination of the histological preparation reveals that the pigeon adrenal is made up of an outer connective tissue capsule and an inner glandular parenchyma composed of intermingled masses of cortical and medullary tissues. Medullary strands are of irregular size and shape, consisting of randomly oriented cells with oval nuclei (Fig. 1). The detailed histological description of this tissue has been presented by SINHA et al. [13].

Histochemical. Dichromate reaction for total catecholamines: The whole medullary patch gives yellowish brown coloration (Fig. 3). Granular nature of variable stainability can be noted under higher magnification.

Iodate reaction for noradrenalin: About 55—65% of medullary cells (Fig. 5) showing brownish yellow colour, indicate the presence of noradrenalin [3].

Treated

Histological. The cortisone treatment has altered the normal histological characteristic of adrenomedullary cells. The cortical region appears highly shrunken and nuclear orientation especially of the cortex becomes almost disorganized. Stainability of both medullary and cortical cells increased to a considerable extent. Simultaneous with the atrophic changes in the cortical strands, a somewhat compensatory hypertrophy of the medullary tissue is also noticed.

An overall medullary hypertrophy is possibly the most remarkable histological feature of the stilbestrol plus testosterone treated group. This treatment also causes an increase in cellular stainability similar to our observations on cortisone group (Fig. 2). DCA and stilbestrol alone also induces chromaffin

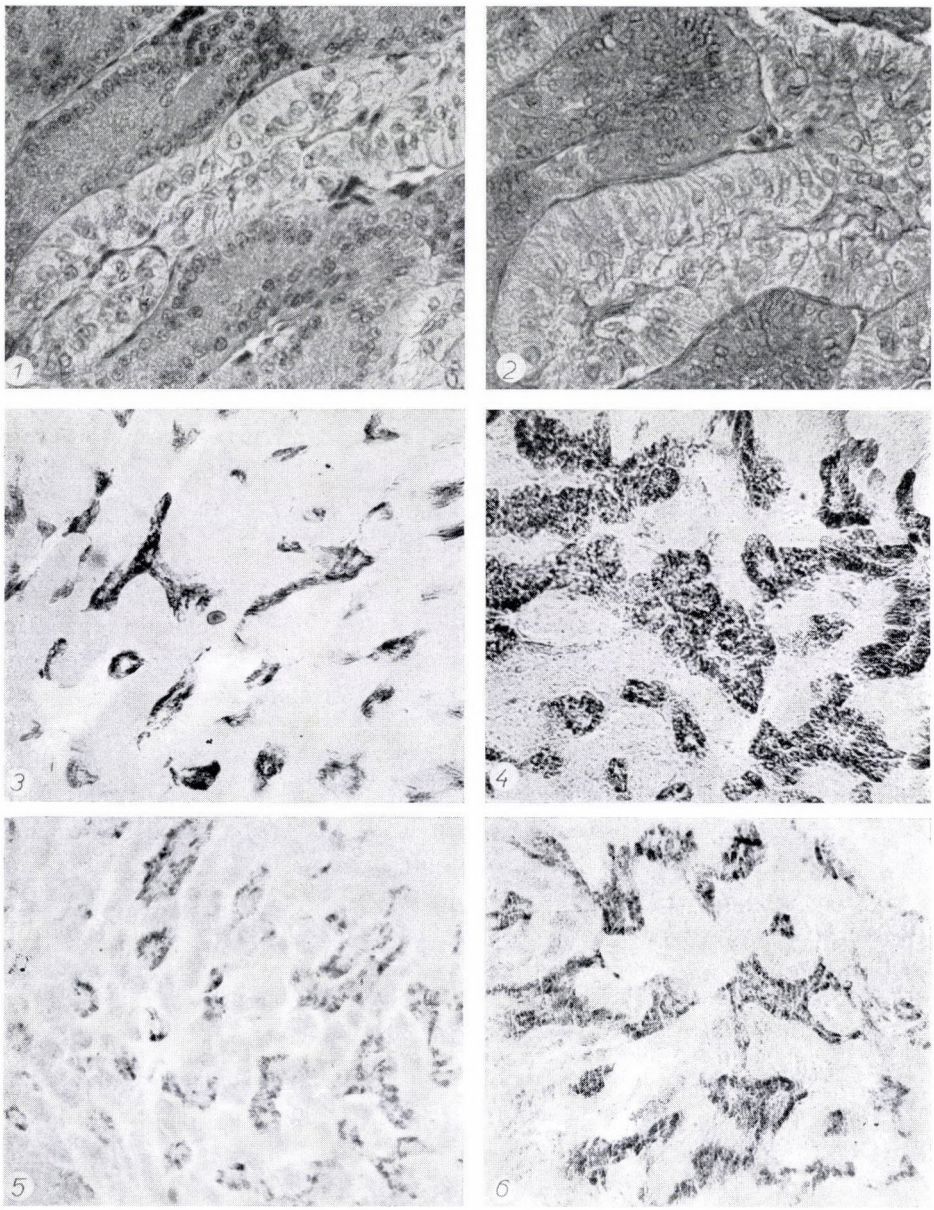

Fig. 1. Adrenal medulla of the control pigeon. Heidenhain's azan, × 400
Fig. 2. Stilbestrol plus testosterone treated birds showing medullary hypertrophy. Heidenhain's azan, × 400

Fig. 3. Adrenal medulla of the pigeon showing chromate-dichromate reaction, × 100
Fig. 4. Hypertrophied medullary tissue of the cortisone-treated pigeons. Note an increase in the total catechol amine concentration, × 100

Fig. 5. Iodate positive noradrenalin containing cells in the control pigeons, × 60
Fig. 6. Hypertrophied iodate positive medullary tissue of the stilbestrol plus testosterone treated pigeons. Staining intensity of the iodate reaction has also been greatly increased, × 60

hypertrophy to some extent. Other steroids practically remain ineffective in producing histologic changes.

Histochemical. Histochemical findings on chromate-dichromate and iodate reactions of the control and treated groups are summarized in Table II. A perusal of the table reveals that cortisone shows a loss of noradrenalin with an increase in total catechol amine reaction (Fig. 4). Concentration of iodate positive material is greatly increased in stilbestrol plus testosterone treated group (Fig. 6), and to some extent in stilbestrol treatment. But testosterone injection only gives a faint iodate positive reaction in large number of medullary cells. With the other steroidal treatment the cytochemical pattern of the medulla practically remains unaltered.

Discussion

It is evident from the present investigation that the glucocorticoid and sex steroids have different action on the medullary catechol hormone concentration in the pigeon. Thus, the cortisone is responsible for the depletion of noradrenalin while the combined administration of stilbestrol plus testosterone elevates medullary noradrenalin content as well as induces chromaffin hypertrophy (Table II).

To explain the cortisone induced depletion of noradrenalin, two alternatives may be suggested. Firstly, the depletion may be due to the release of noradrenalin associated with a concomitant rise of adrenalin and secondly, a conversion of noradrenalin to adrenalin by methylation mechanism (cf. WRIGHT and CHESTER JONES [18] and LEMPINEN [6]) may be held responsible for the noradrenalin loss. Release of noradrenalin may be well explained if we assume that the cortisone is capable of producing hypertension in the pigeon. It may be worth mentioning here that the cortisone-induced hypertension has already been recorded in another avian species *(Gallus)* by STAMLER in 1954 (vide STURKIE and RINGER [14]). In view of this it is quite possible that this high rate of noradrenalin secretion in the pigeon becomes indispensable for the maintenance of homeostasis during glucocorticoid-induced hypertension. It may be mentioned here that the mineralocorticoid used in the present experiment fails to cause any change in the hormone content of the hypertrophied adrenal medulla. An overall increase of noradrenalin becomes the resultant of the combined sex hormone (stilbestrol plus testosterone) therapy. This is evidenced from the relative staining intensity of chromate-dichromate and iodate reactions of the control and the hormone recipients (Table II). It is known that pheochromocytoma can be induced in experimental rats by prolonged administration of estrogenic hormones [8] and it is also reported that noradrenalin becomes predominantly secretory hormone of the adrenal medulla in case of human pheochromocytoma [15]. It seems, therefore, that the increase in norad-

Acta Morphologica Academiae Scientiarum Hungaricae 14, 1966

Table II

Body and adrenal weights, percentage of iodate and dichromate positive areas and staining intensity of Hillarp and Hökfelt's reaction in controls and steroid treated pigeons

Group	Treatment	Days of treatment	Body weight (gm) Mean ± SE		Adrenal weight (mg) Mean ± SE	% of total medullary area	% of Iodate (1 positive area 1)	Staining intensity (average) 2)	
			Initial	Final				Chr-di	Iodate
I	Cortisone (12)	15	289.09 ± 16.40	263.27 ± 21.33	21.32 ± 0.96	40	50	4.5	0.5
	None (12)	—	251.64 ± 6.56	258.5 ± 7.60	20.25 ± 3.87	35	45	4	2
II	DCA (6)	64	256 ± 6.21	261 ± 4.30	21.28 ± 2.12	45	65	4	2
	None (6)	—	267.0 ± 19.3	270 ± 15.22	22.86 ± 1.79	35	60	4	2
III	Stilbestrol (6)	45	238.4 ± 8.93	247.20 ± 12.54	*	38	67	4	2.5
	None (6)	—	266.25 ± 15.5	303.75 ± 21.38	*	33	60	4	2
IV	Stilbestrol + Testosterone (6)	45	281.66 ± 6.03	301.66 ± 19.2	32.88 ± 2.82	45	75	4	4
	None (6)	—	266.25 ± 15.5	303.75 ± 21.38	17.93 ± 2.64	30	50	4	2
V	Testosterone (6)	45	316.7 ± 9.23	318.3 ± 7.22	26.4 ± 3.2	33	80	4	1.5
	None (6)	—	292 ± 16.63	283.3 ± 11.61	31 ± 6.63	38	60	4	2
VI	Estradiol (6)	36	226.6 ± 23.5	298.3 ± 22.4	*	40	65	4	2
	None (6)	—	266.25 ± 15.5	303.75 ± 21.38	*	36	63	4	2

1) Visual estimation; 2) Estimated visually and arbitrarily graded on a scale of 0—5 [11]
* Not recorded.

renalin concentration is possibly associated with the chromaffin hypertrophy leading to pheochromocytoma. This relationship between the high rate of noradrenalin synthesis and chromaffin hypertrophy holds good in case of pigeons, chronically treated with stilbestrol. When the latter was used in combination with testosterone, the concentration of noradrenalin appears to be higher as compared to the pigeons treated with synthetic estrogen alone. It is quite possible that this augmentation of non-methylated hormone of the adrenal medulla in pigeons is due to a synergistic action of the testoid and stilbestrol. The synergistic action of testicular hormone and estrogen is pretty well known in the endocrinological literature [16]. But when natural estrogen (estradiol) was used in the pigeon in place of synthetic one, the medullary noradrenalin content did not increase. This result is difficult to explain. Possibly a more prolonged treatment with estradiol would have caused a higher mobilization of noradrenalin in the pigeon adrenal medulla.

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DIE WIRKUNG VON STEROIDEN AUF DIE KATECHOLHORMONE DES NEBENNIERENMARKS DER TAUBE

D. CHAUDHURI, I. GHOSH und A. GOSH

Die endokrinen Zusammenhänge zwischen dem Nebennierenmark der Taube und einigen Steroidpräparaten wurden untersucht. Zwei Kortikosteroide, namentlich Cortisonazetat und Desoxykortikosteronazetat, ferner die Sexualsteroide Stilböstrol, Stilböstrol + + Testosteronpropionat, Testosteronpropionat allein sowie Estradiolpropionat wurden verabreicht und beobachtet, welche Veränderungen sich unter ihrer Wirkung in den Katecholhormonen dieser Vogelart nachweisen lassen. Die Glukokortikoid- und die kombinierte Sexualsteroidbehandlung (Stilböstrol + Testosteron) übten auf das medulläre Katecholhormon der Taube eine unterschiedliche Wirkung aus. Cortison bewirkt eine Noradrenalinabnahme, während die kombinierte Sexualsteroidbehandlung bei gleichzeitiger allgemeiner Hypertrophie (etwa + 30%) des Nebennierenmarks die Noradrenalinkonzentration erhöht. Stilböstrolbehandlung allein erzeugt ebenfalls eine geringe Hypertrophie der chromaffinen Zellen (etwa + 15%) bei paralleler Zunahme des Noradrenalingehaltes. Testosteron bewirkt eine Abnahme des Noradrenalingehaltes. Estradiol und Desoxykortikosteron erwiesen sich als vollkommen wirkungslos und riefen keinerlei nachweisbaren Veränderungen in der Hormonkonzentration des Nebennierenmarks hervor. Wie aus der Chromat-Dichromat-Reaktion hervorgeht, ruft die Steroidbehandlung bei der Taube im allgemeinen keine signifikanten Veränderungen in der Katecholaminkonzentration hervor.

Die Rolle der Kortikoide in der Methylierung der medullären Katecholhormone, der Zusammenhang zwischen Noradrenalinfreisetzung und Pressor-Homeostase, ferner die Rolle der Sexualsteroide in der Erzeugung künstlicher Phäochromozytome bei Vögeln werden kurz erörtert.

ДЕЙСТВИЕ СТЕРОИДОВ НА АДРЕНОМЕДУЛЛЯРНЫЕ КАТЕХОЛГОРМОНЫ ГОЛУБЯ

Д. ХАУДХУРИ, И. ГОШ и А. ГОШ

Авторы исследовали действие некоторых стероидных препаратов на эндокринологические условия мозгового вещества надпочечников голубей. Два кортикоида, а именно, кортизонацетат и дезоксикортикостеронацетат, и половые стероиды: стильбестрол, стильбестрол + пропионат тестостерона, пропионат тестостерона, а также дипропионат тестостерона применялись в целях выявления изменений катехолгормонов, определяемых цитохимическими методами исследования у этих видов птиц. Дача глюкокортикоидов или комбинации половых стероидов (стильбестрол + тестостерон) вызывает в концентрации медуллярных катехол-гормонов голубей отклоняющиеся изменения. Кортизон вызывает уменьшение количества норадреналина, в то время как комбинированное введение половых стероидов повышает концентрацию его, при общей гипертрофии мозгового вещества (около + 30%). Дача одного только стильбестрола также приводит к некоторой гипертрофии хромаффиновых клеток (около +15%), сопровождаемой повышением содержания норадреналина. Тестостерон вызывает уменьшение концентрации норадреналина. Эстрадиол и дезоксикортикостерон оказались совершенно безэффективными и не вызвали выявляемых изменений концентрации гормонов мозгового вещества надпочечников. В общем, на основании реакции хромат-дихромат, у голубя дача стероидов не вызывает достоверного изменения концентрации общих катехоламинов.

Обсуждается роль кортикоидов в метилировании медуллярных катехолгормонов, связь между освобождением норадреналина и прессорным гомеостазом, а также роль половых стероидов в развитии искусственных феохромоцитом у голубя.

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EFFECT ON GUINEA PIGS OF INTRATRACHEALLY ADMINISTERED ZIEHL—NEELSEN POSITIVE POLLENS

(DATA CONCERNING THE AETIOLOGICAL CORRELATION BETWEEN POLLEN GRANULOMATA AND SARCOIDOSIS)*

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(Received November 25, 1965)

The pollen of two varieties of reed-mace *(Typha latifolia* and *angustifolia)* has been introduced into the trachea of guinea pigs. The pollens were partly intact, partly crushed, and partly administered as their lipid extracts. This treatment failed to induce an epithelioid cell reaction, and all that was observed after eight months consisted in aspecific foreign body giant cell granulation. The supposition that — like pine pollen — other pollens containing Ziehl—Neelsen positive components may induce sarcoidosis-like epithelioid cell granuloma has, thus, not been borne out by the experiments. While the presence of pollen constituents was demonstrated in the lungs and regional lymph nodes, their fat contents and positive Ziehl—Neelsen staining gradually disappeared from the fifth month on. The lipid extract of pollen seemed to be biologically more active than the pollen itself. Pretreatment with DOCA promoted the connective-tissue reaction without changing its quality.

CUMMINGS et al. [2, 3] have shown that the majority of patients with sarcoidosis had had their birth place or domicile in the pine-wood belts of the United States. In their search of possible pathogens they discovered that certain constituents of the loblolly pine pollen took the Ziehl—Neelsen stain like *M. tuberculosis.* The intracutaneous injection of pine-pollen extract suspended in paraffin oil induced epithelioid-cell granulomata in the test animals. By the administration of pine pollen or its lipid extract, VOGEL and THRASH [12], further LINDNER et al. [8] succeeded in evoking local and sometimes even systemic epithelioid cell reactions.

Relying on such evidence, CUMMINGS [1, 2, 3] advanced the theory that sarcoidosis was due to an acid-fast constituent of pine pollen. Since this cannot apply to conditions in Hungary where there are no pine forests, while the number of patients with sarcoidosis is quite considerable [9], it seemed justified to examine the biological effect of the pollen of certain plants widespread in Hungary. There was one among them, the cattail (reed-mace), both varieties of which *(Typha latifolia* and *angustifolia)* were found to have Ziehl—Neelsen-positive pollen. The geographical distribution of these plants may have an aetiolog-

* Part of this study has been submitted to the Third International Sarcoidosis Conference, Stockholm, 1963.

ical significance since they grow all over the country in stagnant or slowly flowing waters, salt lakes, in the ditches of rice fields and in reedy marshes, sometimes separately, sometimes together. Apart from its aetiological aspects, the study was hoped to help in preparing an experimental model of sarcoidosis.

Material and method

After mixing equal amounts of the pollens of *Typha latifolia* and *angustifolia* (Fig. 1) and suspending them in physiological saline, the mixture was homogenized for 30 minutes. Since part of the pollens remained intact in Potter's homogenizer, it was possible to study the effect of crushed and unimpaired pollens simultaneously. The lipid extract of pollen was

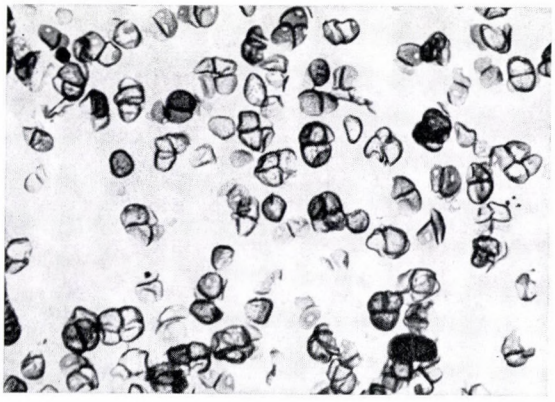

Fig. 1. Pooled pollens of *Typha latifolia* and *angustifolia*. Section embedded in paraffin and stained according to Ziehl—Neelsen. Marked positive reaction in the outer layer (red, but black in the picture), less marked in the inner layers. × 120

employed in a separate series of experiments. To obtain the extract, the pollens were homogenized for one half hour in a mixture of alcohol, ether and chloroform, and then extracted during four days in the same mixture. The extract was filtered and evaporated in a water bath. The viscous residuum was suspended in physiological saline and then administered intratracheally. With a view to preventing secondary infection, 20,000 units/ml of penicillin were given with both the pollen suspension and the pollen extract.

Twenty guinea pigs of both sexes, with body weights between 300 and 500 g, were divided in four groups, with five members per group. After being ether anaesthesized, 0.3 ml of pollen suspension or extract was sprayed into the trachea through a plastic tube mounted on a syringe. Two of the groups received, prior to the treatment with pollen, 0.4 mg of DOCA daily intramuscularly for eight days, in order to promote the connective-tissue reaction. The administration of pollen was repeated a month later. The animals were bled to death in ether anaesthesia between the first and fifth days, on the 46th, 76th, 150th and 240th day. After removing the lungs together with the paratracheal and broncho-pulmonary lymph nodes, they were fixed in a neutral 10 per cent formalin solution of room temperature, and the bronchial tree was likewise filled with formalin. On the average, five pulmonary areas and the lymph nodes of at least three different regions were examined partly for fats and partly in paraffin-embedded 5 μ thick sections after staining haematoxylin-eosin and Ziehl—Neelsen's dye. The periodic-acid Schiff and Gomori's acid phosphatase reactions were also performed in a few instances [6]. Lipids were demonstrated in formalin-fixed frozen sections by means of Sudan black B, alcoholic-acetonic Sudan III—IV solution, oil-red O dissolved in isopropyl alcohol, and by means of Nile blue sulfate. Fifteen animals served as controls. Three were

killed without treatment; four were anaesthetized with ether and allowed to survive for a few weeks; eight received physiological saline without pollen but otherwise under the same experimental conditions as the test animals; four of these last eight were pretreated with DOCA.

Results

The lungs of the control animals showed slight interstitial chronic inflammation with infiltration consisting of a few lymphocytes, plasma cells, occasional eosinophils and — in one case — of foreign-body giant cells. The amount of interstitial connective tissue was slightly increased and enlarged perivascular and peribronchial nodes of lymphoid cells were present. These changes are of

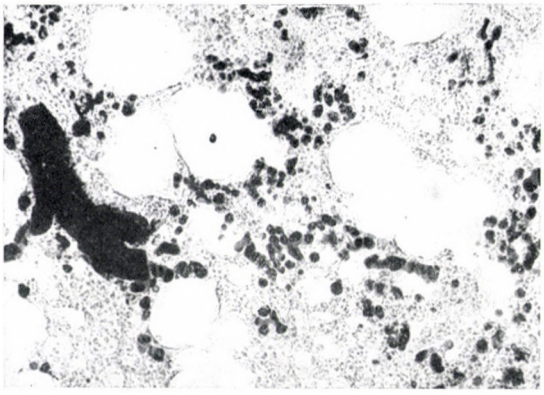

Fig. 2. Pollen lipid extract in the pulmonary vessels and alveoli of a guinea pig which died of shock one day after the intervention. Formol-fixed frozen section. Sudan III—IV-haemalum stain. × 70

unknown aetiology but appear so frequently under physiological conditions that they belong to the normal microscopic picture of the guinea-pig lung [11]. All control animals revealed the same microscopic picture.

Pollens, parts of them and also their extract had passed into the lungs and were demonstrable there by fat stains (Fig. 2). Foreign body giant cells formed around the pollen particles on the very first day, while around intact pollens somewhat later. Part of the lipid extract was demonstrated in the pulmonary capillaries and venules of those two test animals which had died of shock one or two days after the injection. Beside giant cells, infiltration with histiocytes with foamy cytoplasm, accumulation of plasma cells and eosinophils were observed. These changes were well distinguishable from the basic ones, and increased up to the fifth month (Figs. 3, 4). The pollens, their particles and extracts were demonstrated by means of fat stains or according to Ziehl—Neelsen, while the intensity of staining was gradually decreasing. The granulation showed acid-phosphatase activity; the cytoplasm of the macro-

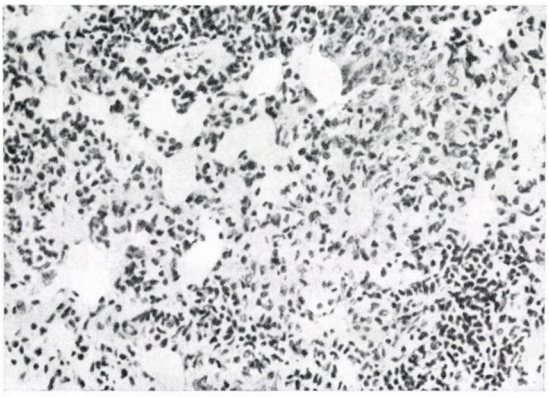

Fig. 3. Conditions at 240 days. Histiocytic granulation in lungs with lymphocytes, plasmacytes and eosinophils. Haematoxylin-eosin. × 120

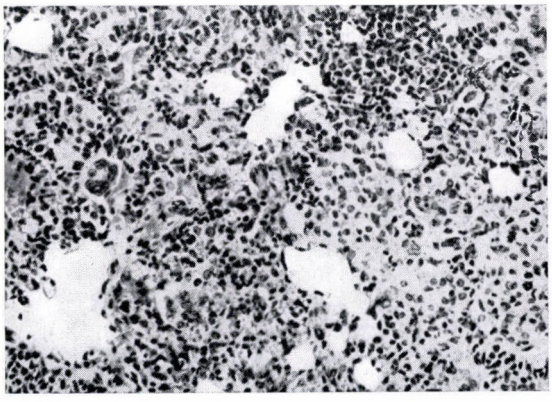

Fig. 4. Conditions at 240 days. Granulation as in Fig. 3; foreign body giant cell on the left side. Haematoxylin-eosin. × 120

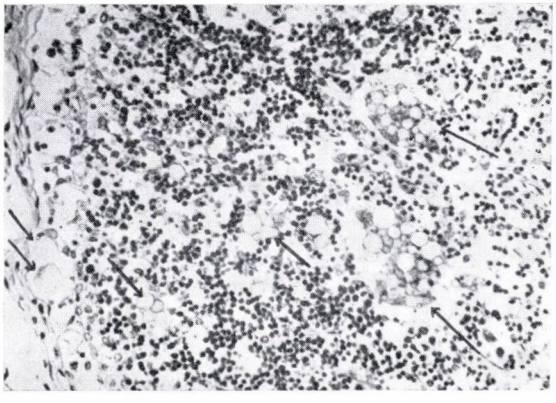

Fig. 5. Fine globules of pollen lipid extract in the marginal and intermediary sinuses of lymph node, indicated by arrows. Ziehl—Neelsen stain. × 100

phages and giant cells contained partly homogeneous and partly slightly granular PAS-positive substance which often formed a ring around the phagocyted lipid globules. The fatty substances of the pollens ceased to take the stain, were split and carried off between the fifth and eighth months. Granulation ceased to progress but remained more or less stationary during that period. Considerable infiltration with eosinophils was observed in some cases; and these were interspersed with histiocytes with a large pale, sometimes foamy cytoplasma, containing large weakly stained nuclei. The pattern was similar to that seen in cases of human histiocytosis X. No epithelioid cell reaction was registered during the eight-month period of observation (Table I).

Table I

	Chronic aspecific inflammation	Foreign-body type inflammation	Epithelioid cell granuloma
Pollen suspension	+	+	—
Pollen + DOCA	+	++	—
Pollen lipid extract	+	++	—
Pollen extract + DOCA	+	+++	—
Controls	+	—	—

The lipid extract of pollen that had found access to the lungs was demonstrable in the marginal and intermediary sinuses of the mediastinal lymph nodes in the very first days, but there was no cell reaction around them. The subsequent microscopic picture of the regional lymph nodes showed the features of chronic sinal catarrh, with a moderate accumulation of eosinophils in some cases, irrespective of whether or not lipid extract was present.

The granular reaction was most pronounced in animals which had been treated with the lipid extract and pretreated with DOCA. The lipid extract caused a stronger reaction than crushed pollen, and the latter a more active one than unimpaired pollen. The connective-tissue reaction was decidedly promoted but not qualitatively changed by pretreatment with DOCA.

Discussion

Animal experiments have so far been concerned with the effect of pine pollen only, and the results were contradictory. CUMMINGS et al. [2] provoked local epithelioid cell reaction by injecting guinea pigs intracutaneously with pollen suspended in paraffin oil. LINDNER et al. [8] injected a benzene extract of pollen into the veins or spleen of rats, rabbits and guinea pigs, and observed as a result epithelioid cell reaction in the liver, spleen, lungs and peribronchial lymph nodes. The chloroform-methanolic extract of pollen produced no granulomata in their experiments. Eight months after injection of pine pollen, the biologically active substances have been broken down to molecular size, lost

their acid fastness and were carried to other organs. HÄGERSTRAND and LINELL [7], BRIEGEL [4] and — in some experiments — CUMMINGS [3] failed to elicit epithelioid cell granulation by treating the animals with pollen or its lipid extract. It follows that sarcoidosis is not induced by pine pollen or its Ziehl—Neelsen positive constituent. Apart from the results of these experiments, another argument against this theory is the fact that — although CUMMINGS demonstrated a geographical connection between pine-woods and sarcoidosis in the U. S. A. and WALLGREN [13] in Sweden, there are other countries, such as Switzerland [10], West Berlin [5] and Hungary [9] where no such interconnection exists. On the other hand, it has been shown that, under adequate experimental conditions, pine pollen and its extracts may produce epithelioid cell granuloma not merely where they are injected but in remote organs as well. This phenomenon has been ascribed by CUMMINGS and HUDGINS [1] to the vegetable-wax and pimelic-acid contents of the pollen and by LINDNER et al. [8] to long-chain fatty acids and to an octadecanol-type alcohol, the latter presumably forming an ester bound with some waxlike vegetable substance. A polysaccharide-like component was also demonstrated in the benzene extract applied by LINDNER et al. [8].

The present experiments have, thus, failed to substantiate the theory that sarcoidosis in Hungary might be due to the Ziehl—Neelsen positive pollen of reed mace. An aspecific chronic granulation of foreign-body character but no epithelioid cell granuloma developed in the guinea pigs during the eight months of the present experiments. Nor are acid-phosphatase activity and the formation of PAS-positive material specific phenomena. The occasional massive accumulation of eosinophils may be indicative of an allergenic component. The microscopic picture suggestive of histiocytosis X, as seen in some cases, points to the possibility that certain fatty substances — those spread by pollens, among others — may promote the development of aspecific granulative processes. Such substances presumably disintegrate and are absorbed; it is therefore impossible to demonstrate them locally after some time, whereas the granulation induced by them persists for long.

It is, of course, possible, that epithelioid cell granulomata can be induced by the Ziehl—Neelsen positive pollen of some other plants in some other species and under other experimental conditions, so that not only pine pollen might elicit the reaction in question. Further researches are necessary to elucidate the problem.

Acknowledgement

We are indebted to the Agricultural College, Debrecen, for the pollens.

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DIE WIRKUNG VON INTRATRACHEAL VERABREICHTEN ZIEHL—NEELSEN-POSITIVEN POLLEN AUF MEERSCHWEINCHEN

J. T. KELEMEN und L. MÁNDI

Nach intratrachealer Verabreichung des ZIEHL—NEELSEN-positiven Stoff enthaltenden Pollens der *Typha latifolia* und *T. angustifolia* ferner des angegriffenen Pollens und des chloroform-äther-alkoholischen Fettextraktes des Blütenstaubes ließ sich am Meerschweinchen in der acht Monate währenden Beobachtungsperiode keine epitheloidzellige Granulation nachweisen. Lediglich eine aspezifische Granulationsreaktion vom Fremdkörperchentyp wurde beobachtet. Somit fand die Annahme, daß ähnlich dem Pollen von Koniferen auch andere, gleichfalls ZIEHL—NEELSEN-positive Stoffe enthaltende Pollen eine der Sarkoidose ähnliche epitheloidzellige Granulation hervorrufen können, keine Bestätigung. In den Lungen und den regionären Lymphknoten konnten die Pollensubstanzen nachgewiesen werden, doch büßten sie nach dem fünften Monat ihre Fett- und ZIEHL—NEELSEN-Positivität allmählich ein. Über die höchste biologische Aktivität verfügte der Fettextrakt. DOCA-Vorbehandlung förderte zwar die Bindegewebsreaktion, doch ohne eine qualitative Veränderung derselben zu bewirken.

ДЕЙСТВИЕ ИНТРАТРАХЕАЛЬНОГО ВВЕДЕНИЯ МОРСКИМ СВИНКАМ ПЫЛЬЦЫ, СОДЕРЖАЩЕЙ ЦИЛЬ—НИЛЬСЕН-ПОЛОЖИТЕЛЬНЫЕ ВЕЩЕСТВА

Й. Т. КЕЛЕМЕН и Л. МАНДИ

В течение 8 месяцев после интратрахеального введения морским свинкам пыльцы разрушенной пыльцы и спирто-эфирно-хлороформного жирового экстракта пыльцы широколистного и узколистного рогоза (Typha latifolia, T. angustifolia) содержащей Циль—Нильсен-положительное вещество, авторы не наблюдали у животных эпителоидо-клеточной грануляции, а лишь неспецифическую реакцию типа грануляции, вызванной инородным телом. Следовательно, предположение, согласно которому, подобно пыльце хвойных деревьев, пыльца других растений, также содержащая Циль—Нильсен-положительные составные части, может вызвать эпителоидо-клеточную грануляцию, напоминающую саркоидоз, не нашло подтверждения. Пыльцевые вещества были выявлены в легких и в регионарных лимфатических узлах, однако, они, начиная с пятого месяца, постепенно утрачивали свою Циль—Нильсен-положительность. Наибольшей биологической активностью обладали жировые вытяжки пыльцы. Предварительная дача ДОКА способствовала соединительнотканной реакции, но не вызвала ее качественного изменения.

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Department of Forensic Medicine (Head: Prof. J. RAEKALLIO), University of Turku

PHOSPHATASE ACTIVITY IN THE INITIAL PHASE OF HEALING OF SKIN WOUNDS

HISTOCHEMICAL COMPARISON BETWEEN THE METAL-SALT AND AZO-DYE METHODS*

J. RAEKALLIO and A. JÄÄSKELÄINEN

(Received January 26, 1966)

Phosphatase activity was investigated histochemically in healing rat skin wounds, comparing the metal-salt and azo-dye methods. The metal-salt technique for acid phosphatase showed more extracellular staining and higher contrast of the final precipitate during the whole experimental period of 1 to 128 hours. The Gomori-method for alkaline phosphatase exhibited a more intense reaction during the initial increase in enzyme activity of the wound periphery from five to eight hours post-operatively. The azo-dye technique revealed more clearly the decrease in activity of alkaline phosphatase in the vicinity of the wound edge.

The activity of phosphatases has previously [9, 10] been demonstrated histochemically in the earliest phase of wound healing, by using the azo-dye methods. Some experiments have recently suggested that the metal-salt and azo-dye methods do not demonstrate activity of identical enzymes [13]. The metal-salt methods for phosphatases have not yet been applied to the investigation of the initial phase of wound healing. It seemed therefore interesting to compare histochemically the results of the azo-dye methods with those of the metal-salt techniques.

Material and methods

24 healthy, male, four-month-old albino rats of approx. 200 g weight were used. Circular wounds, 5 mm in diameter, were cut in a shaved dorsal area. The skin inside the circle was excised, and the wounds were neither sutured nor dressed. The animals were decapitated at intervals of 1, 2, 3, 4, 5, 6, 7, 8, 16, 32, 64 and 128 hours. Squares of skin containing the wounds were removed immediately and frozen with solid carbon dioxide.

Sections were cut in a cryostat at 16 μ and fixed for 5 minutes at +4 °C in neutral 10 per cent formalin. Alkaline phosphatase activity was visualized *a)* by the method of GOMORI [5] as described by BURSTONE [2], and, in alternate sections, by the azo-dye method of GROGG and PEARSE [6], as presented by PEARSE [8]. Acid phosphatase activity was demonstrated *c)* by the method of GOMORI [5], as described by BURSTONE [2], and, additionally, *d)* by the azo-dye method of GROGG and PEARSE [7], according to PEARSE [8]. The sections were incubated as follows: at +37 °C for 1 hour (method *a*) or for 2 hours (method *c*), or at room temperature for 40 minutes (the azo-dye methods *b* and *d*). Fast violet B and Fast garnet GBC were used as diazonium salts for the methods *b* and *d*, respectively. Control sections were incubated without the substrate.

* This work was supported by grants from the Sigrid Juselius Foundation and from the Finnish Medical Council.

Results

The sites of alkaline phosphatase activity stained dark brown and those of acid phosphatase reddish-brown by the azo-dye methods. Phosphatase activity appeared as a black stain in the Gomori-type preparations.

In the intact skin none of the epidermal strata nor the hairs showed any alkaline phosphatase activity by the azo-dye methods. A distinct reaction was

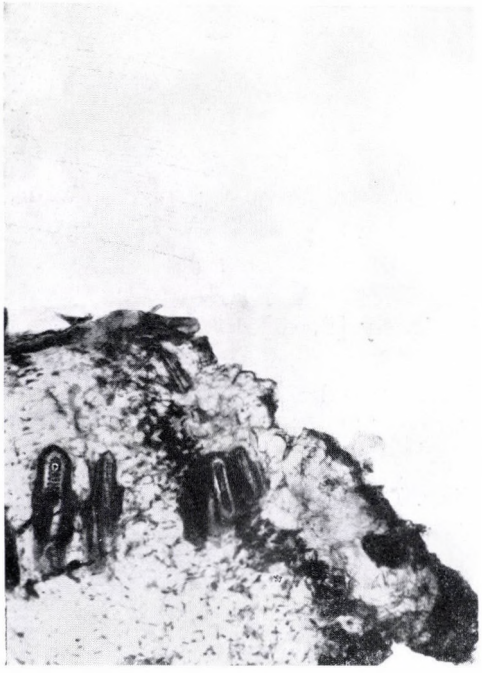

Fig. 1. Alkaline phosphatase activity in a 5-hour wound (Metal-salt method, × 100)

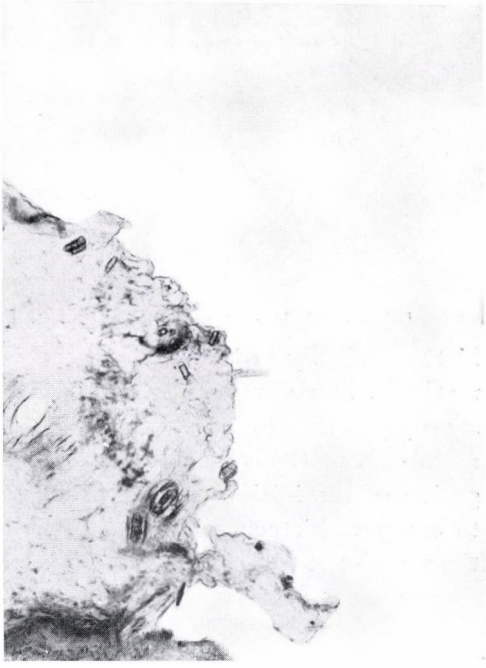

Fig. 2. Alkaline phosphatase activity in 5-hour wound (Azo-dye method, × 100)

observed in the papilla and in the proximal part of the external connective tissue sheath of the growing hair follicles. The capillaries and some fibroblasts of the dermis likewise took the stain. The metal-salt method, too, revealed a slight to moderate activity in these cutaneous appendages. In contrast to the azo-dye technique, the stratum Malpighii of the epidermis, especially the granular layer, was stained by the metal-salt method for alkaline phosphatase.

The intact epidermis, especially the granular layer, abounded in acid phosphatase which was demonstrable by both of the methods. Moderate acid phosphatase activity was revealed in the root sheaths and an intense one in the keratogenous zone of the hair. Dermal fibroblasts showed a varying reaction.

Four to eight hours after injury, two zones could be seen around the wound (Figs. 1 and 2). In the immediate vicinity of the wound edge, a central or superficial zone, 200 to 500 μ in depth, showed decreasing enzyme activity in the connective tissue cells. This was better demonstrable by the azo-dye method for alkaline phosphatase (Fig. 2), whereas there was little diminution in the Gomori reaction for the enzyme (Fig. 1). Neither of the two methods for acid phosphatase revealed any decrease in activity of the central wound zone

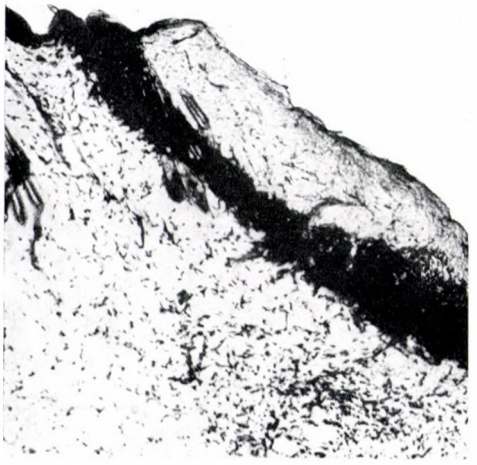

Fig. 3. Alkaline phosphatase activity in a 32-hour wound (Metal-salt method, × 100)

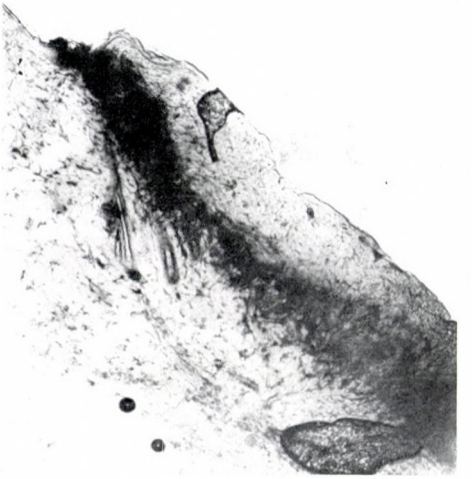

Fig. 4. Alkaline phosphatase activity in a 32-hour wound (Azo-dye method, × 100)

Surrounding the central area, a 100 to 300 μ deep peripheral zone exhibited an increased enzyme activity. This intensification appeared four to eight hours post-operatively. Acid phosphatase activity, demonstrable by both of the methods, increased four hours after wounding and reached a maximum after 16 hours. It remained strong during the rest of the experimental period (Figs. 5 and 6). The first increase took place in and around the local fibroblasts of the peripheral zone. In the same region of 8-hour wounds, active granulocytes appeared also. After 16 hours, they were superseded by mononuclear cells. The immigrating cells showed an intense acid phosphatase activity by both of the methods. There was also an extracellular activity which appeared

stronger by the Gomori method (Figs. 5 and 6). This extracellular reaction, for the most part, contributed to the somewhat stronger staining, typical of the Gomori preparations.

Alkaline phosphatase activity, demonstrable by both the metal-salt and azo-dye methods, increased in the peripheral zone five to eight hours postoperatively (Figs. 1 and 2), reaching a maximum after 16 hours. It remained strong during the rest of the experimental period (Figs. 4 and 5). The distribu-

Fig. 5. Acid phosphatase activity in a 32-hour wound (Metal-salt method, × 100)

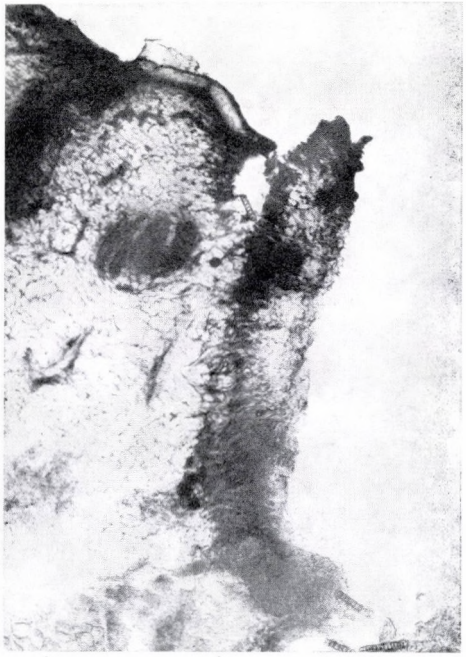

Fig. 6. Acid phosphatase activity in a 32-hour wound (Azo-dye method, × 100)

tion of this enzyme in the cells of the wound periphery was very similar to that of acid phosphatase. The difference between the two methods in the results for alkaline phosphatase was more visible especially during the initial increase in activity of the wound periphery, i.e. five to eight hours postoperatively. The Gomori preparations showed an intense intracellular and extracellular activity in the peripheral zone, whereas the azo-dye method revealed a moderate intracellular activation only in the local fibroblasts and in the immigrating granulocytes (Figs. 1 and 2). Later on, simultaneously with the increasing infiltration of the peripheral wound zone by active leukocytes, the difference became

slighter (Figs. 3 and 4). But also then, the black colour, indicating acid phosphatase activity by the Gomori technique, was more striking than the reddish-brown precipitate by the azo-dye method.

Discussion

The biological interpretation of the appearance in skin wounds of phosphatases and several other enzymes has been previously discussed in detail [9, 12].

Concerning the main differences of phosphatase activity demonstrated by the metal-salt and azo-dye methods, more extracellular staining and higher contrast of the final precipitate characterized the Gomori technique for acid phosphatase. The metal-salt method for alkaline phosphatase showed in the peripheral zone a more intense staining than the azo-dye technique, especially in 5- to 8-hour wounds. On the other hand, the azo-dye method revealed better the decrease in activity which is characteristic of most enzymes in the central, necrobiotic wound zone [11, 12].

The differences in the results could be due to shortcomings of the methods or to the possibility that the metal-salt and azo-dye techniques do not demonstrate identical enzymes.

The main disadvantages of the metal-salt methods consist of false positive reactions and of false localization, due to diffusion artifacts and to adsorption on certain tissue elements [4]. On the other hand, the azo-dye methods are also far from perfect. They often are based upon certain compromises, and all the optimal requirements for the substrate, diazonium salt, and final pigment are seldom fullfilled.

Phosphomonoesterase activity is not specific for the alcohol radical of the substrate, since phosphatases hydrolyze a variety of organic phosphate esters [3]. The differences in localization, using various phosphate esters, have sometimes been interpreted as an expression of a specific phosphomonoesterase [13]. However, differences in histochemical staining reactions do not usually provide a sound basis for distinguishing specific and individual enzymes [1]. Only isolation and biochemical characterization of the enzyme(s) will finally clarify the problem.

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DIE AKTIVITÄT DER PHOSPHATASEN WÄHREND DER ALLERFRÜHESTEN HEILUNGSPHASE DER HAUTWUNDEN

Vergleichende histochemische Untersuchung über die Metallsalz- und Azofarbstoff-Methoden

J. RAEKALLIO und A. JÄÄSKELÄINEN

Phosphatasen-Aktivität der heilenden Wunde wurde am Rattenmaterial histochemisch untersucht, indem man die Resultate der Metallsalzmethoden mit denen der Azofarbstoff-Methoden verglich. Während der ganzen Beobachtungsdauer von 1 bis 128 Stunden wurde mit der Metallsalzmethode für saure Phosphatase eine auffälligere extrazelluläre Färbung und ein mehr kontrastreiches Endprodukt der Reaktion erreicht. Mit der Metallsalzmethode für alkalische Phosphatase konnte man eine stärkere Reaktion in der äußeren Wundzone erkennen, insbesondere 5 bis 8 Stunden nach der Verletzung. Andererseits zeigte die Azofarbstoff-Methode auffälliger die Abnahme der Aktivität der alkalischen Phosphatase in der unmittelbaren Nähe der Wundfläche.

АКТИВНОСТЬ ФОСФАТАЗЫ ВО ВРЕМЯ ПЕРВОЙ ФАЗЫ ЗАЖИВЛЕНИЯ КОЖНОЙ РАНЫ

Сравнительный гистологический анализ методов исследования введением солей металлов и азокрасителей

Й. РАЕКАЛЛИО и А. ЙЕСКЕЛЕЙНЕН

Авторы исследовали гистохимическими методами на крысах активность фосфатазы во время заживления раны, при сравнении результатов исследования с помощью солей металлов или азокрасителей. В течение всего времени наблюдения (1—128 часов) методом исследования кислой фосфатазы введением солей металлов авторы получали более яркое внеклеточное окрашивание и более контрастный конечный продукт реакции. При исследовании щелочной фосфатазы введением солей металлов была получена более интенсивная реакция во внешней зоне раны, в частности в течение 5—8 часов после нанесения раны. С другой стороны, метод введения азокрасителей выявлял в непосредственном соседстве раневой поверхности более выраженное уменьшение активности щелочной фосфатазы.

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SOME METABOLIC CHANGES DURING THE CHICK'S ONTOGENESIS

A PRELIMINARY REPORT

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(Received January 26, 1966)

According to earlier investigations there seems to be a correlation between the intensity of oxybiotic metabolism and the content of tissue mucopolysaccharides (hexosamines). Experimental [1, 3, 9], pathological [4, 5] and phylogenetical [6, 7] observations demonstrate that tissues with high metabolic activity show a significantly high cytochrome oxidase activity whereas their mucopolysaccharide content is relatively slight. On the other hand, bradytrophic tissues (umbilical cord, cartilage, vitreous body, tissues of primitive animals, etc.) beside a slight (or no) cytochrome oxidase activity have sometimes an extremely high mucopolysaccharide content [5].

Mainly on the analogy of our phylogenetical observations [6, 7] we have investigated the correlation between cytochrome oxidase activity and tissular hexosamine content during ontogenesis in the chick. Hexosamine was determined according to BOAS [2] and cytochrome oxidase according to PEARL et al. [8].

Heart, liver, kidney, muscle and brain tissues were studied; in each of them 3 or 4 determinations were made for cytochrome oxidase and 5 for hexosamine. In order to characterize the various developmental stages, the means of the five kinds of tissue are shown in Fig. 1.

Cytochrome oxidase activity was increasing from the 11-day old embryonic to the grown-up stage. At the same time the hexosamine level decreased. Accordingly, embryonic metabolism is characterized by a high mucopolysaccharide content and low cytochrome oxidase activity, whereas postembryonic metabolism is characterized by high cytochrome oxidase activity and low mucopolysaccharide (hexosamine) content.

In this manner the metabolic conditions of embryonic life are similar to those of the primitive animal having an undeveloped cardiovascular system. Thus, this type of metabolism (mucopolysaccharide-type metabolism), characterized by a low cytochrome oxidase activity and a high mucopolysaccharide

* The material of this paper has been presented at the VII. International Embryological Conference, London 1965.

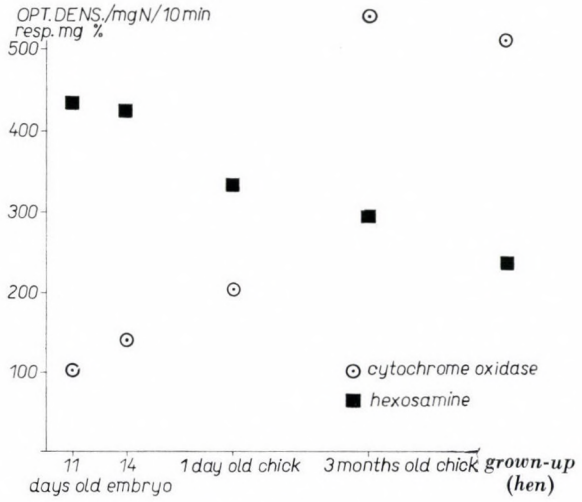

Fig. 1

content, must be more ancient not only phylogenetically but also ontogenetically.

Detailed investigations, including lactic acid determinations and a study of metabolic conditions immediately after birth, are in progress.

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EFFECT OF A SINGLE NEONATAL GLUCOCORTICOID DOSE ON THE LYMPHATIC AND ENDOCRINE ORGANS AND ON THE TRANSPLANTATION IMMUNITY OF RATS

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A single dose of glucocorticoid, administered to rats 24 hours after delivery may induce fatal cachexia.

Homologous skin grafts, applied to 13-day old animals which had received a single injection of hydrocortisone 24 hours after birth, survived considerably longer and their reaction was much weaker than in the untreated controls, while there was no difference between tests and controls in the rejections of heterologous skin grafts.

The number of small lymphocytes in the spleen and lymph nodes was found to have decreased 8 as also 40 days after treatment with hydrocortisone. The megakaryocyte count was conspicuously high in the spleen. The number of PAS-positive granules was reduced in the liver, while that of the Kupffer cells was excessively high. A decreased number of small lymphocytes and an increased number of reticular cells were registered in the thymus.

By repeating the experiment of SCHLESINGER and MARK [1] it has been shown that an adequate single dose of glucocorticoid, injected into rats in the first 24 hours of extrauterine life, led to fatal cachexia [2,28] in most cases, and that the growth of the surviving animals as also the weight of their thymus and spleen were inferior to those of the littermate controls even several weeks after the intervention. The number of small lymphocytes in their blood was greatly reduced [2]. These symptoms were conspicuously similar to those seen after neonatal thymectomy [3—8]. The present experiments had the purpose to establish whether a single neonatal glucocorticoid injection would induce the changes observable in the lymphatic and endocrine organs of neonatal thymectomized rats [9, 10], further a reduction of transplantation immunity (i.e. diminished reaction to heterografts and homografts) indicative of thymic damage.

Material and method

Newborn rats of the Wistar strain were treated intraperitoneally with 1 mg of hydrocortisone acetate (Richter, Budapest) or subcutaneously with 0.25 mg of dexamethazone (Oradexon, Organon), and the control animals with similar doses of 0.9 per cent physiological saline, both groups at the 24th hour of extrauterine life. The organs of animals that had died with cachexia were discarded. Eighteen surviving animals were decapitated on the 8th and seven on the 40th day after the injection, and a corresponding number of the controls was likewise sacrificed. The endocrine and lymphatic organs were fixed in 4 per cent formalin, embedded in paraffin, and 6 μ thick sections were made and stained with haematoxylin-eosin, Kernechtrot, cresyl violet and Schiff's periodic acid (PAS).

A second group received 1 mg hydrocortisone 24 hours after birth; 12 days later, heterologous (from 5-month old mice of the CBA strain) and homologous (from 2-week old rats of the R strain) skin grafts were applied to 6 glucocorticoid-treated and 6 NaCl-treated animals by the pinch-graft method [11] in order to observe the survival time of the grafts and the intensity of their rejection.

Results

Animals injected with a single dose of hydrocortisone 24 hours after birth lagged in growth; their fur coat developed slower and was scarce and ruffled; their skin was thinner and the amount of subcutaneous fatty tissue was subnormal. Many of them had haemorrhagic diarrhoea. About 20 to 30 per cent died with cachexia within 8 days. Changes induced by hydrocortisone (B, E), dexamethazone (C, F) and NaCl (A, D) are illustrated in Figs. 1 to 4.

Hydrocortisone induced moderate hyperthyroidism (Fig. 1/B), further pronounced hypofunction of the adrenal's zona fasciculata which was thinned, richer in cells, more compact and exhibited shrunken nuclei (Fig. 1/E). On the other hand, the zona fasciculata of animals injected with dexamethazone (Fig. 1/F) was wide, the cytoplasm was foamy and the nuclei were enlarged. No essential changes were noted in the zona glomerulosa.

Both glucocorticoid-treated groups exhibited the usual loosening of the cortical and medullary structure of the thymus, a reduction in the number of small lymphocytes, further an increase in the number of reticular cells and connective-tissue elements (Fig. 2/B, E and C, F). In the spleen of glucocorticoid-treated animals (Fig. 3/B, C) the number of Malpighian corpuscles and small lymphocytes diminished while that of megakaryocytes increased (Fig. 3/B). In the lymph nodes loosening of the structure, decrease in the number of small mature lymphocytes and accumulation of reticular cells were registered (Fig. 3/E, F). In the liver the number of PAS-positive granules was decreased (Fig. 4/B, C) and that of Kupffer cells considerably increased (Fig. 4/E, F).

The reaction to heterologous skin grafts was similar in the hydrocortisone-treated animals and the NaCl-treated controls, with homologous grafts rejection began on the 15th day in the controls, and on the 21st day in the test animals. The control animals had completely rejected the skin graft by the 21st day (Table I).

Discussion

The present experiments have confirmed the claim of SCHLESINGER and MARK [1] that a single glucocorticoid injection administered 24 hours after birth induces fatal cachexia, a phenomenon observed by SZEBERÉNYI [12] and also by us [2,28]. The symptoms are conspicuously similar to those of the wasting syndrome caused by neonatal thymectomy [3 to 10]. The two syndromes have many common features, e.g. retardation of growth: early death; consid-

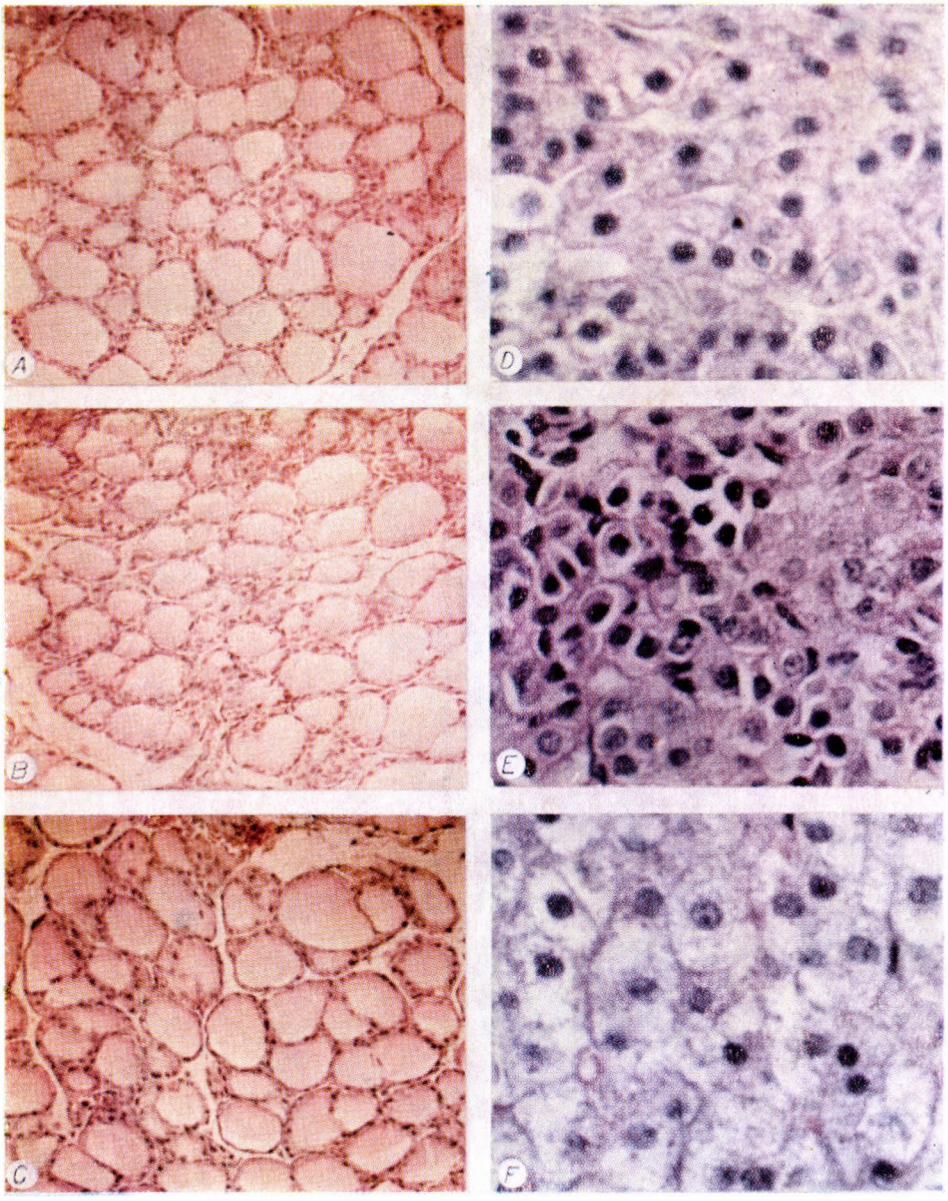

Fig. 1. Effect of neonatal glucocorticoid injection on the thyroid (A, B, C) (Kernechtrot stain, × 150), further on the zona fasciculata (D, E, F) (haematoxylin-eosin × 600)
Control, treated with NaCl after birth (A, D)
Animal treated with hydrocortisone after birth (B, E)
Animal treated with dexamethazone after birth (C, F)

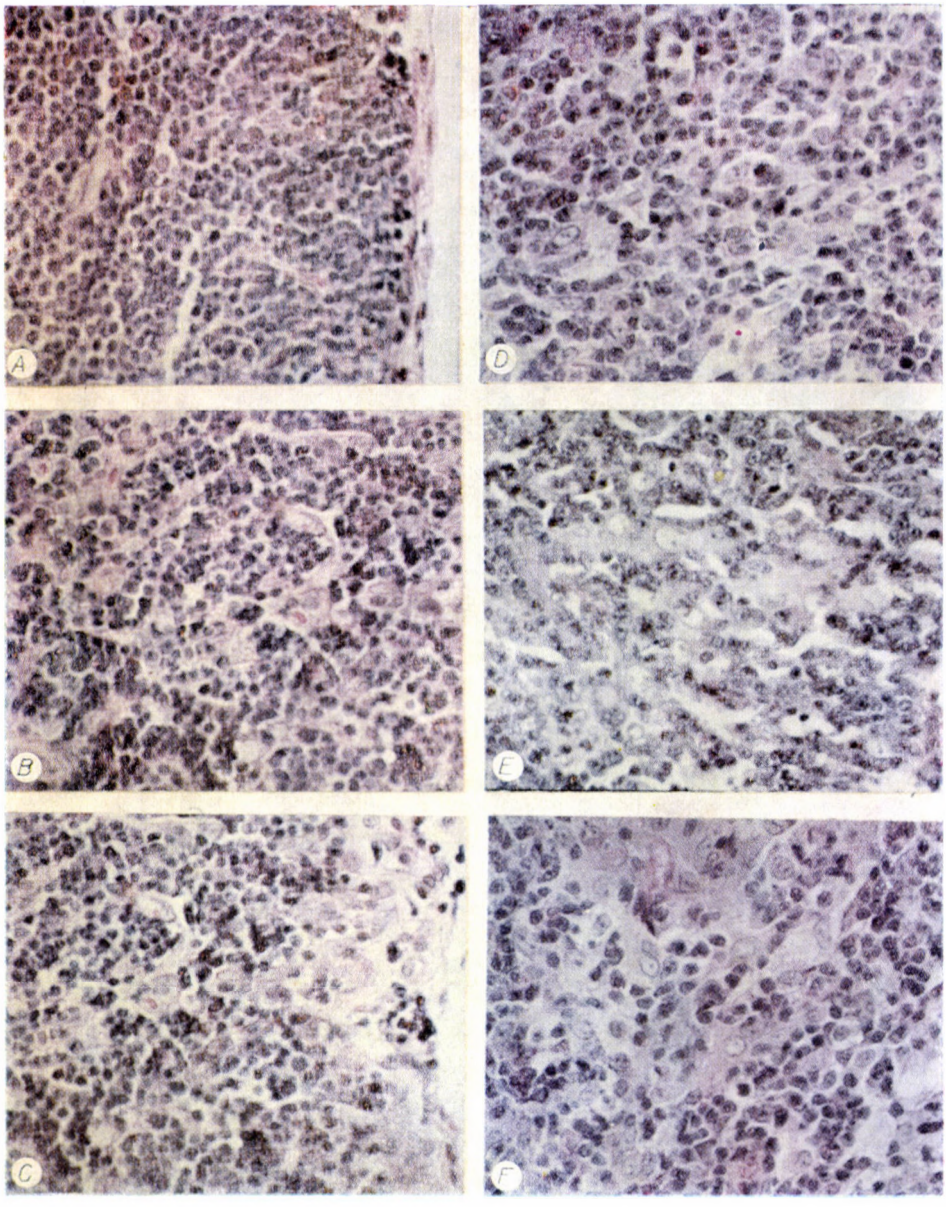

Fig. 2. Effect of neonatal glucocorticoid injection on the cortex (A, B, C) and medulla (D, E, F) of the thymus. Haematoxylin-eosin, × 375.
Control, treated with CaCl after birth (A, D)
Animal, treated with hydrocortisone after birth (B, E)
Test animal, treated with dexamethazone after birth (C, F)

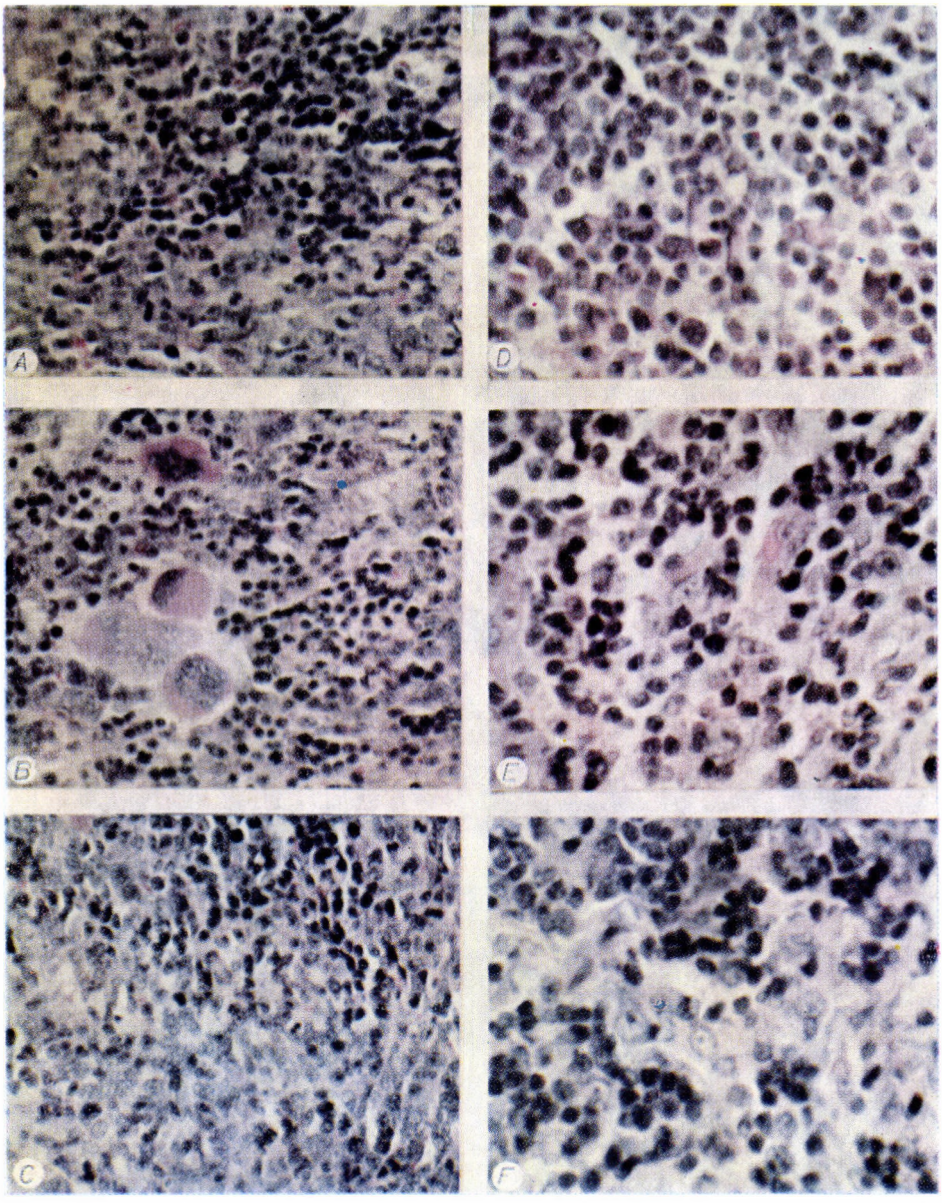

Fig. 3. Effect of neonatal glucocorticoid injection on the spleen (A, B, C) (haematoxylin-eosin, × 375) further on lymph node (D, E, F) (haematoxylin-eosin × 600)
Control, treated with NaCl after birth (A, D)
Animal treated with hydrocortisone after birth (B, E)
Animal treated with dexamethazone after birth (C, F)

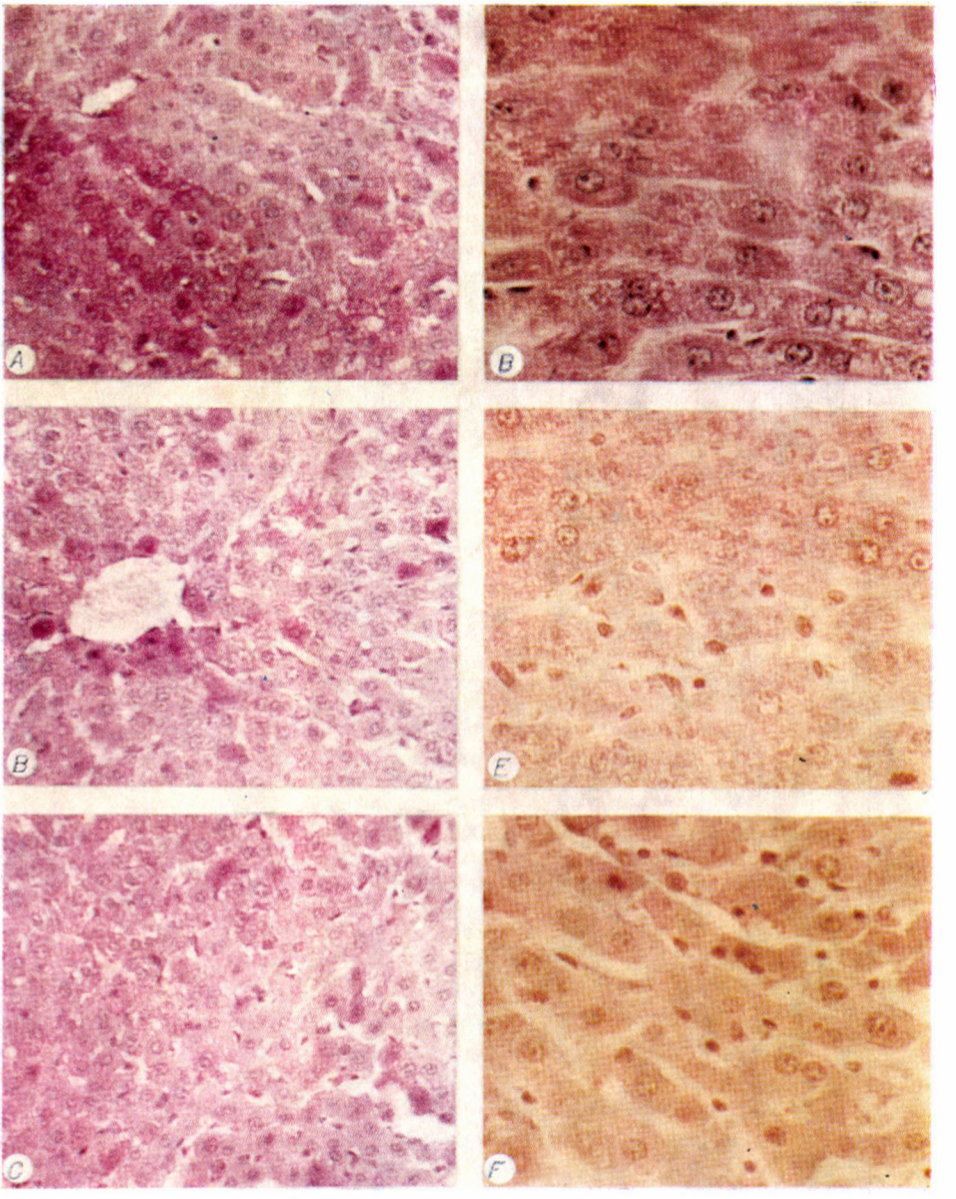

Fig. 4. Effect of neonatal glucocorticoid injection on the liver. Schiff's periodic reaction (A,B,C), × 375. Kernechtrot stain (D, E, F), × 600
Control, treated with NaCl after birth (A, D)
Animal, treated with hydrocortisone after birth (B, E)
Animal treated with dexamethazone after birth (C, F)

Table I

Effect on homologous and heterologous skin grafts of a single hydrocortisone injection, administered to rats 24 hrs after birth

Group	Number of animals	Beginning of graft rejection on day							
		12th		15th		18th		20th	
		homol.	heter.	homol.	heter.	homol.	heter.	homol.	heter.
0.9% NaCl intraperitoneally	6	*0/6	6/6	4/6	6/6	6/6	∅	∅	∅
1 mg hydrocortisone intraperitoneally	6	0/6	6/6	0/6	6/6	0/6	∅	5/6	∅

* = number of animals showing first signs of rejection per total number of animals with skin graf.
∅ = complete rejection

erable decrease in the number of small lymphocytes in blood, spleen and lymph nodes; increased number of reticular cells in lymphatic organs and liver; decreased amount of PAS-positive matter in the liver [2, 9, 10]. In newborn rats glucocorticoid treatment gave rise to marked thymic and splenic involution [2] with a loosening of the structure and a loss of small lymphocytes. Although no thymic deficiency in the hydrocortisone-treated animals could be reckoned with at the time of the examination of transplantation immunity, the rejection of homografts was considerably delayed, a sign of probable thymic damage [4, 13].

The blood of patients suffering from chronic lymphatic leukaemia contains, according to METCALF [14, 15], a lymphocytosis-stimulating factor (LSF) which is demonstrable in the thymus as well; glucocorticoids reduce the activity of this factor or that of some other humoral substance of the thymus [16—19]. Of course, neither the fact that glucocorticoids induce atrophy of the thymus and other lymphatic organs [20, 21] nor their catabolic action in other tissues should be disregarded. It is noteworthy that, while even large doses of glucocorticoids induce but transient lymphocytopenia in the adult animal, their administration to newly born animals gives rise to pronounced lymphocytopenia which often leads to fatal cachexia [1, 2, 21, 28].

The hyperplasia of RES-elements following neonatal glucocorticoid injection may have simply been a sign of repeated infections; yet, reports according to which RES hyperplasia is accompanied by increased elimination of corticoids from the blood should not be disregarded [23—25].

The results of the present experiments suggest that the fact that stress in the first extrauterine days does not cause hypercorticalism [26, 27] may be of biological significance: thymic and lymphatic activity cannot, thus, be impaired by the high blood level of glucocorticoids before the organism's immunological defence mechanism has fully developed.

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DIE WIRKUNG EINER EINMALIGEN GLUKOKORTIKOIDINJEKTION IM NEUGEBORENENALTER AUF EINZELNE LYMPHATISCHE UND ENDOKRINE ORGANE

J. FACHET, M. PALKOVITS und G. PETRÁNYI

In Bestätigung früherer experimenteller Ergebnisse wurde der Nachweis erbracht, daß eine einmalige, 24 Stunden nach der Geburt verabreichte Glukokortikoidinjektion, in Abhängigkeit von der Dosis, eine fatale Kachexie verursachen kann.

Infolge einer 24 Stunden nach der Geburt verabreichten einmaligen Hydrokortisoninjektion wurde das am 12. Tage überpflanzte homologe Hauttransplantat mit bedeutender Retardation abgebaut und die Reaktion war mäßiger als bei den Kontrollen. Der Abbau heterologer Transplantate zeigte gegenüber den Kontrollen keinen Unterschied.

Acht bzw. vierzig Tage nach der Glukokortikoidverabreichung verminderte sich in der Milz und in den Lymphknoten die Zahl der Klein-Lymphozyten. In der Milz wurde die bedeutende Vermehrung der Megakaryozyten beobachtet. In der Leber verminderte sich die Zahl der PAS-positiven Körnchen, während die Zahl der Kupfferschen Zellen erheblich zunahm. Im Thymus ließ sich die Abnahme der Zahl der Klein-Lymphozyten und die Vermehrung der Retikulumzellen feststellen.

ДЕЙСТВИЕ РАЗОВОЙ ИНЪЕКЦИИ ГЛИКОКОРТИКОИДОВ В ПЕРИОД НОВОРОЖДЕННОСТИ НА ОТДЕЛЬНЫЕ ЛИМФАТИЧЕСКИЕ И ЭНДОКРИННЫЕ ОРГАНЫ И НА ТРАНСПЛАНТАЦИОННЫЙ ИММУНИТЕТ

Й. ФАХЕТ, М. ПАЛКОВИЧ и Г. ПЕТРАНЬИ

В подтверждении своих прежних экспериментальных результатов авторы выявили, что однократная инъекция гликокортикоидов, введенная через 24 часа после рождения, в зависимости от применяемой дозы, может вызвать кахексию со смертельным исходом.

После однократной инъекции гидрокортизона, введенной через сутки после рождения, отторжение гомологичного кожного трансплантата, пересаженного на 12-ый день после рождения, произошло с значительным замедлением, и реакция была меньшей, чем у контрольных животных. В то же время отторжение гетерологичного трансплантата, по сравнению с контролями, не показало отклонения.

Восемь или сорок дней после введения гликокортикоидов в селезенке и в лимфатических узлах число малых лимфоцитов уменьшилось. В селезенке наблюдалось значительное нарастание числа мегакароцитов. В печени понизилось количество ПАСК-положительных зернышек, а количество Куппферовских клеток повысилось. В зобной железе наблюдалось уменьшение числа малых лимфоцитов и размножение ретикулярных клеток.

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ELECTRON MICROSCOPIC STUDY OF GUINEA-PIG THYMUS

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The electron-microscopic structure of the epithelial cells contained in the thymic medulla of four to five-day old guinea pigs has been studied. The main object of the study was to elucidate the structural foundation of thymic function. The presence of secretion and secretory cysts makes it obvious that the epithelial cells of the thymus have the character of glandular cells. This is borne out by the endoplasmic reticulum, the well-developed Golgi area and the appearance of "Nebenkern" structures in certain epithelial cells. Apart from the usual desmosomes and zonulae occludentes, the cells are interconnected by labyrinthine, spiked and rivet-like formations. The differentiation of epithelial cells manifests itself through the beginning of secretory activity and the appearance of tonofibrils which, in conjunction with a gradual interlacement of the epithelial cells, leads to the development of Hassall's corpuscles. The existence of an interrelation between epithelial cells and thymocytes is indicated by the phenomena of peripolesis and emperipolesis, the phagocytosis of thymocytes by epithelial cells, and the protoplasmic bridges connecting epithelial cells. These are presumably the structural expressions of the development of thymocytic immunological competence based on information received from epithelial cells.

Light microscopic investigations into thymic structure have left the question open as to whether the thymus should be regarded as a lymph organ or rather as a hormonal gland. Although MILLER [24, 25] and others [2, 21, 23, 29, 34, 35] have demonstrated the immunological competence of the organ, they have failed to explain the mechanism of thymic function. The present observations were based on the theory that the cells of the epithelial reticulum constitute the specific cellular elements of the thymus and that their closer examination might lead to a better understanding of thymic function.

Material and method

The thymus gland of 4 to 5 day-old guinea pigs was used. The vessels of the animals were perfused via the heart with about 1000 ml of 5 per cent glutaric aldehyde, then the removed thymic fragments were fixed in the same solution for 2 hours. The fixing fluid was washed with Millonig buffer at 4 °C for 16 to 20 hrs. A second fixing was performed in osmium tetroxide with 1 per cent buffer for 2 hrs. Ethanol was used for dehydration and araldite for embedding. Semi-thin sections were stained with potassium permanganate, ultra-thin sections were contrast-stained with methanolic uranylacetate and lead citrate solution. The electron-microscope was of the Jem 6C-type.

Results

Epithelial cell junctions are of different kinds in the thymus. The cells are frequently interconnected by simple desmosomes; the connection may assume the form of zonula occludens. An intricate labyrinthine interlacement of the cell surfaces (Fig. 1) can often be observed which results in an irregular, serrated junction of the contiguous cells. It also happens that several long processes intrude themselves between the epithelial cells at various points and in different planes (Figs. 2, 3, 5, 6, 7, 10), forming what might be called a riveted junction. Still another form is marked by protoplasmic processes, which, arising like spikes from the cell, are hooked into the body of the neighbouring cell, forming thus a zipper-like structure.

The epithelial network of the thymus consists of brighter and darker cells. The dark colour of the latter is due to an abundance of ribosomes arranged in rosettes. Some epithelial cells contain much smaller and more densely packed ribosomes. There is furthermore a considerable difference between epithelial cells as regards density and size of the endoplasmic reticulum: there are sporadic cells containing a straight elongated lamellar system (Fig. 3) and others containing distended reticular vesicles (Fig. 2) whence the thin reticular tubules have disappeared. Some cells contain minute (Figs. 4, 8), others very large mitochondria (Fig. 2), while still others include hardly any. The Golgi apparatus is well-developed (Figs. 1, 2, 3, 8, 12); some are vacuolated, others granulated, with larger or smaller lysosomes in their vicinity (Figs. 1, 12). Epithelial cells rich in endoplasmic reticulum of coarse surface were also observed. The reticulum in these cells was arranged as in plasmacytes (Fig. 6); they include large homogeneous globules, the analogues of secretion droplets (Figs. 1, 2, 3, 5, 6, 7, 8, 11). The same cell may contain empty vacuoles and also vacuoles filled with a homogeneous substance (Fig. 5). Cytoreticular formations (Fig. 7) with a concentric structure and many ribosomes are characteristic figures. These so-called "Nebenkerns" contain in the centre a secretory vesicle, the first indication of secretory activity. The concentric structure in question is surrounded by mitochondria. The secretory vacuoles were scattered in some epithelial cells (Fig. 3), while in others they formed a clustered vacuolar system (Figs. 1, 4, 8, 10). Clustered vacuoles may deform the nucleus (Fig. 4). Giant nuclei of bizarre shape were repeatedly observed; their deformation was due to intracellular secretory cysts, among others. On internal surface of the large secretory vacuoles microvillis were rendering the surface uneven. Many microscopic pictures show, consequently, the cross section of long intravacuolar microvilli (Fig. 8). Certain vacuoles appeared to be provided with a well developed distinct and independent wall separated from the cytoplasm by a membrane-like ring of delicate fibrils (Fig. 8). That the wall was not a separate entity follows from the fact that it was seen to fuse at some points with the cyto-

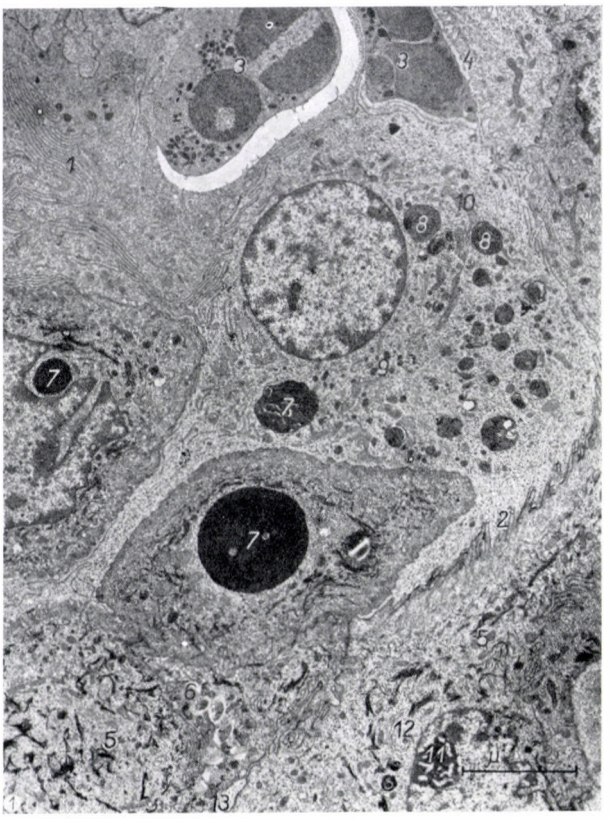

Fig. 1. Medullary detail. 1 = The cells are interlocked and form a tortuous line. 2 = Another form of spiked interdigitation. 3 = Two granulocytes. 4 = Contiguous cells separated by microvilli. 5 = Formation of fibres. 6 = Secretion. 7 = Remnants of phagocyted thymocytes (lysosomes). 8 = Electron-dense and 9 = microbodies of different sizes. 10 = Mitochondrium. 11 = Nucleolus. 12 = Golgi apparatus. 13 = Rivet-like junction of cells

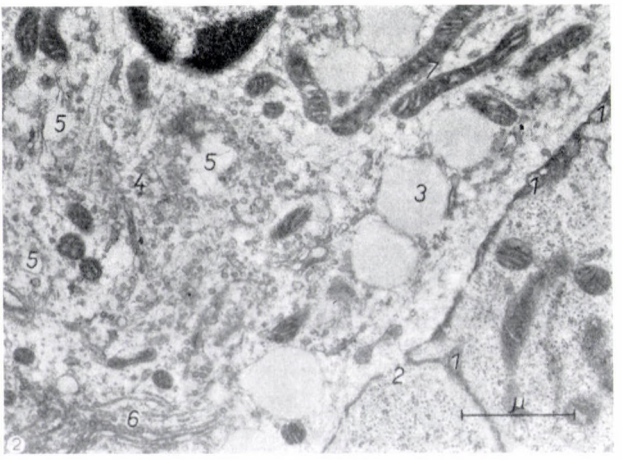

Fig. 2. Boundary between three cells. 1 = Rivet-like cell junction. 2 = Protoplasmic bridge between neighbouring cells. 3 = Droplets of secretion. 4 = Cross section of endoplasmic reticulum. 5 = Distended and fused endoplasmic reticulum. 6 = Golgi area. 7 = Mitochondrium

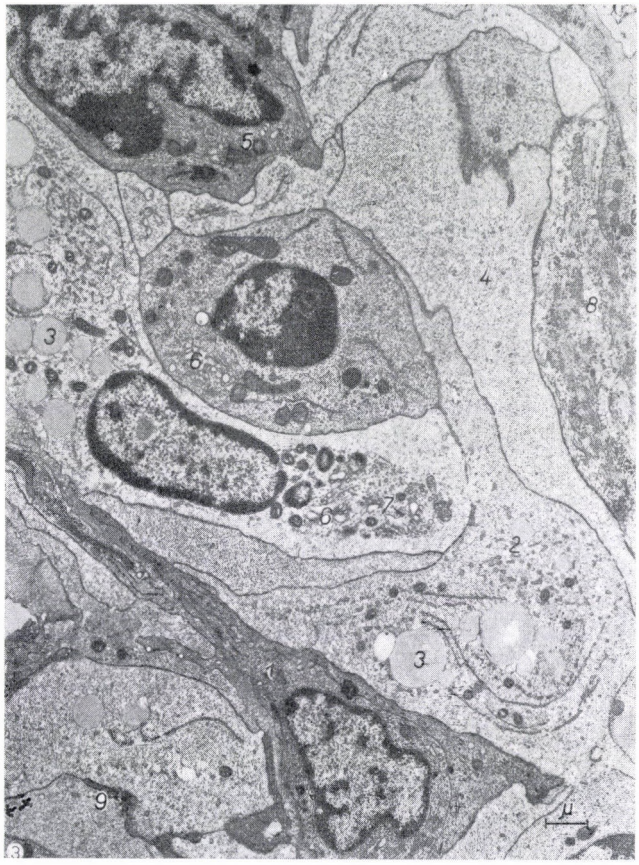

Fig. 3. Group of epithelial cells in the medulla. 1 = Dark cell. 2 = Bright cell. 3 = Droplet of secretion. 4 = Widely extending process of epithelial cell. 5 = Cytocentre. 6 = Golgi apparatus with surrounding area. 7 = endoplasmic reticulum. 8 = Growing striated muscle

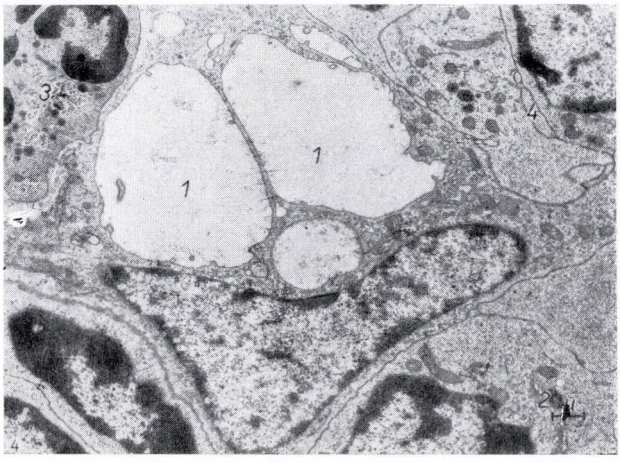

Fig. 4. 1 = Vesicles distended to cysts deforming the nucleus of epithelial cells. 2 = Cytocentre. 3 = Granulocyte. 4 = Electron-dense body; cells connected by rivet-like structures

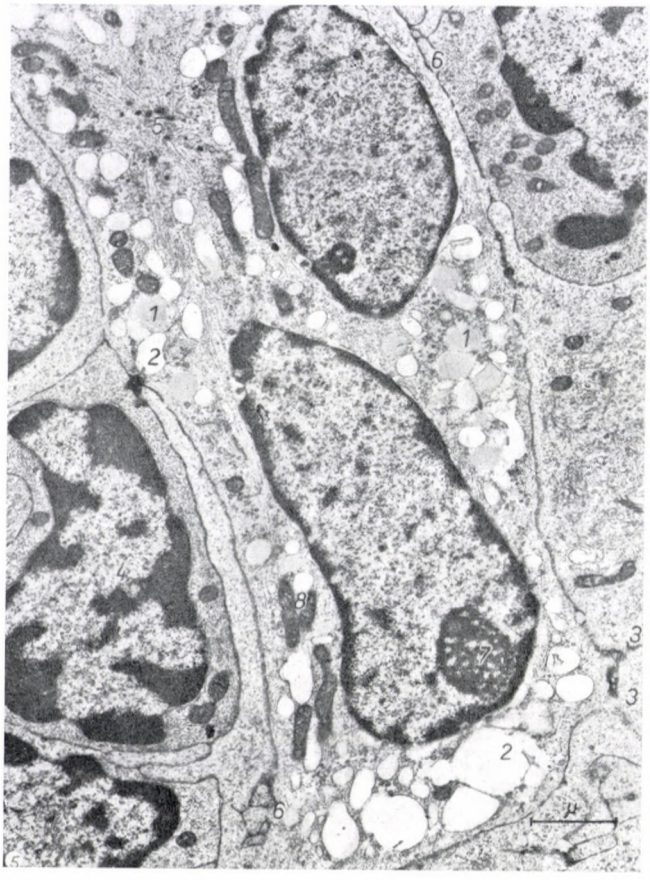

Fig. 5. Secretion in binuclear epithelial cell. 1 = Droplet of secretion. 2 = Emptied secretion. 3 = Intercellular protoplasmic bridges. 4 = Thymocyte. 5 = Microbodies. 6 = Rivet-like cell junction. 7 = Nucleolus. 8 = Mitochondrium

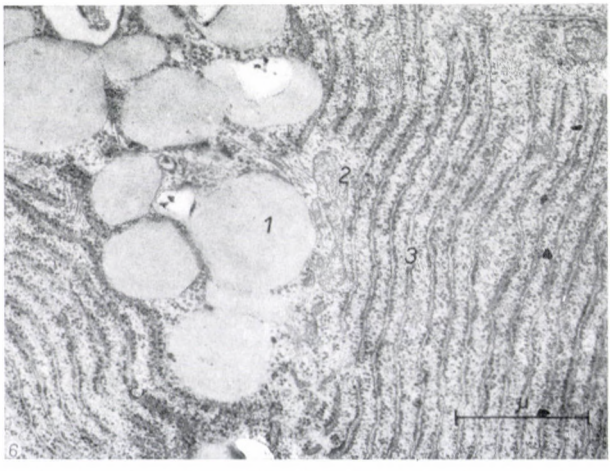

Fig. 6. Coarse endoplasmic reticulum and droplets of secretion in the epithelial cell. 1 = Droplet. 2 = Mitochondrium. 3 = Reticulum

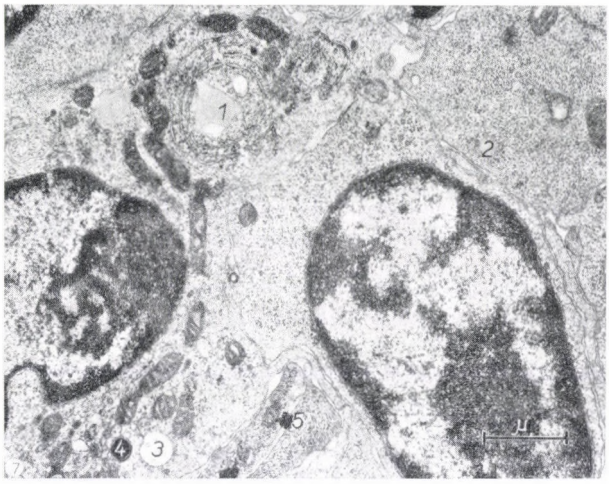

Fig. 7. Formation of "Nebenkern" and secretion. 1 = Droplet surrounded by concentrically arranged endoplasmic reticulum, the latter surrounded by mitochondria. 2 = Droplet surrounded by ribosomal densification. 3 = Emptied secretion. 4 = Dense bodies. 5 = Desmosome

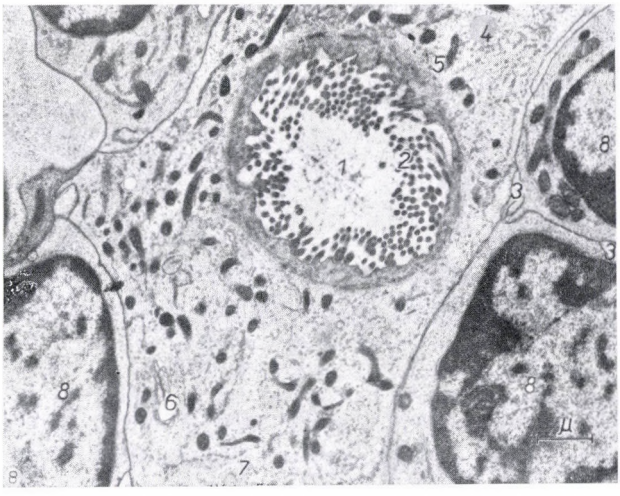

Fig. 8. Secretory cyst with well circumscribed wall and microvilli. 1 = Lumen. 2 = Microvilli. 3 = Rivet-like cell junction. 4 = Droplet. 5 = Mitochondrium. 6 = Golgi area. 7 = Cross section of endoplasmic reticulum. 8 = Nucleus of thymocyte

plasm of the epithelial cells. Although glycogen was not invariably present in the cytoplasm of epithelial cells, patches consisting of glycogen granules were repeatedly observed; these granules were larger than ribosomes and densely packed.

As regards the epithelial elements of the medulla, they are characterized by the presence of fibrils which especially in Hassall's corpuscles are arranged in bundles (Figs. 2, 9, 10, 12, 13, 15). Epithelial cells changing into Hassall's

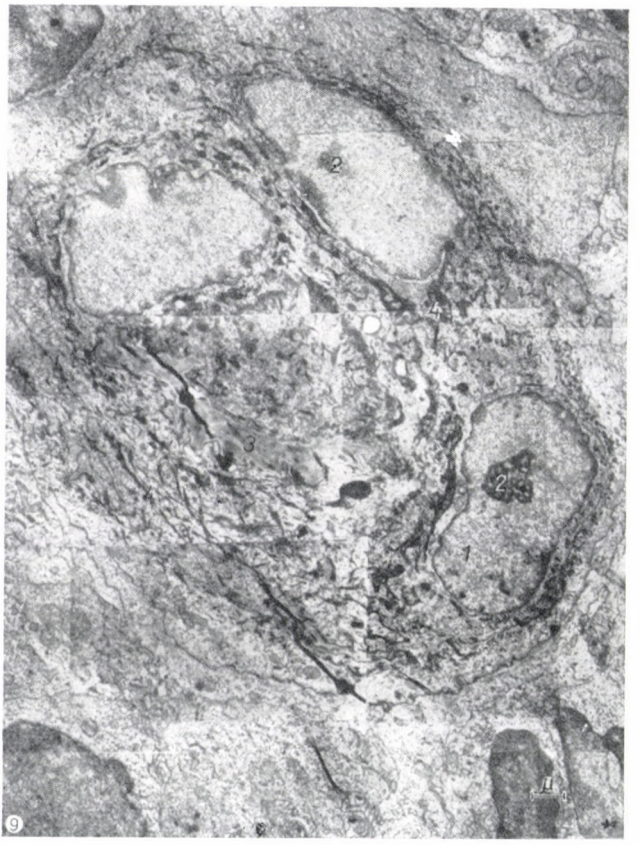

Fig. 9. Young Hassall's corpuscle. Aggregation of three epithelial cells among intact epithelial cells; they form the nucleus of the developing Hassall's corpuscle. The growth of fibrils is advanced; there is degeneration in the centre, while the nucleoli are still active. 1 = Nucleus of epithelial cell. 2 = Nucleolus. 3 = Degeneration. 4 = Growth of fibril

bodies are, therefore, rich in fibrils. Perinuclear fibrils tend to form networks; the nearer the cells are to being converted in Hassall's corpuscles, the coarser their structure and the more readily they assume a reticular arrangement (Figs. 10, 11). Many epithelial cells and thymocytes contained cytocentres, sometimes even two of them in which case they were arranged at right angles. The cytocentres of epithelial cells were usually lying next to the Golgi appa-

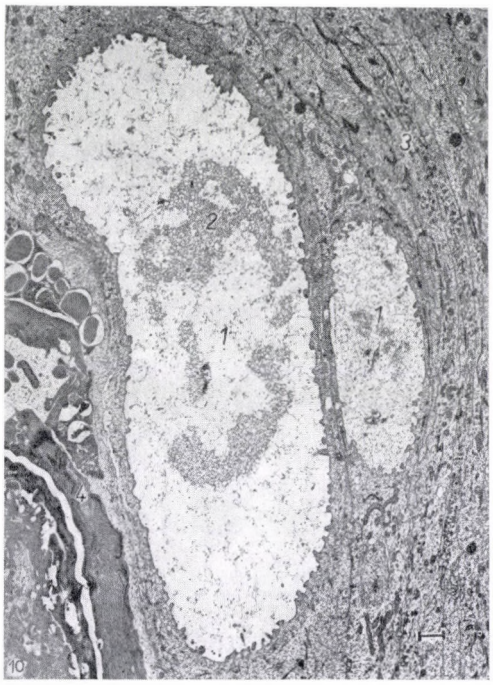

Fig. 10. Secretory cyst in the cortex of Hassall's corpuscle. 1 = Cyst. 2 = Secretion. 3 = Cross section of concentrically stratified cell. 4 = Degenerated nucleus of Hassall's corpuscle

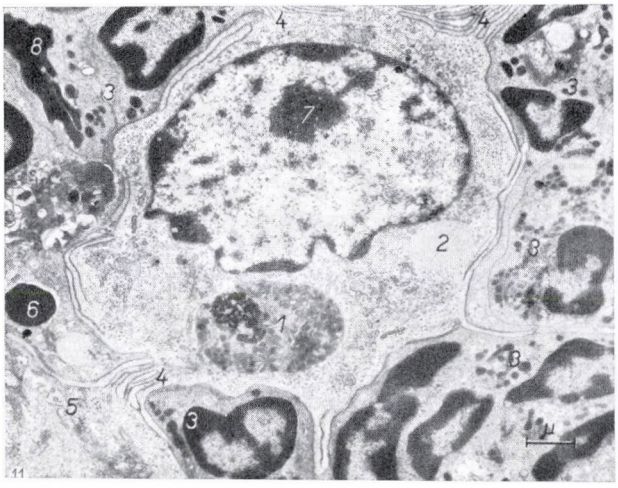

Fig. 11. Epithelial cell including phagocyted thymocyte surrounded by neutrophilic granulocytes. 1 = Remnant of phagocyted thymocyte. 2 = Droplet of secretion. 3 = Neutrophilic granulocyte. 4 = Interlocking of cell surfaces. 5 = Intercellular gap with microvilli. 6 = Remnant of digested thymocyte. 7 = Nucleolus. 8 = Degenerated cellular residuum

ratus. Numerous epithelial cells contained phagocyted thymocytes in various stages of digestion (Fig. 1, 11). The first to be digested and disappear is the cytoplasm of the phagocyted thymocyte; the nucleus, undergoing pyknosis,

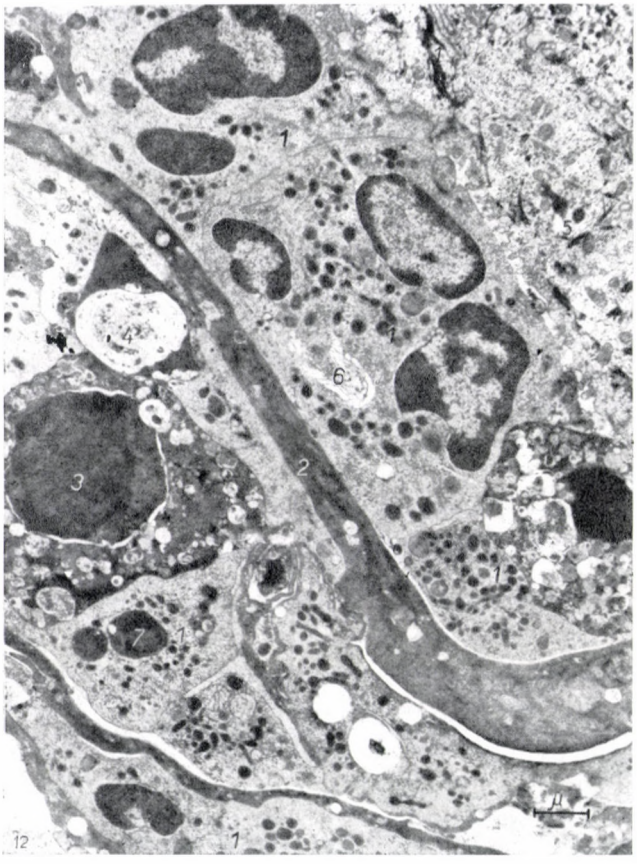

Fig. 12. Massive involvement of granulocytes in cellular breakdown in Hassall's corpuscle. 1 = Neutrophilic granulocytes. 2 = Plate-like structure of elongated, degenerated epithelia processes. 3 = Phagocyted cell with cellular fragments. 4 = Cellular debris occupying the place of disintegrated cell. 5 = Epithelial cell developing tonofibrils. 6 = Golgi area. 7 = Lysosome

persists longer. Even the swollen nucleolus was found in some instances. The medulla of the thymus often contained leucocytes and granulocytes, their neutrophilic (Fig. 12), eosinophilic (Fig. 14) and basophilic (Fig. 13) forms. Hassall's bodies were particularly rich in basophilic granulocytes and mast cells (Fig. 15).

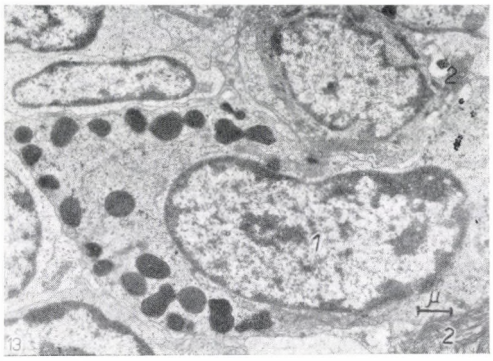

Fig. 13. 1 = Young basophilic granulocyte. 2 = Tonofibrils

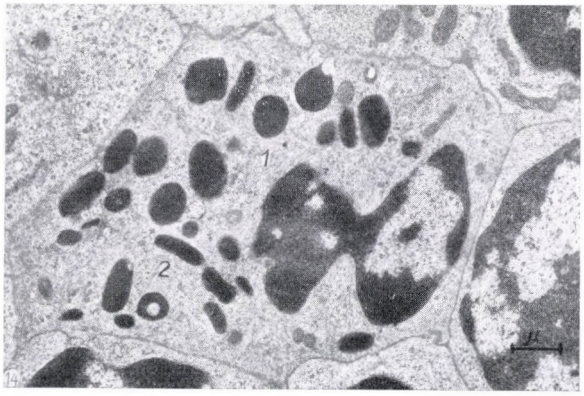

Fig. 14. Eosinophilic granulocyte wedged-in between thymocytes. Note characteristic granular structure

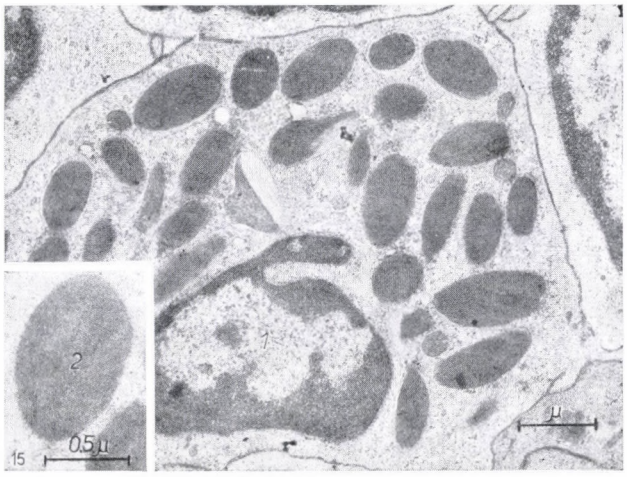

Fig. 15. Mast cell wedged-in between thymocytes. 1 = Nucleus of mast cell. 2 = High-power magnification of granule of mast cell, showing the characteristic structure

Discussion

The thymus of the guinea pig has a highly intricate structure, and its analysis by means of the electron microscope offers new information on thymic function. There are numerous data concerning the ultrastructure of the thymus [2, 7, 8, 11, 27, 34] and KLUG [19] has even analysed the morphogenesis of thymic lymphocytes. The thymus of the mouse [11, 13, 20, 45], the golden hamster [44] and, in certain respects, that of the guinea pig [20], rat and rabbit [16, 17] has been examined in more detail under the electron microscope. The reticular epithelium and the thymocytes form a dynamic unity, and it is necessary to study their cooperation in order to understand the mechanism of thymic function. The epithelial network is constantly moving [41, 42], a movement especially marked in the medullary substance where Hassall's corpuscles are formed. Movement is slower in the cortex where the meshes of the network are widened by the greater number of thymocytes and the mitoses occurring there. Some of the epithelial cells of the reticulum are dark, others bright [14, 22]. Both kinds were encountered in the guinea-pig thymus, and both types — being interconnected by desmosomes — seemed to be epithelial cells (Fig. 3); the bright cells appeared to be more diffuse, the darker ones more compact. The latter were richer in ribosomes and endothelial reticula, and usually more structured. This was confirmed by microcinematographic studies of the pure culture of guinea-pig thymic epithelium [43]. The phenomenon that, with advancing age of the epithelial cells, their coherence and — consequently — the formation of desmosomes and fibres become more pronounced, was likewise observed in such studies. The epithelial cells composing Hassall's bodies are thus more advanced in age [43]. The intricate, labyrinthine interlocking of the cell borders is due to the agglutination and movement of the epithelial cells. This arrangement does not serve the purpose of a closer cohesion of the cells but — by means of larger contacting surfaces — that of intercellular metabolism. Hassall's corpuscles evidently arise from the aggregation of epithelial cells. The development of desmosomes becomes more and more intensive; they form several short segments in some, and a continuous longer segment in other cases. The multiplication of fibres, the digestion of phagocyted thymocytes and the cellular degeneration in Hassall's corpuscles are other noteworthy phenomena. Fig. 9 shows a montage and presents a young Hassall corpuscle. The nucleolus of the epithelial cells displays a distinct nucleolonema, a structural arrangement pointing to nucleolar function. The area of Hassall's bodies reveals rich fibrillarity and an opulence of desmosomes; the topographic arrangement of the fibrils is adjusted to that of the desmosomes. Lysosomes are frequently present in the thymus. A great portion in the centre of aged Hassall bodies is occupied by degenerating cells many of which contain thymocytes destroyed in digestive vacuoles (Figs. 1, 11, 12). The degenerated nuclear part

of the corpuscle is often surrounded by epithelial membranes rich in fibres and arranged in flat concentric layers (Figs. 10, 12). Some cysts in Hassall's corpuscles have a compact wall, their cells contain endoplasmic reticulum and fibres. The cysts are filled with granular substance (Figs. 8, 10). It follows from this structure that epithelial secretion of PAS-positive matter may take place in Hassall's corpuscles. It is noteworthy that granulocytes are involved in the development of Hassall's corpuscles [18], often contain mast cells with large lamellated granules (Fig. 15). Also many nests of neutrophilic, eosinophilic (Fig. 14) and basophilic (Fig. 13) granulocytes are to be found (Fig. 12). The presence of these cells in Hassall's bodies is correlated with the formation of waste products of cellular degeneration as also with the production of substances involved in thymic function. The marginal portion of Hassall's corpuscles contains epithelial cells which bend towards each other like onion leaves; they may be said to constitute the capsule of Hassall's corpuscles, while degenerating cells in the interior present a highly variable picture. Cells of Hassall's corpuscles are doomed to degenerate; the dissolution of the marginal epithelial cells leads to central cytolysis, so that — by the liberation of PAS-positive matter — a "necrohormone" is produced. This concept is supported by the frequent presence of cysts in the eccentric cells of Hassall's corpuscles, the cysts contain an electron-dense substance, and their microvilli point to a process of absorption occurring there. Cysts of this description have been observed also in epithelial cells outside Hassall's bodies. It is in the "Nebenkern"-like structures of the pancreatic cells that the process of secretion starts, and the secreted substance appears to be correlated with the endoplasmic reticulum in the form of homogeneous electron-dense droplets. The formation of these droplets is preceded by a targetlike furling of the membranes of the endoplasmic reticulum, and it is in their centre that, surrounded by mitochondria, the electron-dense globules appear. They may inundate the body of the cells and then be drained one by one. Droplet formation is more frequent in younger, that of cysts in older, epithelial cells. The secretory or digestive activity of the cysts is indicated by the presence of microvilli and pinocytotic vesicles on the inner surface [14]. The vesicles may reach the size of the nucleus, and several large vesicles may even be lodged in some cells. The amorphous substance in the cysts represents PAS-positive matter. A similar process has been observed in tissue cultures where droplets were coalescing into cysts, and small cysts fused to form larger ones. Secretion-like inclusion bodies were found by WEAKLEY et al. [44] in 13-day old golden hamster embryos, where the abundance of endoplasmic reticulum pointed to protein synthesis. Epithelial cells may change there into lymphoid cells, a finding confirmed by TANAKA [33].

Contiguous cells may be connected by protoplasmic bridges (Figs. 2, 5) through which the exchange of ribosomes and macromolecules and so perhaps intercellular transmission of information becomes possible. SCHOENBERG et al.

[30] observed such bridges in lymph nodes and the spleen; the bridges connected macrophages with antibody-forming cells so that ribosomes may have thus passed from the macrophages to the lymph cells and contributed there to protein synthesis. Such bridges were observed by WEAKLEY et al. [44] in the thymus between epithelial cells and thymocytes. A similar purpose may be served by the process in which thymocytes are engulfed by epithelial cells, remain there one or two hours to emerge and pass once more into the epithelial cells, a process that is sometimes repeated several times. The epithelial cell forms a digestive vacuole around the thymocyte, whether it is alive or dead; the thymocyte sometimes escapes but is more often destroyed. Microcinematographic studies have shown that a close relationship and symbiosis may be formed between thymocytes and epithelial cells: the former may adhere to and move or advance with the latter (peripolesis); besides, thymocytes may penetrate into the epithelial cells (emperipolesis). This process may represent the most important phase in the conversion of lymphocytes to thymocytes, i.e. to immunologically competent cells [9]. Similar phenomena have been observed in rabbit thymus cultures by SHARP [31]. Dead thymocytes have frequently been found in the epithelial cells of Hassal's corpuscles; first, the cytoplasm of the thymocyte is digested, then its nucleus becomes pycnotic and disintegrates. Some cysts seem to have developed from the vacuoles digesting thymocytes [15]. The origin of Hassall's corpuscles is connected with the relationship between epithelial cells and thymocytes. The former engulf and digest the latter or their fragments. They form, according to SIEGLER [32], PAS-positive lipochrome and turn into foamy cells, and it is from the enlarged epithelial cells that Hassall's corpuscles develop. Granules will appear in some epithelial cells, and they are then joined by other epithelial cells likewise undergoing granulation so that a concentric aggregation of such cells is taking place. Liquefaction of the innermost cell gives rise to an empty cyst. A process of digestion is, thus, occurring in Hassall's corpuscles.

The observed structural characteristics have confirmed the concept that thymic function is — at least partly — governed by a substance secreted by the thymus itself. According to the latest theory, thymocytes arising in and passing through the thymus receive in this gland information which they carry to the lymphoid tissues, to serve there as the ancestors of cell generations capable of producing immune substances [2, 10, 12, 21, 22, 25, 28, 34, 35]. The mechanism of this potential change occurring in the thymus requires further investigations. It can be taken as proved that thymocytes arise from epithelial cells in the embryo [1], a process which subsequently presumably continues after the birth [19, 34] and constitutes a genetic difference between thymocytes and lymphocytes. These cells are the ancestors of generations of thymocytes which — carried by the circulation to other regions of the organism — are able to assert their immunological competence. This theory gives some information

about the cellular-regulatoric role of the thymus in the immune-biological differentiation of the lymphatic organs. Lymphocytes that have to pass through the thymus in order to receive immunological competence are presumably exposed there to some humoral influence. Such influence becomes possibly operative when the cells come into contact with the substance produced by the thymus. This substance may be the PAS-positive matter originating from the epithelial cells, and the "necrohormone" arising in connection with the breakdown of Hassall's corpuscles. It is conceivable that the nucleoproteins, arising at the phagocytosis and digestion of thymocytes, are involved in these processes, or that — in connection with endocytosis — important factors gain access to the thymocytes. It seems in any case evident that the clue to thymic function lies in the symbiosis of epithelial cells and thymocytes, and that epithelial cells play an essential role in this respect. Although the efforts to demonstrate the existence of a protoplasmic bridge between epithelial cells and thymocytes have remained unsuccessful, bridges of this kind have been found between epithelial cells, a phenomenon pointing to an interaction between the cells of the epithelial reticulum. The activity of epithelial cells in connection with the immunological potency of the thymocytes thus, consists not merely in nutrition but in evocation and completion as well.

It is hoped that further electron-microscopic studies will afford more detailed insight into thymic structure and establish a harmony between function and structure.

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ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNG DER THYMUS DRÜSE VON MEERSCHWEINCHEN

I. TÖRŐ und I. OLÁH

Mit elektronenmikroskopischen Untersuchungen wurde die Mikrostruktur der Epithelzellen der Thymusdrüse von 4—5tägigen Meerschweinchen analysiert, unter besonderer Berücksichtigung der strukturellen Grundlagen der Thymusfunktion. Das Auftreten von Sekretröpfchen und Sekretionszysten sprechen unzweifelhaft dafür, daß die Epithelzellen des Thymus einen Drüsenzellcharakter haben. Auch das endoplasmatische Kerngerüst einzelner Epithelzellen, ihr gut entwickelter Golgi-Apparat sowie das Erscheinen der Nebenkernstrukturen weist in diese Richtung. Die Zellen werden außer den üblichen Desmosomen, den Zonulae occludens durch labyrinthartige, dornige und nagelartige Fortsätze miteinander verbunden. Die Differenzierung der Epithelzellen manifestiert sich in der Sekretabsonderung, aber auch im Auftreten von Tonofibrillen, was zugleich durch die stufenweise Verbindung der Epithelzellen

zur Bildung der HASSALschen Körperchen führt. Die wechselseitigen Beziehungen der Epithelzellen und der Thymozyten offenbart sich im Phänomen der Peri- und Emperiopolese, in der Verdauung der Thymozyten in den Epithelzellen oder in der Bildung von Plasmabrücken zwischen den Epithelzellen. Vermutlich sind dies strukturelle Manifestationen der immunbiologischen Kompetenz der Thymozyten, die auf Grund der während der Zellfunktion vermittelten Informationen entsteht.

ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ ЗОБНОЙ ЖЕЛЕЗЫ МОРСКОЙ СВИНКИ

И. ТЁРЁ и И. ОЛАХ

Авторы исследовали под электронным микроскопом микроструктуру эпителиальных клеток мозгового вещества зобной железы, с особым вниманием на структурные основы функции зобной железы. По их установлениям появление капель секрета и секреторных кист не оставляет никаких сомнений в том, что эпителиальные клетки зобной железы носят железистый характер. На это указывает эндоплазматический остов ядра и хорошо развитый ареал Гольджи отдельных эпителиальных клеток, а также появление «микронуклеарных» структур. Соединение клеток обеспечивают, кроме обычных десмосом и zonulae occludens, лабиринтоподобные, остистые и гвоздиковидные отростки. Дифференциацию эпителиальных клеток показывает, кроме выделения секрета, появление тонофибрилл, в результате чего, одновременно с постепенным спаянием эпителиальных клеток образуются тельца Гассаля. Взаимная связь между эпителиальными клетками и тимоцитами сказывается в явлении пери- и эмпериополеза в переваривании тимоцитов в эпителиальных клетках и в образовании плазматических мостиков между эпителиальными клетками. Это, предположительно, структуральные манифестации развития иммунобиологической компетенции тимоцитов на основе информаций, передаваемых во время функции эпителиальных клеток.

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RECENSIONES

International Conference on Gerontology

Academic Press, Budapest 1965; 939 pages

The volume under review, edited by A. BALÁZS, contains almost the whole material treated at the gerontological conference held in Budapest from October 25 to 27, 1962. After an opening plenary session, the conference continued its work in two separate sections; the volume presents the papers in their chronological sequence.

The plenary session started with an opening address by L. HARANGHY, the then president of the Hungarian Gerontological Society. The world-wide increase in the proportion of aged individuals invests gerontology with a particular importance. While the average life span in Hungary was 24.2 years between 1837 and 1846, it had increased to 65.1 for males and 69.4 for females by 1960, so that 13.8 per cent of the population were older than 60 years. General prolongation of life raises biological, medical and socio-political problems which can be solved only by a close cooperation of state, society and the experts of several branches of science (biology, medicine, demography, social policy). The opening address paid homage to the Hungarian pioneers of gerontology (KORÁNYI, ENTZ, VEREBÉLY, NÉKÁM) and discussed in detail the present multifarious research work of Hungarian investigators which extends to all biological, theoretical and practical problems of this branch of science.

It was likewise at the plenary session that F. VERZÁR gave a paper on the tasks of experimental gerontology, and that GOREV and CHEBOTAREV (Kiev), presented an account of the chief trends of present research carried out in the Soviet Union.

The subsequent papers to be found in the volume are divided into three main categories: (1) biological foundations of gerontology; (2) present problems of geriatrics; (3) social and medical questions connected with gerontology.

In each category numerous clinical and experimental data have been presented. While a number of authors discussed senile changes of the connective tissues and bones, others made comparative analyses concerning the botanical and zoological aspects of senescence.

Geriatrics covers nearly the entire field of medicine so that physicians concerned with the clinical study and treatment of old age and its manifestations have to deal with special diagnostic, surgical and therapeutic problems. The volume under consideration offers a wide survey of geriatric problems encountered in the different spheres of medical science.

The increase in the number of old persons justifies a careful study of the social aspects of old age; studies of this kind require a close collaboration between specialists of medicine, sociology and economy.

The number of papers given at the conference totalled 179 of which 134 are reprinted in the volume, while 45 are referred to in the index only. Lectures held in the Hungarian and English languages are presented in English, the others (Russian, German, French) in the original. It is regrettable that no English summary has been added to non-English texts (to Russian texts in particular).

The volume, of exemplary execution, presents a true picture of international gerontological research work and of the pertaining investigations performed in Hungary.

G. GORÁCZ

J. Botár

The Automatic Nervous System

Publishing House of the Hungarian Academy of Sciences, Budapest 1966. 442 pages, 207 figures
Preface by F. de Castro

In the introduction, the author points out that research is an activity in which the investigator's technical skill, his possibilities as well as the scientific attitude governing his work are of prime importance. Advanced technical facilities make it possible for the modern researcher not only to explore new fields but also to confirm or correct the results of earlier investigators. The former static attitude has been replaced by the more realistic dynamic approach which means that, in evaluating information regarding any examined part of the nervous system, its stage of development, functional condition, the age of the entire organism, the pathogenic or lethal factors have to be taken into account and that, above all, the examined organism has to be known in all its details. By presenting his experimental results, the author wished to demonstrate the truth of these statements and to facilitate the work of investigators engaged in the study of the morphology, physiology and pathology of the autonomic nervous system.

The introductory part describes the theories concerning the vegetative nervous system, divides them into four categories and names their chief representatives.

The first part of the work deals with technical problems. The author analyses the experimental material and surveys the microtechnical procedures, gold impregnation, vital methylene-blue staining, the various methods of silver impregnation and other histochemical techniques as also the role which microscopic inspection and adequate documentation play in the forming of final conclusions by the investigator. In connection with silver impregnation a separate chapter is devoted to the preparation of sections, fixation and the possible sources of error. The chapter (like all other parts of the work) finishes with a concise summary.

The richly illustrated experimental material consists of three main parts.

(1) The definition of preganglionic nerve fibres

(*a*) in the preganglia (innervation of the adrenal medulla in dogs of different ages, and its alterations under the effect of fever, starvation, anoxia and avitaminosis; study of the adrenal medulla of healthy young and old persons and of human autopsy material),

(*b*) in automatic ganglia:

(*aa*) coeliac ganglion in healthy dogs and in dogs with avitaminosis;

(*bb*) tracheal ganglion in young and old dogs.

(2) Autonomic nerve cells of the coeliac ganglion

(*a*) in healthy young persons;

(*b*) in individuals of advanced age.

Separate chapters deal with the various kinds of cells (sympathicoblasts, immature and young cells of the sympathetic nervous system, dividing cells, their changes, degeneration, and the problem of pigment accumulation).

(3) The definition of postganglionic nerve fibres

(*a*) in the myocardium of newly born, young, adult and old dogs;

(*b*) in the smooth muscle of young, adult, and old dogs, further in those of young persons who had died of various diseases.

A bibliography containing papers of 650 authors and teams and an index covering eight pages completes the monographs.

Especial credit is due to the publishers for the careful execution of the book.

E. Somogyi

PROCEEDINGS OF THE ANNUAL MEETING OF HUNGARIAN PATHOLOGISTS AND ANATOMISTS

Szeged, 1966

PLENARY SESSIONS

Acta Morphologica Academiae Scientiarum Hungaricae 14, 1966

INDEX

FORENSIC MEDICINE

Acta Morphologica Academiae Scientiarum Hungaricae Tomus 14 (3—4), pp. 301—391 (1966)

RELATOR

E. Kerpel-Fronius
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ORIGIN AND CLINICAL SIGNIFICANCE OF SOME COMMON DISTURBANCES IN BODY FLUID HOMEOSTASIS

I. Basic Principles of the Regulation of Body Fluid Homeostasis

Acute changes in any parameter of body fluid composition or volume initiate two lines of defence processes aiming at the mitigation of the changes, and then at the final restoration of the initial equilibrium. The peculiar structure of the fluid compartments induces an immediate reduction of the initial changes. Well-known examples of such phenomena are the mitigation of acute hyper- or hyponatraemia by shifts of water between the fluid compartments [1] or the buffering of invading acids. These processes, though sometimes of vital importance, attenuate the immediate shock at the price of new labile and pathologic equilibria. The mechanisms definitively restoring normal conditions are more complicated. They fulfil the criteria for servo systems consisting of information centres that respond to "error signals" of the constants to be regulated. The centres relay the information through a chain of neurohormonal command to the effector organ, the kidneys. The correction of the "error" is fed back to the information centres.

The signal for the hypophyseo-hypothalamic *osmoregulatory* centre is an "error", i.e. a deviation of only a few mEq in the concentration of plasma sodium. This information, analyzed by the osmoreceptors, mobilizes the "agent", the antidiuretic hormone (ADH) acting on the renal tubules. This system adjusting water excretion to extracelullar plasma Na concentration is coupled by the identity of the error signal with the thirst centre, with the system regulating water intake.

The feedback system regulating *extracellular fluid volume* is actuated by an error signal which seems to be a circulatory effect of altered body fluid volume. The chain of command is the renin-angiotensin-aldosterone system regulating sodium excretion [2]. The interplay of the two systems regulating sodium concentration and total body sodium guarantees the constancy of volume and composition.

The first line of defence against *acid or alkali excesses* are the chemical buffers, while the second line is respiratory compensation, a servo mechanism actuated by the "error" of the pH of blood. The final correction of disturbances of acid-base equilibria depends on the kidneys. The excretion of acids, however,

is a slow process. YOSHIMURA [3] found that within 24 hours following administration of an acid load, only 1/4 of the H-ion excess was eliminated by the kidneys. This observation stresses the importance of the immediately acting defence mechanisms: the precisely but slowly working renal mechanism could not guarantee survival in the case of immediate shock due to the liberation of acids in such amounts as occurs for instance in diabetic acidosis.

II. Disturbances in body fluid homeostasis

Disturbances in body fluid homeostasis arise because of 1. overburdening of the normal defence mechanisms, and 2. functional or anatomical lesions of any link of the regulatory chain. In the frame of these introductory remarks we shall briefly discuss dehydration, hyper- and hyponatraemia, and potassium deficiency.

1. *Dehydration*

"1000 parts of cholera serum contain but 860 parts of water", with this sentence in O'SHAUGHNESSY's [4] letter to the Lancet in 1831 begins the history of dehydration. The basis of our modern concepts was laid down 100 years later by GAMBLE [5] who stressed the leading importance of negative sodium

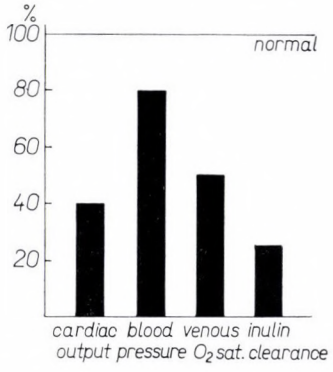


Fig. 1. Shock and renal insufficiency in dehydration. Values in per cent of the normal

balance in the origin of dehydration. Sodium losses accompanied by proportional losses of water lead to the exhaustion of the interstitial fluid reservoir and in turn to oligaemic shock, the clinically decisive event in dehydration.

The haemodynamic changes in clinical [6] as well as in experimental cases [7] of salt depletion correspond to those found in traumatic shock.

Fig. 1 presenting our observations in a dehydrated infant, shows the considerable decrease in cardiac output accompanied by stagnant anoxia and

low renal clearances. The clinical picture at this stage is alarming; the hippocratic face, the frequent pulse-rate, the small heart, the cold, cyanotic extremities, the semicomatous state and the acidotic breathing indicate an emergency case of exceptional severity.

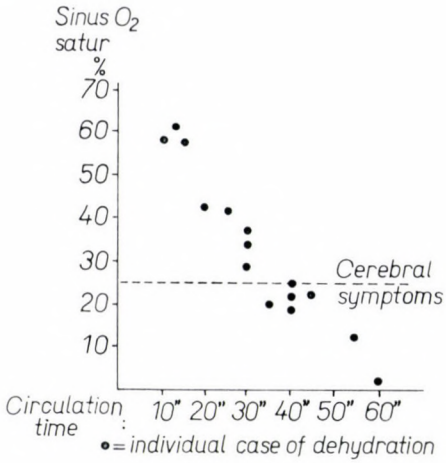


Fig. 2. Diagram of circulation times plotted against cerebral venous oxygen saturations

The central patho-physiologic change is haemoconcentration and oligaemia due to the decrease of plasma volume by 30—40 per cent. This, in turn, leads to the described haemodynamic changes, to extrarenal uraemia, while the stagnant anoxia of the brain is responsible for the cerebral symptoms.

The effect of rehydration is mostly spectacular. However, in exceptional cases renal function does not improve. This is due to anatomical changes superimposed on long standing renal ischaemia.

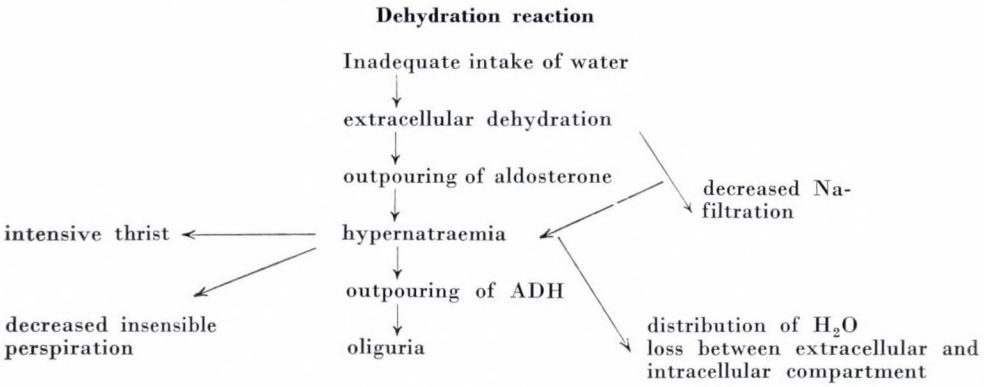
The origin of salt losses. The loss of isotonic digestive secretions containing Na, Cl, HCO_3 and K leads to dehydration. Many details of this mechanism, however, have not yet been clarified. GAMBLE stressed the importance of the shortening of the intestinal transit time due to increased peristalsis inhibiting the reabsorption of intestinal secretions. These isotonic fluids are secreted in daily amounts of 8 L in the upper parts of the gastrointestinal tract, and they are reabsorbed in the lower parts. Any inhibition of reabsorption must cause electrolyte losses. This most valuable didactic conception falls short of explaining daily faecal losses reaching 17 L in severe cases of cholera [8], and the question of heavy losses of bicarbonate and potassium is also left open. According to tracer studies on the movement of water and ions between intestinal lumen and blood, the bowels would receive about 50 L of isotonic fluid in 24 hours [9]. An enteral loss of 17 L/24^h would thus indicate a 35 per cent impairment of the efficiency of the reabsorptive mechanism of the gut. The inhibition

of reabsorption is thought to be caused by damage to an assumed "sodium pump".

An alternative hypothesis was advanced by GREENOUGH[10]who assumed the entrance of a large quantity of fluid into the gut due to the hypersecretion of pancreatic juice. The fluid and electrolyte losses were supposed to be the result of a combination of hypersecretion and defective absorption.

Be as it may, the important practical point is that Na losses amounting to 12—16 mEq/kg, equivalent to a loss of 800—100 ml/kg of e.c. fluid invariably lead to severe oligaemic shock. Losses of this extent may occur in the extreme forms of diarrhoea within 24 hours, while renal or gastric losses proceed for days until negative balances reach critical levels.

To the type of dehydration secondary to sodium depletion described above, Kerpel-Fronius in 1935 contrasted the water depletion type which is due to shortage of water without any significant loss of electrolytes from the body. The main patho-physiologic changes exhibited during thirsting may be summarized in the so-called "dehydration reaction".



Hypernatraemia may be considered the most important life prolonging defence mechanism of the thirsting organism. It induces maximal renal and extrarenal water economy, and by an osmotic shift of water from the cells, plasma volume may be preserved in the face of large losses of water. In this type of water depletion excruciating thirst, dryness of the mucous membranes, nervous irritability and hyperthermia are the main clinical signs, while hypovolaemic shock and extrarenal azotaemia are late events.

2. *Hyper- and hyponatraemia*

a. Hypernatraemia

The common feature in practically all cases of hypernatraemia is cellular dehydration due to the osmotic shift of water from the cells to the extracellular compartment.

Extracellular fluid volume is, however, variable. It may be decreased: "hypernatraemia with global dehydration", or increased: "hypertonic expansion of the extracellular fluid" [11].

Hypernatraemia with global dehydration corresponding to "water depletion" develops when water is not available or when water intake in week or unconscious patients does not cover obligatory expenditures. Special forms may arise in cerebrally damaged individuals because of a higher setting of the cerebral osmoreceptors, or consequent to a damaged thirst centre. Furthermore hypernatraemia with global dehydration may develop in patients with diarrhoea, when the loss of water exceeds that of the salt. The hypertonic expansion of extracellular fluid is mostly a iatrogenic damage referable to the administration of too much salt.

An impressive clinical feature of most cases are *cerebral symptoms*, restlessness, twitches, convulsions. Residuals such as mental retardation have been described in infants suffering from long-standing, unrecognized hypernatraemia. Opinions on the mechanisms involved are divided. The discussed possibilities are osmotic damage of the brain cells, cellular dehydration, cellular acidosis, and "haemorrhagic encephalopathy" [12]. In acute animal experiments as well as in autopsy specimens of 5 newborn victims of an accidental salt poisoning [13], haemorrhagic encephalopathy was a constant finding. Gross anatomical lesions are, however, infrequent in patients who as a result of disease become hypertonic slowly. The rate of the induction of hypertonicity thus seems to be important in the origin of the haemorrhages.

b. Hyponatraemia

The two main forms of hyponatraemia are the "*natriopenic*" and the "*dilution*" type. The first is practically identical with the salt depletion form of dehydration, while in dilution hyponatraemia cerebral symptoms due to water intoxication dominate the clinical picture. Water intoxication is practically always iatrogenic. It arises when salt depleted patients are treated with hypotonic solutions, when excessive amounts of water are "pushed" into individuals postoperatively. It is also seen in anuric patients [14, 15] and in oedematous individuals undergoing forced diuretic procedures. During the last years a peculiar clinical entity described under the heading of "*hyponatraemia due to inappropriate secretion of antidiuretic hormone*" has called the attention of many workers [16, 17]. The salient features of this syndrome, associated with bronchogenic carcinoma, intracranial disease, meningitis and tuberculosis are a hypertonic urine containing significant amounts of sodium in spite of hyponatraemia, and the absence of dehydration and azotaemia.

Potassium depletion

The surprising frequency of secondary potassium depletion in a great variety of diseases is too well known to go into details in this brief summary. We thus intend only to stress a few practical points. *Iatrogenic factors* [18] certainly play an important part in many cases of potassium depletion. As examples we cite the overzealous administration of bicarbonate in diarrhoeal and in diabetic acidosis or in cetonaemic vomiting of children. The chronic iatrogenic forms result from the abuse of laxatives, the use and abuse of diuretics, from abuse of bicarbonate in chronic vomiting.

Attention is actually focussed on cases of chronic renal wasting of potassium due in a number of cases to primary or secondary aldosteronism.

Clinically, the salient features of potassium depletion are related to the three types of muscle in the body, and to kidney function; damage to the skeletal muscles causes an alarming weakness, even muscular paresis. Paresis of the smooth muscles leads to abdominal distension, constipation, to intestinal paralysis. Involvement of the heart muscle is manifested by hypotension, ECG abnormalities, cardiac arrest being the greatest danger in this condition. The leading manifestations of the nephropathy of potassium depletion [19] are hyposthenuria and polyuria. Since in chronic cases the clinical picture is not always easy to interpret, such patients are often transferred to the neurologist, to the gastroenterologist or to the nephrologist before the discovery of low plasma potassium clarifies the situation.

Long-standing potassium deficiency results in anatomical lesions of the myocardium and the kidneys. A frequent finding is secondary pyelonephritis [20]. Since renal biopsies may clarify obscure cases of hypopotassaemia and may establish nephropathy of pyelonephritis, this is the field of electrolyte disorders in which collaboration of the clinician with the pathologist is of the greatest practical importance.

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RENAL CHANGES AND JUXTAGLOMERULAR COMPLEX IN DISTURBANCES OF SALT AND WATER METABOLISM

The primary function of the kidney is to maintain the fluid and electrolyte household, i.e. homeostasis. As the interaction of internal environment and renal function is reciprocal, the disturbances of salt and water metabolism may have the kidney as their place of origin and also the organ they affect. In the present paper we shall not discuss the full range of these functional disorders but limit our inquiries to their most important forms and the morphological changes they involve.

In regulating the water balance, a function which actually can hardly be differentiated from the metabolic disorders of the dissolved electrolytes, the kidney is playing a fundamental part. While physiologists are well aware of the factors determining the quantity and concentration of urine, comparatively little is known about the morphological changes and their sites within the nephron. Obviously none of the early theories which regarded the nephron as a straight tube, was able to explain the processes of dilution and concentration. The countercurrent theory of concentration, which has by now gained general acceptance, has taken into account the morphological fact that Henle's loops and the vasa recta traverse the pyramids in a hairpin form. Evidence in favour of the countercurrent principle is also furnished by morphological examination. So the contrast, visible under the electron microscope, between the poor ultrastructure in the thin descending and the highly differentiated structure in the thick ascending limbs of Henle's loop seems to confirm the assumption that active Na secretion in the ascending limb is an essential feature of the mechanism. Models taking into account the vasa recta have recently replaced the concept of the purely tubular pattern. Our observations by the use of a trichrome dye have shown that the substance inside the vasa recta stains differently in each medullar zone. The darkest staining was obtained, as a sign of highest plasma protein concentration, in the apical portion of the papilla; the diluted contents of the much wider vessels of the external medullary zone hardly took the stain. All this agrees well with the biologically established

fact that a much greater amount of dilute fluid is streaming across the efferent medullary vessels and that in the vasa recta plasma protein concentration increases as the degree of osmolarity in Henle's loops and interstices increases towards the pyramidal tip.

Tests by GINETSINSKY et al. exemplify the difficulties that arise from a wrong interpretation of the morphological conditions. They have namely claimed that the process of concentration was accompanied by apocrine hyaluronidase secretion in the collecting tubules and this would depolymerize the interstitial mucopolysaccharides and make the interstices, the reabsorbing tubules and the vasa recta permeable to water and Na. But the pictures they offered for supporting their hypothesis showed a different situation. What appeared to them as collecting tubules in the state of concentration flattened under the supposed effect of apocrine secretion, was on closer inspection clearly identifiable as a flat endotheloid lining of epithelium in the thin descending limb of Henle's loops.

Tests by my associate I. DÉVÉNYI, to demonstrate the site of the concentrating process in ADH-treated rats, have shown the most intense activity to occur in the collecting channels where the number of mitoses in the treated animals increased to 79.1, as compared to an average of 1.5 in the controls.

Hydropic degeneration of epithelial cells in the proximal tubules, the morphological equivalent to tubular disturbances of water metabolism, may be induced by concentrated parenteral doses of hypertonic sugar or other solutions. ALLEN claimed the kind of degeneration which he termed osmotic nephrosis, to be a process in which the high osmotic pressure of tubular fluid makes water from the intertubular capillaries enter the epithelial cells. Nephrosis, however, is a misnomer for this usually reversible process. KIEF and ENGELBART used solutions at various concentrations and, subjecting the epithelial cells in various points of time to electron microscopic examination, found membranes from the brush border microvilli to demarcate pinocytotic vacuoles which migrate from the lumen across the cytoplasm towards the cell base. They moreover succeeded in demonstrating the dissolved substance itself inside the vacuoles, furnishing evidence that acid phosphatase has a part in this apparently transcellular, yet essentially extracytoplasmatic transport. Although the alteration was found to be reversible, yet, under conditions of excessive transport, the cells are liable to degenerative changes, if not to necrosis. This hydropic swelling should strictly be differentiated from the vacuolar degeneration, although there is much confusion in the literature regarding this point. Relying on the evidence of a large postmortem- and biopsy material, I can assert with confidence that the two kinds of alteration in their pronounced forms never present themselves at the same time and that vacuolar nephropathy is a histological sign of K depletion.

Of the disturbances of salt metabolism we shall now discuss those which involve substances which are secreted by the kidney and which lead to morphological changes. Anomalies due to a shortage or superabundance of Na and Cl make the morphological picture appear extremely meagre because the epithelial cells of the renal tubuli afford in this respect a high degree of compensation.

In the older literature there is frequent reference to renal changes following hypochloraemia. BÜCHNER et al. had claimed that the occurrence of tubular epithelial necrosis and calcification was due to loss of chlorine. KERPEL-FRONIUS in 1936 was the first to show that it was not the hypochloraemia but the simultaneous dehydration that really matters. Examinations by GÖMÖRI and SÁRMAI pointed at dehydration, a drop of the filtration pressure. Morphologically, GÖMÖRI and ROMHÁNYI observed stasis in the renal cortex and the occurrence of alkaline phosphatase which they proved, in connection with the calcifying process, to emerge from the brush border and to liberate calcium from the organic phosphates in the filtrate.

Long known and much studied are the renal changes associated with K depletion. Characteristic of it in humans is the appearance of few, relatively large and sharply demarcated vacuoles in the epithelium of the proximal tubules, while in rabbits and rats experimental K depletion induces changes mainly in the medulla. The epithelium of the collecting tubules present PAS positive droplets, nuclear pyknosis, mitoses and the appearance of multinucleated cells. The epithelial proliferation leads to partial obstruction and the tubules in the internal medulla dilate. Under K shortage of brief duration, the acid phosphatase and esterase activity in the epithelium of the collecting tubules has been found to increase. The changes depend on the duration rather than the intensity of the K depletion and are usually reversible in their clinical as well as experimental forms, although in some grave chronic cases streaky scarring has also been observed.

Of the disturbances of calcium metabolism, hypercalcaemia is the most frequent type to affect the kidney. The process, properly termed nephrocalcinosis, is characterized by tricalcium phosphate deposits in the tubules, but mainly in the interstitium. A classical example is seen in Recklinghausen's osteodystrophy. Here the parathormone superabundance inhibits tubular phosphate reabsorption and leads to hypercalcaemia, whereby Ca is mobilized from the skeleton and keeps the blood and urine Ca levels high. According to electron microscopic evidence, the Ca deposits are not in the normal tissue elements but always in those of deranged ultrastructure. Nephrocalcinosis following hypercalcaemia had been observed in infants suffering from iatrogenic D hypervitaminosis and plasmacellular pneumonia, and in adults in association with plasmocytoma, osteoclastic tumour metastasis and often with Boeck's sarcoidosis. The development of nephrocalcinosis is hard to account

for in connection with bronchial carcinoma, when it does not form bone metastases. Ca deposits in the kidney have been observed also in cases of adrenal cortical insufficiency, thyreotoxicosis, furthermore poliomyelitis and other paralytic diseases of the spinal cord. Similar alterations associate with Burnett's alkalosis syndrome in patients with ulcers who are treated with alkali and a Ca-abundant milk diet. Less frequent is the condition described by CHRISTENSEN and known as D-vitamin resistent primary rickets with an increased phosphate and Ca excretion owing to the diminished capacity of the tubules to reabsorb phosphate. In idiopathic hypercalcinuria (LIGHTWOOD, ALBRIGHT), the kidney is unable to produce acid urine and the calcium salts precipitate in the medulla. RATHBUN observed nephrocalcinosis with congenital alkaline phosphatase deficiency. Na urate, the end product of purine metabolism in the human organism may also precipitate in the kidney, if uric acid formation is increased, as with the uric acid infarcts of newborns, or in adults during the last phase of pneumonia or under radiologic treatment of leukaemia. Gout is a further condition associated with uric acid overproduction, i.e. a state of uricaciduria in which rhomboid and needle crystals of urate first precipitate in the medullary tubules destroying their epithelium, later infiltrate the interstitium where they may cause a variety of disturbances, ranging from reactive inflammation and scarring to renal fibrosis.

A similar mechanism can be operative when calcium oxalate crystals are formed in the kidney. The extreme endogenous form of this process, oxalosis, develops independently of the diet; in this condition first hyperoxaluria occurs, later with rosette or flabellate crystals are deposited in the kidney and the bones. Inflammation, scars and renal atrophy have all been reported in connection with oxalosis. A further condition is ethylene glycol poisoning; this compound is oxidized in the organism into oxaloacetic acid and thus forms a Ca oxalate precipitate in the kidney. A feature of ethylene glycol poisoning is a vacuolar degeneration of the tubular epithelium, closely resembling the vacuolar nephropathy characteristic of K depletion.

Brief mention should be made of the lesions due to disturbances of amino acid metabolism. These are familial for the most part and present themselves with disorders of the tubular reabsorption of certain amino acids, owing to the absence or shortage of one or more enzymes. The most complex form, known as the Debré — de Toni — Fanconi syndrome, consists of infantile glycosuria and hypophosphataemic rickets, associated with dwarfism. CLAY and DARMADY have shown the syndrome to be characterized morphologically by the swan-neck-lesion, presenting a shorter and imperfect development of the proximal tubule, with a very thin initial portion and flattened endotheloid lining, and a dilated distal portion. Some authors called attention to the lack of phosphatase in the underdeveloped tubules. Others distinguished the syndrome from cystinosis in which hexagonal cystine crystals precipitate in the interstitium

without giving rise to an inflammatory reaction. Increased excretion of other amino acids may also occur in familial cystinuria, the principal symptom of which is the development of cystine calculi in the urinary system.

Next, I will deal with the juxtaglomerular (JG) complex, meaning the entity of structural elements which are constantly present in the vascular pole of the glomeruli. Functionally, the complex is closely related to the activity of the kidney as an osmotic and volume regulator, although the mode of interaction between the several components is still far from being fully elucidated. This part of my report is based on the results achieved in our Institute in the last 6 years.

Included as belonging to the JG complex are the pre- and post-glomerular arterioles, and the wedge-shaped area they enclose which contains the group of small cells called Goormaghtigh's organ, now commonly called lacis furthermore the vascular pole of the distal convoluted tubule, the macula densa, called so because it presents a denser agglomeration of epithelial cells with dark nuclei. The mostly studied elements of the complex are the smooth muscle cells in the wall of the afferent arteriole; these are in a state of epitheloid transformation and present a higher or lower number of vividly staining granules in the cytoplasm. The granular cells identifiable in the kidneys of many vertebrates have been regarded since Goormaghtigh as the organ's endocrine elements and the general view today is that they release renin, the substance that accounts for the blood pressure increasing function of the kidney. We used our own trichrome stain for demonstrating the granules and availed ourselves of the Hartroft and Hartroft index to determine their quantity. However, the index only gives a semiquantitative value for the cell group frequency and the intensity of granulation, which it expresses in percentage of the number of examined glomeruli. In our experiments we examined an average of 900 glomeruli per rat. Indices computed on the basis of 100 to 200 glomeruli are not reliable.

The joint occurrence of granules, myofibrils and various transitory forms has furnished electron microscopic evidence for the smooth muscle origin of the studied cells. The formation of granules, according to these findings, is a real secretory process, with the Golgi region and the endoplasmic reticulum involved in it.

In most animals granular cells appear only after birth. In albino rats they appear during the second week of extrauterine life and attain the characteristic value for a full-grown animal by the end of the second month. When grafts void of JGC were taken from the renal cortex of rat embryos and transplanted under homologous conditions into the subcutis, we observed the formation of numerous granulated cells, while all other tissue elements perished. Our conclusion was that in the cells of preglomerular arterioles the potential capacity to form granules must be present during embryonal life and that

this capacity must manifest itself later even under the abnormal conditions of a graft. As to phylogenesis, the view was long maintained that JG cells are absent from the renal arterioles of salt-water fish (with the implied negation of these cells being responsible for renin production), until BOHLE in 1964 demonstrated JG cells, located in glomerular and even in non-glomerular kidneys of various salt-water species. Examinations with our trichrome stain combined with chromium acid and periodic acid preoxidation, revealed fine granular cells in the renal artery wall of the salt-water fish Pleuronectes flesus and of fresh-water carp.

More detailed inquires into the histochemistry of JGC, after HARADA's pioneer work which comprised many errors, were mainly conducted by my associate Sz. GOMBA, who claims that the granules consist of protein and carbohydrate components and contain no lipoid. The carbohydrate component is made up of neutral glycoprotein. The protein component abounds in tyrosine, tryptophane and histidine. Adrenaline, noradrenaline and serotonine were unidentifiable histochemically in the granules. The JG cells in rats and mice present a moderate acid phosphatase activity but, in distinction to the smooth muscle cells of other arterioles, they are void of ATPase. HESS and PEARSE found the granular cells to abound in mitochondrial G-1-PD and the macula densa in G-6-PD. The parallel course of the two enzyme activities seemed to confirm the view concerning the functional unity of JGC and macula densa. Freezing the kidney tissue before fixation to below -10° C, with subsequent thawing, renders the granules unidentifiable by staining, probably because the treatment destroys the lipoprotein membranes. Thus care is needed when estimating the histochemical reactions of freezing and thawing kidney tissue; GOMBA and other authors devoted separate studies to the vital staining properties of the granules. The selective colour reactions of the granules to neutral red, cresyl blue, Nile blue sulphate, and acridine orange seem to be attributable to the physical binding of these dyes.

The specific fluorescence of granules observed by EDELMAN and HARTROFT in sections treated with fluorescein-labelled antirenin serum allows the inference that those granules correspond to renin or its precursor. It remains to be cleared whether renin is present in the renal cortex independently of the granules. In our experiments pressor activity, this generally accepted indicator of renin was practically identical in the granule-free kidneys of rat embryos, the kidneys of full-grown rats and in JGC rich mouse kidney extracts. These findings apparently contradict the existence of a direct proportion between the JGC count and the degree of pressor activity. A possible alternative would be that non-granulated types of kidney tissue also contain renin or some other kind of pressor substance. The presence of isorenin — a substance of similar effect — was demonstrated by WERLE in the salivary gland and by GOULD in the vascular walls of mice.

Accepting the JGC as the source of renin, the exact site of production still remains doubtful. HARTROFT et al., TOBIAN et al. and many other authors including ourselves, claim renin to be a product of the modified muscle cells. BING et al. regard the macula densa as the primary site of renin formation, assigning to the granular cells the function only of taking up, storing and discharging the substance into the blood current. In our experiments, on the other hand, there was no macula densa to perform any function, and granulated cells still made their appearance in the transplanted kidney tissue, although there was no enzyme reaction and extensive destruction presented itself in the tubules — all this seeming to indicate that the JG cells are the primary site of granule production. Examinations by GOMBA have placed beyond doubt the pressor activity, i.e. the renin producing function, of such transplantates.

Much disputed in connection with the JG complex is also the stimulus that releases the secretory function. There is a view, supported mainly by DUNIHUE, also by HARTROFT, that in proportion as the serum Na or mineralocorticoid level falls — e.g. as a result of adrenalectomy — so do the granules multiply, and vice versa as these levels rise, so do the granules decrease in number. This chemical theory contrasts with TOBIAN's experimental results which indicate, in agreement with ours, that the granule count varies inversely with blood pressure in the afferent arterioles. If this assumption of a mechanical stimulus proves true, then it would be proper to describe the JG cells as structures corresponding to baroreceptors — or stretch receptors, as TOBIAN called them — located in the renal vessel walls.

When repeating our experiments on larger material, we were unable to confirm the finding that destruction of the anterior hypothalamus would increase the granule count. Neither did we notice any change in the granulation index under the effect of drugs affecting the sympathetic and parasympathetic nerves or the smooth vessel musculature. Nevertheless the possibility can not be excluded that the nervous system exerst some influence on the JGC function. This, at least, seems to appear from the electron microscopic studies of BARAJAS who described a rich network of medullary unmyelinated fibres among the lacis cells, in close contact with the ordinary and the granular smooth muscle cells. These assumptions do not necessarily exclude one another and might perhaps be brought to a common denominator, the consideration being that rises in the Na and mineralocorticoid levels alike involve an expansion of the intravascular space and thus result in degranulation, owing to a stretching of the arterioles. According to TOBIAN, the extra- and intracellular Na gradients in the muscular layer of the renal arterioles would in final analysis condition the secretion of renin.

The question whether an increased granule count really involved an increased secretion had been long disputed because with other glands the situation was just the opposite. The finding that higher granule production went

together with intensified secretory activity was not a surprise for the investigators, including ourselves, considering that modified muscle cells, and not gland cells differentiated to perform a secretory function, were the object they had studied. GROSS, too, established a parallelism between the amount of renin the kidney contains and the amount it secretes.

More important than all this is to discover the way how renin works within the organism. Produced in the JG complex, renin acts as an enzyme, turning the plasma angiotensinogen into active angiotensin, the most efficient pressor substance known. The remarkable thing is that angiotensin in the kidney decomposes thousand times as fast as in other organs or in plasma. Thus renin and the destroying agent of its product — angiotensinase — are not only present together in the kidney, but are present at the highest concentration. Many authors ascribe a role to the renin-angiotensin system mainly in blood pressure regulation and in the development of hypertension. Following the experiments by DAVIS and associates, furthermore by GENEST and his team, the renin-activated angiotensin has been held to be the trophormone of the zona glomerulosa and thus to act as a regulator of aldosterone production and, through it, also of Na metabolism. If this is the case, there must be a correlation between the functions of the JGC and the zona glomerulosa, and of course also between their structures.

Questions of interdependence between the zona glomerulosa and aldosterone production do not concern us here in detail. Neither shall I discuss the role that the JGC system is assumed to play in the development of hypertension. In the light of recent results it seems more and more likely that renin acts on blood pressure not primarily but in some indirect way. Nevertheless, an analysis of some cases we shall discuss in a separate paper, seems to indicate that a pathologic constriction of the renal artery may sometimes induce JGC hyperactivity, leading through increased aldosterone production to grave forms of electrolyte disorder and hypertension (secondary aldosteronism).

It adds to the difficulty of discovering the action mechanism, that renin from the JG complex and the angiotensin activated by it, exert effects which vary from one species to the other and differ even in normotensive and hypertensive human subjects. HARTROFT's antirenin serum administered to Na-deficient dogs inhibited the previously increased tubular Na reabsorption without affecting diuresis, K excretion and serum electrolyte concentration. According to FASCIOLO, under physiological conditions renin acts solely on the kidney but in emergency states such as renal ischaemia the greatly increased renin production is likely to influence the entire organism.

Considering BARTTER's hypothesis in relation to the connection of hypervolaemia and aldosterone secretion; furthermore TOBIAN's and our group's conceptions concerning the connection between the stretching of the arteriolar wall and the granulation of JG cells, we may outline the following hypothesis.

If the stretch at the receptor site is diminished, the granulation of JG cells increases, more renin is produced and more angiotensin is activated. As a result more aldosterone is produced by the zona glomerulosa, which leads to sodium retention and an increase of plasma volume. The increased plasma volume exerts an increased stretch upon the arteriolar wall, consequently the granulation of JG cells diminishes and the process outlined above takes place inversely.

LEYSSAC's examinations of the kidney in vivo combined with micropuncture tests showed that angiotensin exerts an influence on sodium and water metabolism by diminishing tubular sodium reabsorption. According to THURAU's important experiments there is an inverse relation between sodium content of the macula densa region and the GFR; this mechanism would regulate the RBF, GFR and tubular sodium load, respectively.

Thus, the JG cells may be considered chemoreceptors or stretch-receptors, but at the same time secretory elements, and by the production of renin they play an important role in the regulation of sodium and water metabolism.

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THE ROLE OF THE ADRENAL CORTEX IN THE REGULATION OF FLUID AND ELECTROLYTE BALANCE*

The adrenal cortex has a decisive role in the maintenance of electrolyte and water balance. Although the homeostasis is influenced by glycocorticoids, their importance is secondary as compared to that of aldosterone. The site of action of aldosterone is the renal tubule, where it causes sodium retention and enhances the excretion of potassium. The regulation of aldosterone secretion is a matter of dispute. It has been suggested that the pituitary, hypothalamus, eventually the pineal body are involved in the control of adrenal aldosterone production. Data concerning the problem of regulation are, however, contradictory. Great importance is being attached to the kidneys in the regulation of aldosterone secretion. Receptors in the kidney, susceptible to changes in the pressure and ionic composition of the blood, enhance the production of renin in case of hyponatraemia or hypovolaemia. Renin stimulates the formation of angiotensin which in turn leads to augmented production of aldosterone.

Diseases of the adrenal cortex are generally associated with disturbances of electrolyte and water metabolism. Sodium depletion and hyperpotassaemia are well-known symptoms in Addison's disease. Isolated hypoaldosteronism is characterized by significant alterations in electrolyte and water metabolism. Enzyme defects in the adrenal cortex give rise to salt losing syndrome. Cushing's

* Paper will be published later.

syndrome is often accompanied by disturbances in salt and fluid equilibrium. The most pronounced defects in water and electrolyte metabolism are usually observed in primary and secondary hyperaldosteronism. Primary hyperaldosteronism or Conn's syndrome is a pathological entity, while secondary aldosteronism may accompany a variety of diseases: cardiac failure, hepatic cirrhosis, the nephrotic syndrome, etc. Pathological aspects of hyperaldosteronism have been discussed.

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HISTOPHYSIOLOGY OF THE ANTIDIURETIC CENTRES*

It has long been known that the supraoptico-neurohypophysial apparatus plays a decisive role in the maintenance of water balance. The antidiuretic hormone was first thought to be secreted by the pituicytes of the pars nervosa hypophyseos, the activity of the latter being regulated by the supraoptic nucleus (RANSON et al., 1938—39). Subsequent morphological investigations carried out after the revival of the neurosecretion principle (SCHARRER, 1928; BARGMANN, 1949) made it clear that the neurohypophysial hormones were produced in the ganglion cells of the supraoptic and paraventricular nuclei, gained access to the posterior pituitary by means of the azoplasmic stream and were stored there, whereas the presumable function of the pituicytes was to pass the hormones into the circulation. These discoveries threw a new light on the existing notions regarding the neurohypophysis and the pathogenesis of diabetes insipidus, and encouraged investigations concerning the so-called hypophysiotropic substances.

In accordance with its secretory and storing functions, the anterior hypothalamo-neurohypophysial system responds to lesions with special histological reactions, is provided with a particular blood supply and has a special enzymatic pattern. While both SH and S—S groups are demonstrable in the protein of the ganglion cells, only S—S groups have been observed in the neurophypophysis; tagged thioamino acids were found to be incorporated in the central part of the system.

It was suggested at the 1951 Pathological Congress that the histologically demonstrable neurosecretion might represent glycolipoprotein (BACHRACH et al.), a notion arrived at by SCHIEBLER (1951) as well. After the chemical structure of the so-called neurohypophysial hormones had been elucidated (DUVIGNEAUD et al., 1953) it was found by several investigators that carbohydrates and lipids were not permanent components and that the protein fraction was decisive so that the secretion of the supraoptico-neurohypophysial

* Paper will be published later.

system represented the hormones themselves and not only some hormone-carrying substance (SLOPER, 1955; HOWE, PEARSE, 1956). The results of recent investigations allow a combination of the two concepts: it seems that the proteinaceous secretion of the ganglion cells is first bound to carbohydrates and lipids which then become detached (BARA, B. SZABÓ).

The neurones of the supraoptic and paraventricular nuclei are particularly suitable for histophysiological investigations: one is dealing with well-circumscribed magnocellular nuclei whose activity is indicated by secretion; the neurones respond to adequate stimulation (dehydration) intensively, while their other activities — save the secretion of oxytocin — seem to be negligible. Information regarding the functional condition of the neurones is supplied not only by the classical methods (Gomori's chrome-alum haematoxylin and aldehyde-fuchsin staining) but also by the histochemical demonstration of SH and S—S groups (i.e. the sulphur-containing amino acids of the proteins), further by other indications of protein synthesis (interproportion of cellular components, amount of RNA) as well as by enzymatic-histochemical and electron-microscopic observations.

By following the various phases of dehydration and rehydration in white rats, the functional cycle of the neurones has been drawn up. Findings made in this connection may serve as a guide to further histological conclusions concerning the functioning of other — non-neurosecretory — ganglion cells.

Finally, some recent results concerning the role of the epiphysio-epithalamic system in the maintenance of salt and water balance is discussed.

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Consequences of the Disturbed Acid-Base Balance

Disorders of the acid-base balance may give rise to various morbid changes in the human organism. On the basis of his studies of ammonia poisoning in humans and experimental animals FAZEKAS established that ammonium hydroxide, despite its alkaline nature, is apt to induce severe acidosis and lipaemia. Examinations at that time in the forensic and pathologic institutes of Szeged have shown that ammonium acidosis destroys the elastic fibres of the arteries and mobilizes fat.

HALDANE stated ammonium chloride to lead to a similar state but subsequent studies have made it evident that the mechanism of severe acidosis is different. Postacidification of the blood has shown that a destruction of red cells by ammonia is responsible for the acidosis. Tests by FAZEKAS in 1939 demonstrated that adrenal hypertrophy is one of the various consequences of acidosis.

A few recently discovered facts are quoted, referring to disorders of the acid-base balance.

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Changes in Water Content and in Solid Concentration of Cells Measured by Interference Microscopy and Microcinematography

The refractive indices of various biological material such as cell cytoplasm can be measured with high degree of accuracy. From such a refractive index measurement cytoplasmic solid concentration and water content may be determined. The total dry mass of most cells is accounted for by the protein components. By the effect of cytotoxic agents(tragacanth, mustard derivatives, Vincaleucoblastine) the dry mass may be altered withont any perceivable change in the solid concentration.

Changes of cellular solid concentration were followed by colour interference microscopic cinematography in tissue culture (HeLa cells). It has been established that Vincaleucoblastine induces an increase in the solid concentration.

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The Effect of Electrolytes on Dry Weight and Microstructure of Cell Nuclei

Resting cell nuclei show double refraction after treatment with trypsin or salts. In the present experiments, the effect of different salt concentrations has been studied on the dry content, nucleic acid and protein content of the thymic lymphocytes isolated in sucrose. The dry content was determined by interference microscopy, while the amount of nucleic acid and proteins was established by the cytophotometric method, after staining with gallocyaninchrome alum and fast green, respectively. Changes in cell microstructure were followed by polarization microscopy, using anisotropic toluidine blue staining.

In a medium with low ionic concentration, development of nuclear anisotropy runs parallel with the increase in surface, while dry weight and total protein decrease and nucleic acid content do not alter significantly. Nuclear anisotropy is assumed to be due to changes in the linkage between protein and nucleic acid.

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Fluid, Electrolyte, and Protein Balance in 200 Cases of Major Thoracic Surgery

The paper has dealt with some practical aspects of controlling fluid and electrolyte household in patients undergoing major thoracic surgery. Experience concerning treatment of impaired balance and measures applied in the department are discussed. Emphasis is layed upon the special problems arising at pulmonary surgery. Disturbances of protein household. common when treating tuberculotic cavities by the open method, are also discussed.

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(Department of Pathology, Pál Heim Children's Hospital, Budapest)

Pathological Aspects of Electrolyte Therapy

A survey of post-mortem findings and histologic studies in infants and children displaying electrolyte disturbances suggests that it is the general condition rather than the basic disease which determines the severity and course of the process. The organic changes developing during electrolyte therapy are discussed.

A. Haraszti, L. Török

(Department of Pathology, County Hospital, Eger)

Potassium Content of the Liver in Acute Circulatory Failure and Secondary Shock

Potassium content of the liver has been studied in necropsy material. In most cases, shock was accompanied by diminished potassium contents in the liver. Histological examination revealed in most of the potassium-depleted livers signs of structural lesions variable both in form and extension. The histological finding was in more or less satisfactory correlation with the amount of potassium found in the liver. No correlation, however, could be demonstrated between hepatic and serum potassium. Determination of hepatic potassium content appears to be a valuable method supplementing the routine histological study.

A. Sárdy

(Central Research Institute for Physics, Budapest)

Activation Analysis: a Sensitive, Rapid, and Reliable Method for the Study of Salt Metabolism

Activation analysis is growing continuously in importance as a method suitable for the study of mineral exchange in biological materials, thus of the electrolyte household of the human organism. Principally, the method is based on the exposure of the sample to radiation capable of inducing a nuclear reaction (usually neutron radiation); from the activity thus induced, the original constituents of the sample can be established. The main advantage of the method lies in its sensitivity which exceeds by several orders of magnitude that of any other method (10^{-9} to 10^{-12} g). The method is equally reliable in the case of very low concentrations, since contamination of the reagents does not interfere with the results. It is suitable for the study of quite small amounts of material such as needle biopsy samples. Matrix elements (carbon, hydrogen, oxygen, nitrogen) are not activated. In addition, the method is simple, there is often no destruction of the biological material, the procedure is rapid and can easily be automatized which always means reduction of costs. In contrast to the isotope methods, it is suitable for studying conditions of equilibrium, and as the material is activated after it has been removed from the organism, no radiation injury occurs. Sources of activating radiation may be not only reactors, but also neutron generators and other accelerator devices.

Sodium can be estimated with quick neutrons in 30 sec. With slow neutrons, the sensitivity of the method is at the 3 μg level; which can be determined together with 0.5 μg of potassium in an aliquot of 1 μl, separated without any destruction from a sample of 10 μl. The sensitivity of the method with regard to sodium, potassium and phoshorus attains 10^{-10}, 10^{-9}, and 10^{-10} g, respectively. Calcium and magnesium can be determined in quantities amounting to a few μg, within 20 min. Chlorine, sulphur and iodine can be estimated in amounts of 10^{-9}, 10^{-6} and 10^{-9} g, respectively. The method of activation analysis allows the quantitative determination of some further biologically important elements.

K. Lapis, I. Benedeczky, G. Szilágyi

(Section of Pathology, Postgraduate Medical School, Budapest)

Ultrastructure and Function of the Various Cells of Human Parathyroid Adenoma

The electron-microscopic identification of cell types seen in the parathyroid under the light microscope is still problematic and the correlation between functional activity and morphological picture is not fully elucidated either in the normal gland or in parathyroid adenoma.

The morphology of mixed-cell adenomas has been studied under the light and the electron microscope. It was possible to identify under the electron microscope the main, oxi-

philic and the so-called transitional water-clear cells, while attempts to recognize typical water-clear cells under the electron microscope have failed.

Clinically confirmed extreme hyperfunction (serum Ca 14 to 16 mg per 100 ml) of the examined adenomas was accompanied by the predominance of bright cells which showed signs of secretory activity (numerous secretory granules, abundant endoplasmic reticulum of coarse surface, presence of Golgi apparatus). It seems, therefore, that earlier notions regarding the inactivity of these cells (RÓTH, 1962) have to be corrected. It is suggested that the dark cells containing many secretory granules should be regarded as glandular storage cells, rarely present in mixed-cell adenomas.

Aranka László, Edit Kocsárdi, D. Bara

(Institute of Pathology, University Medical School, Szeged)

The Feed-Back Regulation of Antidiuretic Hormone

Most authors believe that the blood ADH level does not affect the secretion and release of ADH. It is known, on the other hand, that the effect of ADH is more pronounced if the hormone is injected into the ventricle or intranasally than following parenteral administration. Thus, nervous structures seem to be involved in its action. According to the classical and often cited observations of BIGGART, resistance to hormonal treatment in diabetes insipidus develops when the lesion destroying the supraoptico-hypophyseal tract has reached the tuberal hypothalamic nuclei.

In the present study it has been investigated whether electrolytic lesions in the middle, posterior or lateral area of the hypothalamus interfere with the inhibition of water diuresis by posterior pituitary extract in hydrated albino rats. According to preliminary observations, such lesions do not impair the neurosecretory function of the hypothalamus. The observed antidiuretic response was normal in most animals. Thus, BIGGART's hypothesis has not been confirmed by the present experiments.

J. Skaliczki, D. Bara

(Institute of Pathology, University Medical School, Szeged)

Enzym and Histochemistry of the Anterior Hypothalamus and the Pituitary

Phosphatase, succinic dehydrogenase and diaphorase activity has been examined in the magnocellular nuclei of the anterior hypothalamus and the pituitary gland of white rats. The ganglion cells of the supraoptic and paraventricular nuclei showed pronounced acid phosphatase and DPNH-diaphorase, further less marked succinic dehydrogenase and TPNH-diaphorase activity. Only the capillary network gave a positive reaction with alkaline phosphatase, the reaction of the ganglion cells was negative. But for succinic dehydrogenase, enzymatic activity was considerably less pronounced in the neurohypophysis than in cerebral nuclei. DPNH- and TPNH-diaphorase reaction appeared to be more intensive in the cells of the intermediate lobe than in those of the adenohypophysis. Acid-phosphatase and TPNH-diaphorase activity was notably increased in the anterior hypothalamic nuclei of animals made antidiuretically hyperactive by dehydration. On the other hand, the activity of succinic dehydrogenase and DPNH-diaphorase showed no perceptible increase, and did even decrease in certain cases.

Sára Koritsánszky

(Institute of Histology and Embryology, University Medical School, Budapest)

Number and Arrangement of Gomori-Positive Glia Cells and their Relations to Secretory and Non-Secretory Nerve Cells in Invertebrates

The central nervous system of both the vertebrates and the invertebrates contains numerous Gomori-positive glia cells. While age-conditioned changes in the glia cells of rats were described in an earlier communication, the present experiments had the purpose to compare the system of glia cells in different species of the genus *Lumbricus* and also the relations of these cells to secretory and non-secretory nerve cells. Gomori-positive glia cells were found in characteristic arrangement; they were encountered around both secretory and motor neurones, especially around the cytoplasm and the axon. The number of Gomori-positive glia cells varied according to species: it was high in some, moderate in other species, while no such cells were found in a third category. Topographical differences are presumably due to differences in metabolism, while species differences may have oecological reasons.

P. Lantos, D. Bara

(Institute of Pathology, University Medical School, Szeged)

Rare Tumours in the Hypothalamo-Hypophysial System

The results of two postmortem examinations are described.

1. Highly placed choristoma of the hypophysial stalk was encountered as an accidental finding at the autopsy of a 62-year old female. The neurosecretory fibres of the supraoptico-hypophysial tract were swollen in certain parts of the choristoma, and the stalk showed moderate storage of secretion. No diabetes insipidus had developed because part of the fibres of the tract had been able to pass between the tumour cells into the neurohypophysis, and the area of the median eminentia also remained intact. There were no signs pointing to disorders of adenohypophysial activity.

2. Postmortem examination of a female, aged 40, revealed — in connection with a moderate degree of Recklinghausen's disease — a neurofibroma which occupied the greatest part of the adenohypophysis and a dedifferentiated spongioblastoma which had destroyed the entire hypothalamus. The patient had had amenorrhoea six, and diabetes insipidus four, months prior to death. The diabetes had improved in the terminal phase.

G. Gorácz, P. Baranyai

(Department of Pathology, Pál Heim Children's Hospital, Budapest)

Electrolytes in Experimental Myocardial Necrosis

The necrosis-inhibitory effect of certain electrolytes has been studied in rats subjected to treatment inducing myocardial necrosis. Histological and biochemical studies were performed to establish whether the loss of ions was the initial process of muscular necrosis or else a secondary phenomenon accompanying the disintegration of tissues.

Mária Keller, D. Tanka

(Department of Pathology, National Institute for Rheumatology and Balneology, Budapest)

Enzyme Activity in the Parenchymal Organs of the Rat Kept on Hypo- and Hyperpotassaemic Diets

The connection between electrolyte household and the various enzymatic systems has so far received poor attention.

The present study has been devoted to the changes evoked by hypo- and hyperpotassaemic conditions in the activity of enzymes in the parenchymal organs; for this purpose experiments were performed in rats. Hyper- and hypopotassaemic conditions were induced by appropriate diets. Observations were carried out between the first and fifteenth days after starting the diet. Changes in the morphology of the parenchymal organs were followed by the usual methods; enzyme activity in these organs was detected by biochemical and histochemical techniques.

The oxido-reductase system showed considerable impairment as early as 24 hours after starting both the hypo- and hyperpotassaemic diets; the latter appeared more effective in this respect. Hydrolase activity showed less marked changes.

The initial depression was followed in some of the organs by an increase of enzyme activity. For instance, in hypopotassaemic conditions acid phosphatase in liver and kidney, and succino-dehydrogenase in cardiac muscle showed increased activity on the 6th to 8th days. In the spleen, carboxyl esterase activity increased, and large amounts of haemosiderin pigment could be found, indicating enhanced destruction of erythrocytes.

Regeneration capacity after an initial rise seemed to diminish in the subsequent period of observation and a further deterioration of the enzymatic pattern of the various organs could be observed.

I. Törő

(Institute of Histology and Embryology, University Medical School, Budapest)

Nuclei and Mitochondria of Thymus Cells

Electron microscopic examination revealed peculiar phenomena on the nuclear mitochondrial and cell membranes of the rat and guinea pig thymus. The mitochondrial changes are suggested to be connected with immunobiologic changes at birth, the formation of nuclear vacuoles with nucleic acid synthesis accompanying thymocyte production, and the presence of cytoplasmic bridges with information transfer.

B. Bukulya, I. Ökrös, I. Törő

(Department of Morphology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest)

Submicroscopic Structure of the Thymus in 18 to 21-Day-Old Rat Embryos

In addition to variously matured thymocytes, the subcapsular zone of the thymus of 18 to 21-day-old rat embryos contains many "undifferentiated" ovoid epithelial cells, with comparatively few cytoplasmic ribosomes, smooth-surfaced endoplasmic reticulum, and cristate mitochondria. Their ovoid nucleus is poor in chromatin, which is evenly distributed and granular.

The cortex consists mainly of thymic "units" composed of a central reticular cell and thymocytes surronding it. The most characteristic "units" were encountered near the cortico-medullary boundary in 18-day embryos. In older embryos, different interposing cell types shatter the "unit".

Apart from a limited number of thymocytes, the medulla contains three types of reticular cells readily distinguishable against their background.

It is suggested that thymocytes derive from "undifferentiated" epithelial cells in the subcapsular zone and probably continue to mature in the thymic "units" of the cortex.

The medulla contains, apart from a limited number of thymocytes, three kinds of well distinguishable reticular cells. The long processes of the dark cells often form networks or are arranged around vessels.

It is suggested that thymocytes derive from undifferentiated epithelial cells in the subcapsular zone and undergo a process of ripening in the thymic units of the cortex.

E. Bácsy, I. Ökrös, D. Szabó, G. Rappay

(Department of Morphology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest)

Ultrastructural Changes in the Rat Thymus Following Exposure to X-Rays

By means of electron microscopy, rats exposed to a sublethal dose of x-rays have been studied 4, 24, 96, 168, and 264 hours after irradiation for changes in the thymus and the localization of acid phosphatase activity.

While most of the mature thymocytes were destroyed within a day, many reticular cells remained intact. The degenerated cells were phagocytosed, and this process was accompanied by increasing acid phosphatase activity. Regeneration was indicated by the increase in the number of cell divisions. In the cytoplasm of some reticular cells comparatively large vesicles were seen, mostly some time after irradiation, which were apparently unrelated to endocytotic activity.

P. Müller

(Institute of Pathology, Friedrich Schiller University, Jena)

Pathogenesis of Hepatic Eclampsia

On the basis of post-mortem findings in numerous cases of perinatal maternal death and of the results of animal experiments, some new points are raised in connection with the pathogenesis of toxaemic liver injury. The condition is ascribed to embolization of thromboplastic substances (amniotic fluid, retroplacental haematoma) across an uteroportal by-pass circulation.

H. David

(Institute of Pathology, Humboldt University, Berlin)

Changes in Kupffer's Cells Due to Actinomycin

Actinomycin interferes with RNA synthesis by combining with guanine and so counteracting the effect of RNA-polymerase. This property of actinomycin has repeatedly been demonstrated in numerous kinds of cells (including liver cells) both *in vivo* and *in vitro*.

Mice and rats were treated intraperitoneally with 0.5 to 1 μg of actinomycin C (Sanamycin, BAYER) or actinomycin D (Cosmegen, MERCK), and killed 3 to 48 hours later.

Apart from the repeatedly described changes in the parenchymal cells, changes of Kupffer's cells dominated the picture in numerous cases. These cells were diffusely swollen, markedly enlarged and contained large vacuoles. The nuclei displayed bizarre shapes and were pushed towards the periphery. Most organelles were destroyed. The appearance of cytolysomes, sometimes larger than the nuclei, was observed. They contained homogeneous osmiophilic matter as well as filiform and crystalloid structures separated from the surrounding vacuolar cytoplasm by a simple membrane. The cytolysomes were presumably phagocyted cells.

The swelling of Kupffer's cells obstructed the sinusoids partially or entirely at numerous points and so induced a decrease of blood supply and hypoxia in the adjacent parenchymal cells. This phenomenon may be of considerable significance when the effects of actinomycin are examined *in vivo* and also when hepatic injury is observed following treatment with the antibiotic.

B. Szende, J. Juhász, G. Kendrey

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Effect of Chronic Isoniazid Treatment on the Liver of White Rats

There exist several reports on experimental and clinical observations on hepatic injury caused by isonicotinic acid hydrazide (INH). Therefore, the effect of chronic INH treatment on the parenchymatous organs of white rats has been studied. Seventy animals of both sexes, kept on a standard diet, were treated with oral doses of 100 mg/kg daily. The rats died between the 70th and the 700th day of treatment. Microscopic examination revealed the following alterations 1. Fatty degeneration of various degrees. 2. Focal hepatic necrosis and consequential cellular reaction. 3. Variations in the size of liver cell nuclei and increase of the number of multinuclear hepatic cells.

These phenomena were encountered in the majority of the animals; the changes became more frequent and severe towards the end of the experiment.

In four animals, died in the last phase of the experiment, there were tumours originating from the cells of the hepatic parenchyma. The morphological features of the tumours are described.

G. Kendrey, J. Juhász, B. Szende

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Ultrastructural Changes in the Rat Liver Following Isoniazid (INH) Treatment

White rats of both sexes, kept on a standard synthetic diet, were treated orally with 100 mg/kg of INH daily for nearly two years. The ultrastructural phenomena observed in the liver cells and the hepatocellular tumours during the later phase of the treatment are reported.

Changes were most pronounced in the endoplasmic reticulum and the mitochondria. The rough surfaced endoplasmic reticulum was disorganized in most liver cells; many cisterns were distended, and partial degranulation of the membranes could be observed. Vesicular transformation of this system along with the disappearance of ribosomes was frequent. The membranes of the smooth surfaced endoplasmic reticulum forming vesicles and tubules were accumulated in larger and smaller foci; the glycogen had disapperaed. The mitochondria showed signs of regressive changes; abnormal enlargement with decrease of the density of the matrix and loss of the cristate were prominent alterations. Large numbers of osmophilic granules were matrices frequently observed in Golgi apparatus.

Giant mitochondria of varying shapes with serious degenerativ changes were frequently seen in the hepatocellular tumours. Most tumour cells included an extreme accumulation of smooth membranes which correspond the cytoplasmic acidophilic areas seen under the light microscope. These membranes showed a concentric or lamellar arrangement in some cells. In other tumour cells well developed rough surfaced endoplasmic reticulum and large numbers of free ribosomes could be demonstrated.

I. Bartók, V. Totovic

(Institute of Pathology, University Medical School, Szeged, and Institute of Pathology, Medical University, Marburg)

Autophagous Vacuoles (Cytolysomes) in the Liver Cells Following Partial Hepatectomy

A considerable accumulation of autophagous vacuoles (cytolysomes) has been observed in the hepatic parenchyma of rats 1 to 3 hours after partial (2/3) hepatectomy. The vacuoles were situated between the hyperplastic Golgi complexes and the bile canaliculi. Some of them, bounded by unit membrane, contained different cytoplasmic components or their remnants.

Some autophagous vacuoles contained a double cavity: in the vacuole, bounded by the unit membrane and containing a homogeneous matrix, there was a second cavity surrounded by concentric annular membranes. This inner cavity contained sequestered but still recognizable cytoplasmic fragments.

The formation of autophagous vacuoles is discussed on the evidence of many transitory forms. It is suggested that, since partial hepatectomy intensifies the activity of the liver, the accumulation of autophagous vacuoles is due to an increased physiological turnover of the cell components. The accumulation of autophagous vacuoles does not, therefore, necessarily mean a predominance of catabolic processes in the hepatic cells: it may even be due to an increased anabolic activity.

G. Varga, L. Varga, I. Piukovich, F. Szontágh

(Institute of Pathology, Second Department of Medicine, and Department of Obstetrics and Gynaecology, University Medical School, Szeged)

Hepatic Activity in Rats Treated with Gestagen

Rats with body weights between 150 and 250 g, kept on a standard diet, were treated orally with gestagen preparations over six to ten days and then examined for the concentration of serum proteins, protein-bound hexose, hexosamine and neuraminic acid. Glutamic-pyruvic transaminase activity of the serum and that of liver tissue were determined in some cases. The biochemical examinations were supplemented by a histological study of the liver. The possibility of hepatic damage by the gestagen, a controversial subject, is discussed.

Márta Balázs

(Department of Anatomy, Postgraduate Medical School, Budapest)

Extrahepatic and Intrahepatic Occlusion of the Biliary Tract

Hepatic lesions induced by the administration of α-naphthyl [isothiocyanate show much resemblance to human biliary cirrhosis.

The successive stages of intrahepatic biliary occlusion induced by the said drug have been studied in rats and compared with the findings in extrahepatic biliary occlusion induced by the ligation of the bile duct. Rats with body weights between 150 and 180 g were divided in four groups. The first received acute, the second subacute, the third chronic oral treatment with α-naphthyl isothiocyanate, while in the fourth group the common duct of the animals was ligated. Acute poisoning caused swelling, vacuolation and then necrosis of the cells of the interlobular ducts. The changes reached a peak on the third day. Subacute and chronic treatment caused proliferation and fibrosis of the biliary ducts. The picture after ligation was different from that observed in the acute and subacute stage of poisoning, whereas ligation and chronic poisoning gave rise to identical results.

P. Oszwald, L. Fónyad, Anna Skodnicz

(Tétényi Hospital, Budapest)

Hepatotoxic Action of Fluothane

The fact that recent studies on the hepatotoxic effect of fluothane have presented contradictory descriptions of the clinical and anatomical picture, justified to investigate the problem on a large material.

The results of microscopical inspections and post mortem findings made in the course of ten years, have been surveyed. The surgical and anaesthetic records, the postoperative observations and laboratory studies of the last five years have been subjected to a detailed scrutiny, and particular attention was paid to commonly known hepatotoxic factors. Results were compared with data from the period 1956—1960 when the currently employed methods of anaesthesia were not yet in use.

P. Kapp

(Department of Pathology, University Veterinary School, Budapest)

Development and Sequelae of Acute Infectious Hepatitis in the Dog

Dogs have been infected with hepatitis virus, and development of the disease was followed by biopsy. Changes of the sinusoid walls and increasing capillary permeability, the first hepatic changes, were promptly followed by those of the parenchyma such as vacuolation, acidophilic necrosis, decrease of glycogen and ribo-nucleoprotein content of the cells, etc. Inflammatory infiltration of the periportal spaces was usually moderate and occurred in the terminal phase only. Nuclear inclusions, characteristic of canine infectious hepatitis, were observed first in Kupffer's cells, then in the endothelial cells of the sinusoids, and terminally in the parenchyma. Similar but slighter changes were observed in the animals surviving the infection.

Acute changes developing in the course of infectious hepatitis were found not to lead to cirrhosis. Some of the surviving animals developed chronic cholecystitis and subacute or chronic interstitial nephritis.

F. Szarvas, Éva Horváth, F. Biliczki

(First Department of Medicine, and Institute of Pathology, University Medical School, Szeged)

Effect of Experimental Hepatic Cirrhosis on Leydig Cell Hyperplasia Following Subtotal Orchidectomy in the Rat

It has been shown earlier that, after subtotal orchidectomy, in the testicular remnant nodular hyperplasia of the Leydig cells takes place which in a few months leads to the formation of adenomas. The phenomenon has been attributed to increased pituitary ICSH-activity. The present experiments have shown that the reactivity to exogenous ICSH-like preparations decreases in rats with liver cirrhosis induced by thioacetamide. Steroid-3β-ol-dehydrogenase activity in the testicle did not increase to such an extent as in the control animals, and the growth of the accessory glands also remained behind that of the controls. Subtotal orchidectomy failed to elicit nodular Leydig cell hyperplasia in the cirrhotic rats, although local administration of thioacetamide had caused no direct testicular injury. It is suggested that, as a consequence of the metabolic disturbance accompanying hepatic lesions, the reactivity of Leydig's cells to endogenous ICSH is also decreased.

G. Varga, Éva Horváth, Irma Korom, Zsuzsa Korpássy

(Institute of Pathology, University Medical School, Szeged)

Effect of Continued Regenerative Stimulation on the hepatocarcinogenesis

Posthepatectomy serum (PHS) provokes regenerative phenomena in the liver of healthy rats. Its effect has been studied in the early stage of hepatocarcinogenesis. Young adult male rats were fed dimethylamino-azobenzene (butter yellow) and another group of test animals received, in addition, PHS every third day. Untreated and PHS-treated animals served as controls. The experiments were continued for four months.

Combined PHS and butter-yellow treatment provoked more pronounced lesions than butter yellow alone. While precancerous symptoms were less striking after treatment with butter yellow alone, combined treatment with butter yellow and PHS was frequently followed by a complete breakdown of the hepatic structure, diffuse oval-cell hyperplasia, pronounced basophilia and even malignancy (hepatoma). Continuously applied regenerative stimuli seem to promote hepatocarcinogenesis.

L. P. Szabó, A. Haraszti, Klára Kis

(Department of Pathology, County Hospital, Eger)

Lipofuscin Contents of the Liver of Aged Individuals

The liver of 401 autopsied patients who had lived more than 60 years has been studied for lipofuscin content. Lipofuscin content was increased in 80 per cent of the examined livers (against 61 per cent in the liver of younger individuals). Low liver weight is not necessarily associated with an increase in lipofuscin contents. Patients who had died of malignant tumours showed the same ratio of normal and increased hepatic lipofuscin as old persons. Acute cardiac failure did not affect the lipofuscin level, while chronic insufficiency was accompanied by increased lipofuscin concentration.

P. Endes, S. Gomba, G. Krasznai

(Institute of Pathology, University Medical School, Debrecen)

Behaviour of the Juxtaglomerular Apparatus in Renal Hypertension, Primary and Secondary Aldosteronism

Four — clinically grave — cases of hypertension were accompanied by secondary aldosteronism, while certain branches of the renal artery (unilaterally or bilaterally) were stenosed. The juxtaglomerular apparatus showed extreme hyperplasia in the ischaemic areas of the renal cortex, while no juxtaglomerular granular cells were seen in the cortical areas with patent arteries where hypertensive vascular and parenchymal changes were evident. On the other hand, granular cells were absent from the renal cortex in two cases of primary aldosteronism.

The granular and agranular forms of the epitheloid smooth-muscle cells of preglomerular arterioles and their functional significance are discussed.

J. Simárszky, P. Endes, Margit B. Soltész, G. Dauda

(Institute of Pathology, University Medical School, Debrecen)

Behaviour of the Juxtaglomerular Apparatus in Shock Induced by Duodenal Ligation

Increase in the number of juxtaglomerular granular cells (JGC) was found in the kidney of patients who had died of renal failure subsequent to shock. It has been attempted to create in the rat a condition similar to human renal failure following upon shock by ligating the duodenum. In such cases the loss of water leads to dehydration and consecutive peripheral circulation failure.

The JGC was observed 24, 36 and 48 hours after ligation at the duodeno-jejunal boundary, and also one and two weeks after a similar ligation for 36 hrs. The Na, K and NPN levels, as well as blood pressure were determined two weeks after duodenal ligation for 36 hrs. In contradiction to clinical observations, the JGC index was found to have decreased at 24 and 48 hrs; it became more or less normal one and two weeks after the release of the ligature. Serum Na and K underwent no essential change; NPN increased 36 hrs after the ligation, and blood pressure decreased.

S. Gomba, Margit B. Soltész, V. Szokoly

(Institute of Pathology, University Medical School, Debrecen)

Histochemical Study of the Phosphatases in the Juxtaglomerular Apparatus

The juxtaglomerular granular cells in the kidney of mice and rats contain acid phosphatase which is presumably localized in the secretory granules. There is, in the rat, an interstitial alkaline-phosphatase positive band between the macula densa and the Goormaghtigh

cells; the band is connected with the outer surface of both the afferent and efferent arterioles and often reaches into the interstitial space around the Goormaghtigh cells. The ATP-ase activity registered there is not due to aspecific ATP splitting by the alkaline phosphatase, and it extends to the lateral membranes of the macula densa cells. No such phenomenon has been observed in mice and humans. Human macula-densa cells show strong acid-phosphatase activity. No glucose-6-phosphatase activity has been encountered in the juxtaglomerular cells of mice and rats. Human organs have not been tested for this enzyme.

L. Szűcs, G. Krasznai

(Institute of Pathology, University Medical School, Debrecen)

Juxtaglomerular Apparatus and Adrenal Gland in Rats Treated with Homogenized Homologous Muscle and Heterologous Renal Tissue

Administration of exogenous renin affects the juxtaglomerular granular cells (JGC) and blood pressure. Renin, presumably a product of the JGC, may be regarded as the trophic hormone of the zona glomerulosa. Earlier investigations have shown the JGC to be involved in the so-called autonomous regulation of renal blood circulation, whereas certain metabolites of the striated muscles are responsible for the isoaemic autoregulation of muscle.

It has been studied whether the administration of exogenous mouse kidney rich in granular cells would affect the JGC-index of the rat, and whether this index was affected by treatment with homogenized muscle tissue rich in vasoactive substances. Changes in the adrenocortical cell nuclei have also been determined, and the results have been compared with the changes occurring in the JGC-index.

J. Stolarczyk

(Department of Pathology, Medical Academy, Gdansk)

Micropuncture Studies of the Effect of Lymphatic Occlusion on Renal Function in Rats

Acute experiments were performed to study the effect of lymphatic occlusion on renal function. Urine was collected bilaterally, the right kidney served as a control. Lymphatic occlusion produced a marked increase in urine flow with an increased osmolality but GFR was not altered. There was an elevation in total medullary solutes, a moderate rise in intratubular pressure, normal tubular fluid osmolality, and normal proximal insulin ratios but significantly lower inulin ratios in the distal tubule. Thus, the restricted lymph flow increased medullary and urine osmolality. It is concluded that changes in lymph flow could play a role in determining medullary osmolality and in the regulation of the concentrating mechanism.

D. J. Davies

(Department of Pathology, University of Liverpool)

The Effect of Ethylene-Imine on the Kidney

Ethylene-imine (formerly called vinylamine) is a volatile organic liquid which has the specific property of destroying the renal medulla when administered in doses of about 0.05 ml/kg.

A description is given of the lesions seen in the kidney and the urinary tract of rats and rabbits following administration of nephrotoxic doses of this substance. The effects on urinary volume and concentrating capacity are also described.

Ethylene-imine produces lesions which are quite strictly localized to the inner part of the medulla, the renal pyramid, and do not affect the intermediate zone except for occasional prolongations along the bundles of vasa recta. Lesions are also seen in the walls of the pelvis and in the bladder.

The functional effect of ethylene-imine is to produce a type of nephrogenic diabetes insipidus.

The probable mode of action of this substance is discussed particularly in relation to the newer concepts of the physiology of the renal medulla.

J. Justus

(Institute of Pathology, Medical Academy, Dresden)

Osmotic Nephrosis in Familial Juvenile Nephrophthisis

The morphological changes in familial juvenile nephrophthisis are described in connection with three cases occurring in siblings. One child died from meningo-encephalitis, so it was possible to observe the early stage of the renal disease. A feature observed in all the three cases was osmotic nephrosis. Hydropic swelling of the tubular epithelium was probably due to hyperhydration by infusions, whereby the basic disease might have been as a significant disposing factor.

B. Szende, L. Urai, K. Gyenge

(First Institute of Anatomy and Experimental Cancer Research; Second Department of Medicine, University Medical School, Budapest)

Juvenile Hypertension Pointing to Conn's Syndrome

Although primary hyperaldosteronism was recognized by CONN as far back as 1956, only a single case has so far been described in Hungary (GLÁZ et al.).

The 21-year old male patient had had hypertension for five years. The condition had been attributed to chronic nephropathy, and it was possible only the day before death to determine the blood Na and K levels. The results pointed to Conn's syndrome.

Death was caused by ventricular haemorrhage; both the brain and the kidney showed signs of hypertensive vascular disease. The cortex of the left adrenal contained a tumour bright yellow in colour and 1.5 cm in diameter, a hormone-producing adenoma originating partly from the zona glomerulosa and partly from the zona fasciculata. The heart muscle revealed lesions characteristic of electrolyte-steroid cardiopathy. Histological changes in the hypothalamus pointed to chronic inflammation.

The history, the histochemical and postmortem findings made it safe to assume that the case was one of Conn's syndrome.

Éva Horváth, P. Lantos, K. Kovács

(Institute of Pathology, and First Department of Medicine, University Medical School, Szeged)

Histochemical Aspects of Experimentally Induced Zona Glomerulosa Necrosis

The intravenous administration of 5 mg hexadimethrine bromide (Polybrene, ABBOTT) to rats induces total necrosis in the zona glomerulosa and a considerable decrease in the production of aldosterone. Histochemical analysis revealed a considerable diminution of steroid-3,3β-ol-dehydrogenase activity over the entire area of the zona glomerulosa one hour after the administration of hexadimethrine and there was no sign of activity after two hours. In the zona fasciculata and the zona reticularis remained normal activity. The activity of succinic dehydrogenase ceased after 24 hours only. The two inner zones showed no change in this respect.

Although in some cases focal necrosis has been observed also in the zona fasciculata and the zona reticularis, such necroses have no toxic character: both histological and histochemical examination revealed their ischemic nature. In the infarct no enzyme activity was demonstrable but their disappearance was slower than in the zona glomerulosa.

Postnecrotic regeneration was rapid. Development of a histochemically normal new zona glomerulosa took two-three weeks.

L. Kovács, Edit Baumgartner

(Medico-Radiological Research Group of the Hungarian Academy of Sciences; Departmen of Roentgenology, University Medical School, Budapest)

Histological and Functional Changes in the Adrenal Cortex Caused by Supralethal Doses of Ionizing Radiation

White rats have been exposed to 1300 R total body irradiation and examined 1, 3, 4, 48 and 72 hours after exposure.

The adrenal cortex was poor in lipids at one and three hours, and only the zona glomerulosa contained fats. The organ became once more rich in lipids at 24 hours. Their amount diminished and was chiefly demonstrable in the zona glomerulosa at 48 hrs. After 72 hrs., it was only the zona glomerulosa which still contained a modest amount of lipids. The cortex was congested and the cells were swollen.

As to the blood corticosterone level and corticosterone and aldosterone synthesis *in vitro*, the hormone secretion had two phases; a first one immediately after the irradiation, and a second 48 hrs. later. Both phases were more marked in the case of corticosterone.

Edit Beregi, H. v. Mayersbach

(Department of Pathology, Semmelweis Hospital, Budapest and Institute of Histology, University Medical School, Nijmegen)

Immune-Fluorescence Study of Experimental Glomerulonephritis

Cases of Masugi nephritis and pilocarpine nephritis have been studied with Coons' immune-fluorescence method. The glomerular binding of nephrotoxic serum was observed at 10, 30, 60, 90 and 120 minutes, 24 hours, 6, 8, 12, 16 and 20 days, further after 3 1/2 months. Glomerular changes occurring in pilocarpine nephritis were estimated after 8, 12, 16 and 20 days by means of immune-fluorescence. The morphological studies were supplemented by simultaneous immune-electrophoretic examinations.

Klára Németh, A. Bajtai

(First Institute of Pathology and Experimental Cancer Research, University Medical Schoo Budapest)

Mannitol Nephrosis

Mannitol, an osmotic diuretic, is extensively used for the treatment of postoperative anuria, and has proved beneficial in certain chronic nephropathies. It is, on the other hand, not commonly known that the compound induces hydropic degeneration in the kidney.

Three cases have been observed in which patients, treated with different doses of mannitol following cardiac surgery, developed hydropic degeneration. The severity of the damage was proportional to the dosage.

Experiments were made in Wistar rats of both sexes. The findings corresponded to the clinical observations.

K. Bella, P. Rutkai

(Department of Pathology, Postgraduate Medical School, Budapest)

Mannitol Nephrosis

In recent years mannitol has been adopted for the prevention of postoperative oliguria. Many recent reports omit to point to the fact that osmotic diuresis can only be induced by the drug as long as no organic damage had developed. Few papers only call attention to the fact that mannitol may cause osmotic nephrosis. Treatment with mannitol may give rise to a grave hydropic degeneration of the proximal convoluted tubules in the same manner as do other hypertonic sugar solutions.

P. Rutkai

(Department of Pathology, Postgraduate Medical School, Budapest)

Pancreatic-Renal Syndrome or Electrolytic Nephropathy?

Symptoms of renal failure may dominate the clinical picture in cases of both acute haemorrhagic and chronic pancreatitis. The condition is usually termed pancreatic-renal syndrome in the clinical reports. Clinical and pathological data allow the conclusion that different factors may give rise to the syndrome. Shock, toxic metabolites and disturbances of the electrolyte household may be responsible for the renal injury.

The kidneys have been subjected to a study in 15 cases of pancreatitis. Degenerative tubular lesions were found to predominate. In a case associated with renal failure, hypochloraemic calcium nephrosis was revealed.

It is suggested in harmony with literary data that it is useless to differentiate the syndrome at issue: it is not one of uniform pathogenesis but a non-specific complication of the primary disease so that other conditions, too, may produce complications revealing the same anatomical and histopathological features.

G. Kelényi

(Institute of Pathology, University Medical School, Pécs)

Comparison of the Acid Thiazole Fluorescent Microscopic and Congo Reb Anisotropic Stainings with Special Regard to their Specificity for Amyloid

The fluorescence microscopic staining effects of the acid thiazole fluorochromes, primuline and thioflavine S on secondary and senile amyloidosis have been studied. These methods seem to visualize the same tissue structures as the Congo red anisotropic staining. Quantitative analysis of the dichroism of the structures stained anisotropically by Congo red (renal glomerular amyloid, senile plaques, Alzheimer fibrils) showed that, although the rate of absorption of the linear polarized light by these structures is different, their maximal dichroism occurs at the same wavelength (at about 540 mμ). In electron micrographs of choroid cells with rings on Biondi the presence of fibrils of about 240—280 Å thickness was observed (thickness of amyloid fibrils is about 100 Å). Based on the present findings and literature data the specificity for amyloid of the acid thiazole fluorescent microscopic and Congo red anisotropic methods is discussed.

G. Deák, G. Romhányi

(Institute of Pathology, University Medical School, Pécs)

Thermal Shrinkage of Collagen Fibres

Up to now direct methods have only been available for the estimation of the thermal shrinkage (Ts) of collagen fibres. Changes in the anisotropic staining of fibres induced by heat (i.e. their conversion from stenocollagen to porocollagen) offer possibility of studying the Ts in their natural environment.

The thermostability of collagen fibres has been studied in skin samples from 60 subjects of various ages. Thermostability was found to increase until puberty when a heat of 62° C has to be applied for 10 minutes in order to obtain porocollagen transformation. This value remained constant after puberty.

The method is suitable for the study of local thermal effects applied *in vivo or in vitro*, and furthermore it allows to observe the effect on thermostability of different salts at various concentrations. Potassium thiocyanate, potassium iodide and carbamide tend to loosen, sodium chloride and potassium chloride to stabilize the micellar structure of collagen.

G. Dashev

(ISUL-Clinic of Endocrinology, Department of Pathology, Sofia)

Histochemical and Immunohistochemical Studies of the Nodular Forms of Endemic Goitre

The morphologic peculiarities of the nodular forms of endemic goitre have been studied by histochemical and immunohistochemical methods. Substantial morphological alterations were observed. A considerable percentage of the glands exhibited fibrosis and destruction accompanied by an autoimmune reaction.

A. Hecht

(Institute of Pathology, Humboldt University, Berlin)

Enzymatic Histochemistry of Necrosis

In connection with enzyme histochemical examinations concerned with the problem of necrosis, two questions are of a major pathologic importance, *viz.*

1. Is it possible to establish disturbances of cell metabolism by means of enzyme histochemical methods before structural changes would present themselves?
2. Is it possible to locate the primary point of attack of a pathogenic agent on the basis of enzyme histochemical changes?

Genetically different necroses of the heart muscle and liver have been studied, such as experimental heart infarction and disseminated necroses elicited by cardiotoxic substances, and in the liver central necroses and necroses induced by papain.

The experiments showed that the enzymatic changes do not invariably precede the morphological ones but coincide with them, and that the ferment-histochemical findings are practically of the same character in genetically different necroses. Thus, they offer little information about the primary point of attack. There is no specific ferment-histochemical sign referring to a particular type of necrosis.

W. Zschiesche

(Institute of Microbiology and Experimental Therapy, Jena)

Experiments to Influence Induced Amyloidosis in Mice

The object of earlier studies was to observe activity changes of the RES in the course of experimental amyloidosis. The present experiments had the purpose to test the effect of various factors (BCG, antibiotics, cytostatics, splenectomy) on the development of amyloidosis. Inhibition was most pronounced under the influence of BCG. Probable pathogenic interrelations between experimental amyloidosis and RES activity are discussed.

W. Kühne

(Institute of Pathology, Friedrich Schiller University, Jena)

Forms and Causes of Pulmonary Emphysema

The collective term emphysema covers a wide variety of morphologically, pathogenetically and functionally different changes in the lungs, with distension of the intrapulmonary air spaces beyond the terminal bronchiole, as the one and only common feature. This purely descriptive anatomic definition is unsatisfactory, as a different pathologic value attaches to each form of emphysema. Therefore the problems of pathogenesis and the functional structures of a few clinically important forms of emphysema are discussed, with particular stress on the little known centro-acinous type.

S. Elias

(Research Institute for Normal and Pathologic Embryology, Roumanian Academy of Sciences, Timişoara)

Growth of the Chicken Embryo in Early Stages

Since length and weight of the chicken embryo in the early periods of development do not characterize growth in an unambiguous and statistically reliable manner, it is suggested to accept the surface of the embryo's projection as a characteristic measure of development. The practicability of the proposed data to establish growth dimensions has been proved statistically on both normal and treated samples.

H. Timmel

(Institute of Pathology, Medical University, Halle)

Cytomegalic Inclusions

The cytomegalic inclusions in the nucleus and the cytoplasm differ not only in localization but also in size, structure and staining reaction. From their study by light and electron microscopy it has been concluded that the change in the nucleus probably corresponds to the virus formation centre (viroplasm) or an inclusion body in the strict sense. The cytoplasmic inclusions are clusters of mature virious collections at the cell surface.

H. J. Reiss

(Institute of Pathology, Medical University, Halle)

Pathologic Anatomy of Listeriosis

The clinical pattern and the morphological findings in cases of listeriosis are widely varying. On the basis of several cases the important changes in listeriosis of adults (meningoencephalitis, malignant hepatitis) are discussed. The congenital form of the disease presents itself almost invariably as a septic granulomatosis, with the development of characteristic granulomas in all organs. The route of infection and the placentar changes are of especial importance.

A. Schwartz

(Department of Pathologic Anatomy, Plzeň)

Allergic Lymphadenitis

Despite the important part played by the tissue in immunity processes, lymph node affections of allergic origin are rarely observed. The cause of allergic lymphadenitis often remains obscure, sometimes, however, it can be brought into relation with the administration of some drug.

Seven cases of histologically confirmed allergic lymphadenitis are reported. The condition was induced in two cases by mephenytoin, in one by sulphamethopyridazine treatment, in one case it developed after administration of anthrax serum. Of the remaining three patients, who had not been subjected to drug or serum treatment, one suffered from polyarteritis, one from uterine carcinoma, and in one every effort failed to detect a pathogenic factor.

Histologic examination of the enlarged lymph nodes revealed a blurring of the usual structure. The node consisted mainly of proliferating reticular, eosinophilic and plasma cells. The allergic reaction presenting itself after hydantoin treatment, could hardly be distinguished from lymphogranulomatosis or other types of malignant reticulosis. Important in such cases

is to know the history and the kind of drug. Discontinuation of the drug will be followed by a quick regression of the lymphadenitis.

Administration of hydantoin drugs to patients suffering from epilepsy combined with some other disease of allergic character, requires utmost precaution lest their allergic condition exacerbate in the course of treatment.

L. Józsa, G. Lusztig

(Department of Pathology, County Hospital, Kecskemét)

Mucopolysaccharide Contents of Organs in Experimental Cholesterol Sclerosis

It has been shown earlier that, in cholesterol sclerosis the amount of hyaluronic acid, heparin-heparitin and chondroitin sulphate B increases in the aortic wall in the first three months of cholesterol treatment, to decrease gradually thereafter.

The present experiments have shown that in the

1. *liver* the amount of heparin and chondroitin sulphate increased in the first three months, but dropped below the normal level by the end of the fifth month;

2. *kidneys and myocardium* the concentration of chondroitin sulphate increased over the 5 months period of observation, while that of heparin and hyaluronic acid did not respond to treatment;

3. *lungs and spleen* the level of chondroitin sulphate increased, that of heparin decreased;

4. *skin* there was no connection between the mucopolysaccharide contents and the degree of cholesterol sclerosis.

O. Szücs, L. Kovács

(Department of Pathology, State Railways Hospital, and Research Group for Medical Radiology of the Hungarian Academy of Sciences, Budapest)

Determination of the Sulphate Groups of Acid Mucopolysaccharides

To the current methods for the demonstration of acid mucopolysaccharides (Hale-PAS, alcian blue, methylene-blue extinction, metachromasia, digestion, blockade) two new procedures have been added for the elective demonstration of sulphate groups: TAKOUCHI's acriflavine method and STEMPIEN's sodium-rhodanate method.

White rats were given ^{35}S sodium sulphate, killed after 24 hrs, and their costal, laryngeal and auricular cartilages were worked up. Results yielded by the histochemical methods have been compared with microautoradiographic findings.

Conclusions have been drawn as to the specificity of the above mentioned two methods of assay.

J. Sebők, J. Juhász

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Mesenteric Xanthomatosis

Mesenteric xanthomatosis, a focal storage of lipoid at the root of the mesentery, often presents a tumour-like appearance. Its gross and microscopic features are described on the evidence of 12 cases. The condition must be differentiated from retroperitoneal xanthogranulomatosis, mesenteric xanthofibrogranuloma, Whipple's disease, Weber—Christian's non-suppurative nodular panniculitis.

The composition of the mesenteric fatty tissue has been analyzed chemically in normal subjects and in cases of mesenteric xanthomatosis. Differences were pronounced in the composition of cholesterol lipids.

It is suggested that mesenteric xanthomatosis is partly due to lymphatic congestion and partly to quantitative and qualitative changes in cholesterol esters.

Z. Csapó

(Institute of Pathology, University Medical School, Szeged)

Eosinophilic Infiltration of the Stomach

In three cases biopsy revealed eosinophilic infiltration in the stomach. Two were submucosal, the third — connected with a chronic peptic ulcer — was diffuse. The first and second were characterized by a proliferation of connective tissue fibres, eosinophilic infiltration and a sharp delimitation from the muscular layer and the submucosal connective tissue. The third case showed marked eosinophilic infiltration which separated the individual muscle fibres.

The two forms of the condition must be different as to pathogenesis, and different aetiological factors may be at work even within the diffuse form. Most frequently, there is some allergic process in the background. The condition is not exceptional and certainly benign.

Magda Frank

(Department of Pathology, John's Hospital, Budapest)

Aetiology and Differential Diagnosis of Necrotizing and Pseudomembranous Enterocolitis

Four bacteriologically authenticated cases of necrotizing enteritis are presented. In the first case the condition has developed postoperatively in a male patient of middle age, the second in an aged female, the third in an aged female patient who had moreover Addison's disease and diabetes, the fourth in a male patient of advanced age who had bronchiectasis and emphysema. Pseudomembranous enterocolitis was diagnosed in 6 aged patients who were simultaneously suffering from sclerosis, necrotizing granulomatosis on pulmonary tuberculosis. In the case of an aged female ulcerative enteritis developed after the patient's pulmonary tuberculosis had healed and proved to be amyloidosis of the small bowel.

The problems of differential diagnosis have been discussed.

L. Kovács, Gy. Jánossy

(Medico-Radiological Research Group of the Hungarian Academy of Sciences, Budapest)

Morphological and Functional Changes in the Small Intestine Caused by Supralethal Doses of Ionizing Irradiation

Morphological changes in the small intestine of white rats exposed to 1500 R total body irradiation have been compared with those observed in perfused small intestines.

Mucous secretion was intensive, and alkaline phosphatase and aspecific esterase activity pronounced 24 hrs after irradiation. At 72 hours, the oedematous swelling of the intestinal villi was marked, the epithelium was flattened and became at some points detached, while mucous secretion and enzymatic activity had almost completely ceased.

Functional experiments had the object to observe the absorption of potassium, sodium and water at different points of time. Absorption was increased 24 hours after irradiation, dropped below the control level after 48 hours, and ceased completely in the 72nd hour.

The respective results of the histological and the functional examination showed good agreement.

J. Vajda, Z. Herpai, T. Raposa

(Institute of Anatomy, University Medical School, Budapest)

Arterio-Venous Anastomoses in the Small Intestine

The vessels of the mesentery and the small intestine have been studied in corrosion preparations, further by filling with Indian ink and also by Gross—Schultzer's silver impregnation. Whereas SPANNER observed anastomoses between the arteries and veins of the small

intestine at the mesenteric border of the intestine only, the present studies revealed three points of anastomotic communication. 1. In the mesentery where the arterial leg of the anastomosis was thickened or its initial portion was spiralled. 2. In the intestine next to the mesentery (as described by SPANNER). 3. In that segment of the intestinal wall where the subserous vessels are piercing the muscular layer.

The three different communications may be of decisive significance in nutritional processes and in cases of heavy blood loss.

J. Balogh

(Department of Pathology, County Hospital, Szekszárd)

Histochemical Studies in Neurogenic Hypertension

Experimental neurogenic hypertension is accompanied by well-defined vascular changes. Besides those demonstrated by staining reactions, alterations of the hydrolytic enzymes have been observed. These present a characteristic pattern in the initial phase of hypertension and also during the development of definitive changes, and are connected with the vascular lesions.

Similar phenomena have been observed in the fibres of the vessel walls and the secondarily developed intercellular substance.

Margit Zombori, A. Tóth, H. Jellinek

(Second Institute of Pathology, University Medical School, Budapest)

Renal Injury by Mercury Bichloride

The kidneys of rats and dogs have been examined by histological and histochemical methods in the acute and chronic phases of mercury bichloride poisoning.

Glomerular changes such as hyperaemia, swelling of the loops and circumscribed capillary dilatations dominated the histological picture in the acute phase. These phenomena were followed by lesions of the tubular epithelium. Signs of regeneration and interstitial accumulation of connective tissue elements appeared in the chronic phase.

As regards histochemical reactions, alkaline phosphatase activity was proportional to the extent of the lesions, while changes in the activity of non-specific esterase and acid phosphatase were in harmony with those observed earlier under anoxic conditions; viz. the initial increase of diffuse cytoplasmic activity was followed by a predominance of granular activity, and — in the chronic phase — the regenerated tubular epithelium displayed increased cytoplasmic or granular activity.

M. Palkovits, I. Munkácsi

(Institute of Anatomy, University Medical School, Budapest)

Comparative Study of the Thin Segments of Henle's Loop in Normal and Desert Mammals

The size of Henle's loops and the distribution of thin segments in the pyramidal zones were studied in desert and other mammals. Length, number and mass of Henle's loops in the deep nephrons were greater in animals of the desert; in these the number of thin segments in the internal medullary zone was twice as much as in other mammals. This finding is well in keeping with the countercurrent hypothesis of the urine concentrating mechanism which is most efficient in the kidneys of desert animals.

I. Munkácsi

(Institute of Anatomy, University Medical School, Budapest)

Comparative Study of the Vascular Structure in the Renal Medulla

The tubulo-vascular structure of the renal medullary substance has become a favoured subject of study since the countercurrent concentrating activity of Henle's loops has been discovered. The urine concentrating ability of desert mammals is many times greater than that of other animals. Comparative tests in the two groups yielded the following results. In the desert animals:

1. the cortical arteries are short and the medullary vessels long, in consequence of the relatively thin cortex and long pyramid;
2. the juxtamedullary glomerules are large and posses efferent arterioles of muscular type. These control the blood supply of the medulla according to need;
3. owing to the special structure of the nephron the vasa recta bundles lie widely apart and contain an increased number of vessels;
4. the vascular system in the outer and inner zones, of the medulla has a peculiar structure, reflecting the greater concentrating capacity of the desert animal.

L. Molnár, T. Mészáros

Second Department of Surgery, and Institute of Anatomy, University Medical School, Budapest)

Morphologic Features of the Renal Vessels, Responsible for Acute Renal Ischaemia in Shock

Irreversible shock was induced by Fine's method in dogs, by 90-minute clamping of the superior mesenteric artery. The shock period varied from 1 to 8 hours. The renal vessels were studied in PVC corrosion preparations and after India ink injections and in histologic sections stained with various dyes.

Spasm of the interlobular and afferent arterioles was found to be the cause of the patchy cortical ischaemia. Four types of spasm could be differentiated morphologically: 1. conical and 2. needle-like locking spasms, depending upon the vascular tonicity, 3. local spindle-like circular or annular spasm of the interlobular arteries, 4. locking and local spasm of the afferent arterioles.

Coinciding with these vascular reactions, the parenchyma presented the characteristic histologic picture of the shock kidney; these changes were relieved by spasmolytic treatment.

G. Sótonyi, E. Mátyus

(Section of Pathology and Section of Urology, Szentpéteri Hospital, Miskolc)

Segmental Ischaemic Changes in the Kidney

Human kidneys with aberrant vasculature, selected from a surgical material, have been subjected to histological examination. In segments with a slightly deficient blood supply during protracted periods, chronic ischaemic changes were observed. These were a thickening of the basement membrane of Bowman's capsule and the glomerular loops, in more pronounced cases periglomerular fibrosis, hyalinized glomeruli, and also lesions in the arteries and arterioles. Signs of inflammation were absent in these cases, chronic pyelonephritis was rare. On the basis of these findings, a great, though not exclusive, pathogenetic importance is attached to chronic segmental ischaemia in certain conditions, e.g. chronic pyelonephritis.

G. Ungváry, Jolán Demeter, J. Faller, Aranka Hudák

(Institute of Anatomy, University Medical School, Budapest)

Renal Infarction in Nephrectomized Rats

The right kidney has been removed from 100 white rats, weighing 200 to 250 g each. Thirty animals, divided into groups of 6, were killed 2 hours, 1, 4, 7 and 28 days after the operation. In the rest of the animals, on the 28th day infarction extending to one half or three quarters of the left kidney was induced by partial ligature of the branches of the left renal artery. Following this second operation, the animals were killed by bleeding, at 2 hours, 1, 3 and 10 days, 6 weeks and 12 weeks.

The changes presenting themselves in the left kidney were studied by weighing, glomerulometry and nuclear volumetry, histochemical reactions and by the injection-corrosion method. The blood was tested for NPN.

The findings indicate that the injured organ and in the first place the glomerules are regenerating rapidly. The juxtamedullary glomerules decreased as the cortical glomerules increased, in volume. The glomerules in animals presenting high NPN values were larger than in those with a normal NPN value.

L. Varga, A. Schäfer

(Second Department of Medicine, University Medical School, Szeged, and University Medical Shool, Würzburg)

Changes in the Serum Level of Protein-Bound Hexosamine and Neuraminic Acid in Experimental Nephroso-Nephritis in the Rat

Protein-bound hexosamine and neuraminic acid showed a biphasic reaction in the serum of rats with nephroso-nephritis. The glycoprotein components showed a moderate reduction in the first hours after the administration of nephrotoxin. The level of both components then rose considerably on the first to third day, to decrease slightly thereafter. Another rise was registered on the 8th to 9th day; it was more pronounced than that observed between the first and the third day.

The possibility of a correlation between the changes in the glycoprotein level and the immunological phenomena is discussed.

E. Szalay

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Widening of the Capsular Epithelium of Glomeruli

This phenomenon, mostly associated with severe hepatic lesions, occurs but rarely in clinical practice. In order to be able to study it under experimental conditions a combination of merthiolate and casein has been administered subcutaneously and intraperitoneally to white mice for prolonged periods. Merthiolate alone was less efficacious. As regards histological picture and histochemical reactions, the widened capsular epithelium of the glomeruli seemed to be identical with the epithelium of the proximal convoluted tubules; in some planes the former appeared to be continuous with the tubular epithelium.

It is suggested that chronic hypoxia of the tissues may play some part in the development of the condition.

S. Gomba

(Institute of Pathology, University Medical School, Debrecen)

Renin Contents of Autotransplanted Renal Cortex in the Rat

It has been shown earlier that the tubular elements of autologous renal grafts of rats undergo destruction or suffer grave damage, whereas after an initial decrease the granular cells of the juxtaglomerular apparatus increase in number. The granules of these cells presumably contain renin which is probably produced in the cells themselves. On the other hand, it has been suggested by BING and other authors that renin is produced in the macula densa and only stored in the said cells.

Direct and indirect examination of 30-day old rat kidney cortex grafts revealed marked renin activity and a great number of juxtaglomerular granular cells. On the grafts the tubules were gravely damaged. Since histochemical examination failed to reveal glucose-6-phosphate dehydrogenase activity, characteristic of the macula densa, it has been concluded, it is in the juxtaglomerular cells that renin is formed.

J. Balogh, E. Kelemen

(Department of Pathology and Surgery, County Hospital, Szekszárd)

Pathological Aspects in the Surgical Treatment of Renovascular Hypertension

Data obtained from renal biopsy have been compared with the functional conditions, specially as regards postoperative changes in renal activity. Some pathological aspects and the characteristic features of surgical complications have been discussed.

B. Horányi

(Department of Neurology, University Medical School, Budapest)

Histopathology of the Pineal Body

With a view to adding to the scanty knowledge regarding the histopathology of the pineal body, more than 200 glands of persons who had died of neural and other diseases were examined, with the following results. 1. The pineal body is seldom involved in the inflammatory processes of the nervous system and is usually not affected even by inflammations in the adjacent meningeal tissues. 2. Grave vascular diseases of the nervous system and the entire organism (diffuse arteriosclerosis, hypertensive vascular disease) cause no damage to the vessels of the corpus pineale. 3. Senile changes in the nervous system affect the pineal body but exceptionally. 4. There is usually no metastatic neoplasm in the pineal gland even if the organism and the brain are full of metastases. It seems, therefore, that the corpus pineale is biologically more or less independent of the rest of the nervous system.

B. Flerkó, G. Sétáló, F. Hajós

(Institute of Anatomy, University Medical School, Pécs)

Ultrastructural Aspects of Ovarian Steroidogenesis

The existence of a specific connection between the ovarian production of steroid hormones and the so-called steroidogenic organelles is substantiated by the observation that these organelles (1) appear in the parenchyma of the ovary simultaneously in the beginning of hormonal activity; (2) they disappear after hypophysectomy (i.e. with the lack of specific tropic hormones), and reappear folloving administration of gonadotropic hormones. Granulosa

cells of an oestrogen producing small follicles do not contain steroidogenic organelles, so that these cells are no sources of oestrogen. Their ultrastructure points to a trophic function in the growing follicles. Since it is only in the luteinizing granulosa cells of follicles with large cavities that steroidogenic organelles appear, they seem to be responsible for the preovulatory secretion of progesterone. It appears that the oestrogen-synthesizing cells of the ovary are of thecal, and those producing progesterone of granulosal, origin.

F. Hajós, T. Szomor, B. Flerkó

(Institute of Anatomy, University Medical School, Pécs)

Ultrastructural Differentiation of Rat Ovary

The ovaries of foetal, 6, 25 and 30-day old rats have been studied. Ultramicroscopic structures, typical of steroid producing cells, (agrinular endo-plasmic reticulum composed of vesicles and ovoid tubovesicular mitochondria) appeared in the parenchyma cells at the time of beginning hormonal activity.

H. Knolle

(Institute of Pathology, Medical University, Halle)

Testicular Feminization

Determination of nuclear sex and chromosome analysis have fastly contributed and to some extent corrected our knowledge about the various forms of pseudohermaphroditism. The extremest among the masculine types is known as testicular feminization. A case is reported and attention is called to the problems of interphase nuclear examination and chromosome determination, and their significance in intersexuality research and clinical investigations is stressed.

B. Halász, R. A. Gorski

(Institute of Anatomy, University Medical School, Pécs, and Department of Anatomy, University of California, Los Angeles)

The Role of the Afferent Nervous Pathways to the Hypophysiotrophic Area in the Control of Gonadotrophic Hormone Secretion

The neural connections of the rat medial basal hypothalamus (hypophysiotrophic area) have been partially or totally severed by means of a small knife fixed in the holder of a stereotaxic instrument. Complete deafferentation of the hypophysiotrophic area inhibited further ovulation, and unilateral oöphoerctomy did not induce compensatory ovarian hypertrophy. On the other hand, bilateral oöphorectomy was followed by increased secretion of luteinizing hormone and the development of castration cells in the hypophysis. Interruption of the nerve fibres running to the area from the two sides, from above and from behind, did not interfere with ovulation. After interruption of the anterior afferent pathways the animals ceased to ovulate, but still developed compensatory ovarian hypertrophy and hypophyseal changes following castration.

H. Ueberberg, M. Reimer

(Dr. Karl Thomas GmbH Laboratory for Experimental Pathology, Biberach/Riss)

Electron Microscopic Appearance of the Cell Nucleus in the Anterior Pituitary of the Rat

The cell nucleus of the anterior pituitary in the normal rat has been subjected to electron microscopic examination. A light and a dark type is roughly distinguishable. The nucleus is mostly round or oval, sometimes reniform or of bizarre shape. It is surrounded by a double

membrane, presenting pores in some places. Most nuclei have one nucleolus, a few have two. The nucleolus is usually round and of granular structure. The differences revealed by the examination do not permit of any inference regarding the interplay between nucleus and cytoplasm and the related hormone production.

Sz. Virágh, K. Kovács, T. Tiboldi

(Department of Histology and Embryology, University Medical School, Budapest, and First Department of Medicine, University Medical School, Szeged)

Electron Microscopic Study of Adenohypophysis Necrosis Caused by Hexadimethrine-Bromide in the Rat

Intravenous administration of hexadimethrine-bromide gives rise to necrosis in the anterior pituitary of rat. 30 minutes after the injection of the drug, dilatation of some capillaries and ruptures corresponding to their endothelial fenestrae were found. Simultaneously intravascular fibrin precipitation, inter- and intracellular edema occured at some places. One hour after the treatment some capillaries were obstructed by thrombi. At two hours diapedesis of erythrocytes, increased interstitial edema and numerous thrombi characterized the alterations. At three hours ruptures of the cell membrane, karyolysis and karyorrhexis was observed in a part of the parenchymal cells. Later the above alterations increased per field and complete necrosis of the parenchymal cells and blood vessels were found in large areas.

T. Tiboldi, K. Kovács, M. Kurcz, A. Orosz

(First Department of Medicine, University Medical School, Szeged, and Institute for General Zoology, Loránt Eötvös University, Budapest)

The Effect of Hexadimethrine-Bromide upon Pituitary Blood Circulation in the Rat

Previous examinations have shown that hexadimethrine-bromide induced infarction in the adenohypophysis of the rat. Light and electron microscopy revealed the vascular origin of the lesions. Therefore, pituitary circulation has been studied in hexadimethrine-bromide treated rats, by means of the India-ink method. The observed changes were compared to those appearing in rats with infundibular injury. India ink was injected 1 to 24 hours after hexadimethrine-bromide dosage. Subsequent examination revealed in the adenohypophysis necrotic areas which in the initial stage already turned a vivid red and took up no India ink. Similar changes were observable in the prenecrotic phase. Absence of India ink from the necrosed parts was obvious in the histologic preparations. The defects observed in the adenohypophysis closely resembled the changes occurring after complete or partial infundibular injury. The results seemed to indicate that the arrest of blood circulation by hexadimethrine-bromide must play a decisive part in the pathomechanism of adenohypophysial necrosis.

Éva Horváth, Gy. Varga, K. Kovács

(Institute of Pathology and First Department of Medicine, University Medical School, Szeged

Histochemical Examination of Adrenocortical Ischaemic Necrosis

The vessels in the left adrenal of white rats have been clamped by a specially devised instrument for 60, 90 and 120 minutes, and the consequent changes observed at intervals of one hour to one month. The signs of necrosis became obvious 4 to 6 hours after release of the ligature, although enzymatico-histochemical changes appeared earlier.

A focal decrease of steroid-3-3-ol-dehydrogenase, a specific indicator of adrenocortical function was perceptible after the first hour already. After six hours it completely disappeared

from the infarcted area. Succinic-dehydrogenase activity diminished markedly by the fourth hour; acid phosphatase and non-specific esterase activity ceased after 24 hours. The histologically unimpaired areas showed no changes except for an increased non-specific esterase activity around the infarction. Capillary lesions were already present in the prenecrotic phase. The time of postnecrotic regeneration depended on the extent of the lesions. The newly formed cells presented a normal histochemical picture.

D. Szabó, E. Stark, B. Varga

(D artment of Morphology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest)

Electron Microscopic Study of the Functional Changes and the Acid Phosphatase Reaction in the Fasciculate Zone of the Rat Adrenal

The ultrastructure of the adrenocortical fasciculate zone in the rat was examined in various functional states of the pituitary-adrenal system. Treatment with ACTH caused the mitochondria to enlarge and increased the number of lipid granules and microbodies.

After hypophysectomy these structures decreased in size and number. The acid phosphatase reaction revealed enzyme activity in the microbodies which structures are considered to be lysosomes.

G. Kup

(Section of Pathology, Municipal Hospital, Sopron)

Reaction of Six Month-Old Foetus to the Mother's Cushing Syndrome

In the foetus, the hypophysis and the epiphysis were perfectly normal. The weight of the thyroid was four times, that of the thymus five times, the pancreas four times the normal; the islets of Langerhans were normal. The weight of the adrenals was three times, that of the testicles was more than four times the normal.

Thus, practically the whole foetal endocrine apparatus was involved, as shown by the increased weight of the glands as also by their fourfold enlargement. The adrenals were enlarged threefold only.

The foetal reaction shows that the maternal hypothalamic centre was counteracting the disorder. This explains why neuroendocrine diseases are not inherited.

S. Varga

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Ethionine Injury

The effects of ethionine, especially those on the pancreas, have been studied in rabbits. Administration of the compound induced loss of weight, pancreatitis, fatty necrosis of the pancreas, and fatty degeneration of the liver.

It has been proved by a great number of biochemical, autoradiographic and electron-microscopic investigations that ethionine causes grave damage to cellular metabolism, especially in organs with intensive protein synthesis, and that its chronic administration may give rise to tumour formation.

Deficiency of methionine, and later that of choline was regarded as responsible for the damaging action of ethionine, whereas recently it has been ascribed to an inhibition of protein metabolism. The mechanism of ethionine action requires further investigations.

I. Hüttner, T. Kerényi, H. Jellinek, Klára Szentágothai

(Second Institute of Pathology, University Medical School, Budapest)

Fine Structure of Subendothelial Plasmic Substances Accumulating in Experimental Hypertension

Hypertension has been induced in white rats by the compression of both kidneys in order to study the fine structure of the fibrinoid matter accumulating in the mesenteric arteries between the internal elastic lamina and the endothelial cells. First, there appeared non-birefringent plasmic substances together with leukocytes in the subendothelium; later fibrinoid was seen to accumulate which narrowed the lumen of the vessels and showed in polarized light a topochemical reaction characteristic of fibrin. At this stage, the electron microscope revealed typical fibrin bundles of 240 Å and clumps displaying 1000 to 1500 Å periodicity amidst the disintegrated subendothelial cells. No such structures were seen in the media. Part of the fibrin bundles was phagocyted by monocytes. Later there appeared connective tissue and muscle cells in the subendothelial space which gave rise to intimal proliferation at the site of the fibrinoid. At another place, simultaneously with the appearance of hyaline staining reaction the place of subendothelial fibrinoid was occupied by homogeneous areas in which the structure of the fibrin bundles of regular periodicity and the periodically structured bodies became gradually less distinct and then disappeared. At the time of hyalin formation, the adjacent endothelial and subendothelial cells displayed signs of intensive pinocytosis on their surface contacting the hyalin.

A. Kóczé, B. Veress, H. Jellinek

(Second Institute of Pathology, University Medical School, Budapest)

Hypertensive Changes in Elastic and Major Muscular Vessels

Hypertension had been induced by the Lőrincz—Gorácz method in white rats and the thoracic and abdominal segments of the aorta as also the major muscular vessels have been studied. 1. The mechanism of changes is the same in the major and minor vessels with the difference that changes take longer to develop in the former than in the latter. 2. Elastic fibres in the major vessels prevent or diminish the extent, and delay the process, of fibrinoid necrosis as seen in minor vessels. 3. Development of subendothelial fibrinoid in the thoracic and abdominal portions of the aorta has been observed in chronic cases (40 days), a stage at which the minor vessels exhibit fibrinoid necrosis, extensive intimal proliferation and hyalinosis. 4. Lesions of the abdominal aorta are much graver than those of the thoracic portion.

Klára Szemenyei, L. Haranghy, J. Hintalan

(Second Institute of Pathology, University Medical School, Budapest)

Pulmonary Vascular Changes in Senile Emphysema

Pulmonary vessels of individuals who had suffered from senile emphysema have been compared with vessels obtained from the lung of accidentally deceased healthy subjects of different ages.

In most cases of senile emphysema the pulmonary vessels were dilated and tortuous, and the amount of periadventitial connective tissue increased. The elastic fibres were thickened and more readily digested by elastase than those of the controls. Before elastase digestion only a small amount of argentophile reticular fibres was visible in the vessel walls; after digestion, Foot's silver impregnation revealed numerous delicate longitudinal and transverse argentophile fibres of undulating course.

It follows that not only the alveoli but also the elastic fibres of the vessels undergo changes with age; their digestibility by elastase increases, and — as in the alveolar walls — argentophile fibres are increasing which reduce elasticity.

Dilatation and tortuosity of the vessels are presumably due to the atrophy of the pulmonary parenchyma, the loss of elasticity and the increase of argentophile reticular fibres.

L. Nagy

(Second Institute of Pathology, University Medical School, Budapest)

Effect of Cholesterol Treatment on Injuries of the Aortic Intima

The abdominal aorta of white rats has been exposed and its inner surface was injured by means of a thin wire. The created intimal defect healed with regeneration and scarring.

A group of animals was fed with cholesterol during the healing period, and the incorporation of cholesterol by the intima in the injured area was observed. The serum cholesterol level at which cholesterol began to invade the vessel wall has been estimated.

L. Wallacher, J. Balogh

(Department of Pathology, County Hospital, Szekszárd)

The Bronchial Arteries in Pulmonary Hypertension

The bronchial arteries have been studied in chronic pulmonary hypertension due to primary destruction of the lung parenchyma.

The nature of mural changes, the correlation between age, duration of the disease and clinical manifestations, and those observed between the pulmonary and bronchial arterial systems have been discussed.

G. Lusztig, B. Góg

(Department of Pathology, County Hospital, Kecskemét)

Heparinocyte Reaction and Serum Cholesterol Level

It has been shown earlier that the serum heparin level as determined on the basis of thrombin activation, rose after cholesterol feeding in rabbits.

In the present experiments the heparinocyte reaction associated with changes in the serum cholesterol level has been studied.

Feeding of cholesterol elicited marked quantitative and qualitative changes in the heparinocytes in the myocardium and the skin of rabbits but none in the abdominal muscles and the lungs.

No change in heparinocyte count was observed after combined or successive treatment with thiouracyl and thyroid.

The cellular reaction arising under the effect of cholesterol treatment caused morphological changes in the heparinocytes of the heart and skin which manifested themselves chiefly in a change of the granular pattern and the increase of orthochromasia. These phenomena pointed to increased heparin secretion.

The fact that the reaction occurs only in cases of pronounced hypercholesterolaemia admits of the following conclusions.

a) There is a threshold concentration of cholesterol below which no heparinocytic reaction occurs.

b) Exogenous cholesterol as a chemical and physical stimulus produces a non-specific irritative effect on the heparinocytes.

I. Nagy, Katalin Nagy

(Institute of Anatomy, Histology and Embryology, University Medical School, Szeged)

Lipid Contents of Leukocytes in Tuberculous Individuals

It has been demonstrated most convincingly by SEHRT that lipoid granules are physiological constituents of leukocytes.

Since a certain degree of lipaemia is common in tuberculosis, it has been studied whether the lipaemia is accompanied by a rise in the fat contents of the leukocytes, and if so how the elevated lipid level responds to combined treatment with PAS, isoniazid and streptomycin. The Sudan III method of BACSICH was used for the estimation of fat.

White blood cells were obtained from patients with positive sputum and pharyngeal swab. The examination was repeated every month during treatment. Number and size of lipoid granules were found to increase in the granulocytes of most patients suffering from pulmonary tuberculosis. It took three to four months of combined treatment until the elevated level of lipids had begun to fall.

I. Kádas, I. Péley, M. Németh-Csóka, Mária Simon

(Department of Pathology, County Hospital, Children's Hospital, and First Department of Surgery, University Medical School, Pécs)

Familial Occurrence of Glycogen Disease

Glycogen disease was revealed in a 9-month old female baby at necropsy. The infant's two living siblings, a boy of 2 years and a girl of 9 months were suffering from the same disease. The two living children were examined clinically; biopsy specimens from the liver and striated muscles were studied histologically, histochemically, biochemically (among others, enzymatically) and cytogenetically, under the light and the electron microscope. The results pointed to glucose-6-phosphatase deficiency as the pathogenic factor.

V. Szokoly, L. Megyeri

(Institute of Pathology and First Department of Surgery, University Medical School, Debrecen)

Mucopolysaccharides in the Female Breast under Different Conditions

The stroma of female breasts has been studied histologically and histochemically. Part of the females was healthy, others were suffering from some kind of mastopathy or mammary carcinoma. The special argentophile stroma of both the normal and the cases of mastopathy was found to contain acid mucopolysaccharides, while neutral mucopolysaccharides or mucoproteins were present in the connective tissue containing young collagen fibres. These substances were lacking or demonstrable in traces in aged hyaline connective tissue. Both acid and neutral mucopolysaccharides were present in the stroma of carcinomatous breasts. Histochemical changes in the stroma of involutional breasts have been discussed.

P. Ladányi, I. Krompecher, Mária László

(Institute of Anatomy, Histology and Embryology, University Medical School, Debrecen)

Changes of Tissue Metabolism in Embryonal and Postembryonal Life

The embryonal and postembryonal development of chicken has been studied from the viewpoint of tissue metabolism. Cytochromoxidase activity was considered as the characteristic parameter of oxidative metabolism, the lactic acid content to characterize glycolytic metabolism, and the hexosamine content of the tissues to characterize mucopolysaccharide-type metabolism. Compared to findings on the 11th day of embryonal life, postembryonal cytochromoxidase activity showed a 600 per cent rise, mucopolysaccharide-type metabolism reduction by approximately 30 per cent. Glycolysis showed a slight increase. Intensive mucopolysaccharide metabolism and low cytochromoxidase activity are characteristic of embryonal life, while increased oxidative and slow mucopolysaccharide metabolism of the postembryonal stage.

I. Törő jr.

(Institute of Histology and Embryology, University Medical School, Budapest)

The Endocytosis, Endoplasmic Reticulum and Golgi Complex Related to the Lysosome Formation in the Trophoblast Cells of Rats and Mice. An Electron Microscopic Study

The so-called *coated* (fuzzy) *vesicles* averaging 1000 Å in diameter were described (at first by ROTH and POTTER in 1962) in certain cell types to be responsable for the uptake of protein-substances. In the present paper similar vesicles are reported in trophoblast cells with the aim to follow their intracellular fate.

According to the observations of the author these special pinocytotic vesicles occur usually in the vicinity of the Golgi complex and of the smooth-surfaced endoplasmic reticulum. *Tubular elements* originating from both latter systems and containing a substance fuse with the vesicles resulting in a condensation of some dense material within their cavities.

On the other hand the tubular elements seem to transport and empty their content into absorption vacuoles from which then lysosome-like structures develop.

I. Ökrös

(Department of Morphology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest)

Digitonin Reaction Adapted to Electron Microscopy

The digitonin reaction, known in polarization microscopy as a means by which to demonstrate free cholesterol has been modified with a view to providing a direct electron-microscopic method for determining the intracellular localization of cholesterol. Underlying the procedure is the fact that the crystal needles of the digitonin-cholesterol complex are highly osmiophilic. The method may be of value in the study of steroid-producing tissues and in throwing light on certain polarization phenomena connected with the digitonin reaction.

J. Lackó, S. Keresztury

(Department of Pathology, Semmelweis Hospital, Miskolc)

Postmortem Findings in 70 Cases of Measles

Seventy infants, who had died of measles in the course of the 1964—1965 epidemic, have been studied. The most frequent complication, and cause of death in about 80 per cent of the material was haemorrhagic, purulent, necrotizing bronchopneumonia. Bacteriology in most of these cases revealed Staphylococcus aureus and pyogenes, Proteus, and E. coli. In a few cases Hecht's giant-cell pneumonia was present. The complication next in frequency was with an incidence of about 70 per cent acute suppurative otitis media; the incidence of enterocolitis was nearly as high. The incidence of encephalitis was nearly 20 per cent. The majority of the deceased patients had had no debilitating chronic disease.

S. Keresztury, J. Lackó

(Department of Pathology, Semmelweis Hospital, Miskolc)

Changes in Lymphatic Tissue and Bronchial Mucosa in Measles with Complications

In patients died of measles necropsy revealed grave changes especially in the hilar and paratracheal lymph nodes, the tonsils and the spleen. The chief features were the accumulation and swelling of reticular cells and the appearance of giant cells of reticular origin. The pattern

was suggestive of grave reactive reticulosis and even malignancy. The giant cells were similar to the Sternberg—Reed type rather than to the Warthin—Finkeldey type.

The bronchial and bronchiolar mucosa showed extensive squamous epithelial metaplasia in nearly every case. It is suggested that, although lymphatic changes and the epithelial metaplasia of the respiratory tract are not symptoms specific of measles, they nevertheless present useful diagnostic clues.

A. Gellért, Mária Poberai, Márta Kozma, Erzsébet Husztik

(Institute of Histology and Embryology, University Medical School, Szeged)

Innervation of Lymph Vessels: Fine Structure and Histochemistry

The nerves reach the lymphatic wall in the company of blood vessels. In the adventitia and media they form interwoven plexuses of a more or less coarse appearance, whose finer terminal fibres adhere closely to the smooth muscle cells and innervate them. The stratified arrangement described by Kytmanof has not been confirmed. According to some preliminary examinations, the lymph vessels present a varied structure, not always permitting to differentiate an arrangement of the wall into intima, media, and adventitia.

The noradrenaline test was used for determining the chemical mediator operative in the process of impulse transmission from the terminal vegetative plexuses innervating the lymph vessel wall. Part of the varicose terminal fibres has been found to show fluorescence, which apparently indicates that they belong to the sympathetic part of the vegetative nervous system.

The findings presented a morphological support to the biological observation that sympathetic inhibitors bring about relaxation of the lymph vessels.

A. Ábrahám

(University of Sciences, Szeged)

Electron Microscopic Studies of the Human Glomus Caroticum

The material removed at surgery was fixed in toto in 4 per cent formalin. A few days later 100 μ thick frozen sections were prepared, placed in sugar-solution, then fixed in os mic acid and set in araldite. Part of the sections was studied in the Electron Microscopic Laboratory of Middlesex Hospital Medical School, London, and the rest in Szeged. In the pictures the structure of glomus cells and the terminal connection between these cells and the nervous system has been studied. Although the electron microscopic picture of all glomus cells was identical, certain signs have lead to the conclusion that there are two or three different types to be discerned. The endoplasmatic reticulum with the ribosomes is well visible in the cytoplasm as well as a great number of osmiophilic granules lysosomes in some places large round tubular mitochondria, Golgi-apparatuses. Among the above components, the lysosomes are considered the most important, not having previously been observed in the glomus caroticum; they have a conglomerate structure and are of considerable size. It was remarkable that in some parts there was a large number of mitochondria, in other parts they were hardly detectable. Where there were many osmiophilic granules, mitochondria were scarce and where mitochondria were in abundance there were few osmiophilic granules. As to the nervous connection of the glomus, the studies failed to furnish reliable information. On the glomus cells and the impregnated material surrounding them there were many terminal nerve rings, which, however, could not be detected by electron microscopy, in spite of the fact that the nerve fibres passing through Schwann-cells, as well as the neurotubules could well be seen. This, however, was only natural since hundreds of sections are obtained from one terminal ring and these themselves are not sufficient to demonstrate how the receptors are attached to the cytoplasm of glomus cells.

T. Donáth

(Institute of Anatomy, University Medical School, Budapest)

Adrenergic Innervation of Extra- and Intracerebral Vessels

The adrenergic innervation of extracerebral vessels has been demonstrated in leptomeningeal preparations by Falck's fluorescence-microscopic method.

These nerves follow the vessels entering the brain along a short segment only, and terminate with a free ending. Adrenergic fibres belonging to intracerebral vessels have not been encountered. Adventitial cells with characteristic cytoplasmic fluorescence have been observed around intracerebral vessels. On the basis of different neuropharmacological experiments it seems obvious that these cells contain catecholamine precursors. It is suggested that the chemical components of these cells are involved in the regulation of intracerebral vessels that have no adrenergic innervation.

G. Botár

(Department of Neuromorphology, State Institute of Neurology and Psychiatry, Budapest)

Branchial Nerves in Cyclostomata

The dorsal nerves make their appearance in the *Urochordata* and become segmented in the *Cephalochordata*. They are of mixed character. Their efferent components innervate the striated musculature of the atrium in *Chordata* and that of the pharynx in *Cyclostomata;* their efferent centres have various locations; for all these reasons the dorsal (branchial) nerves are not identical in *Chordata* and *Cyclostomata*.

In *Cyclostomata* even the most rostral branchial nerve, the premandibular nerve has an efferent component. The two rostral branchial nerves, the premandibular and mandibular nerves unite in the *Cyclostomata* to form the trigeminal nerve. The afferent and efferent components of the said two nerves, as that of the trigeminal nerve, run a separate course in the initial portion or from beginning to end.

B. Csillik, F. Joó, Erzsébet Knyihár

(Institute of Anatomy, Histology and Embryology, University Medical School, Szeged and Electron Microscopic Laboratory, József Attila University, Szeged)

Nerve Fibre Degeneration and Transmitter Depletion in the Vegetative Axons

In the normal autonomic terminal innervation apparatus there are 3 to 8 unmyelinated postganglionic axons in each bundle of Schwann's cytoplasmic processes, which almost covers these axons. At 36 hours after removal of the superior cervical ganglion, noradrenaline disappears from the adrenergic postganglionic nerve fibres, as could be established by Falck's fluorescence technique. Electron microscopy, however, showed the axon degeneration to take a longer time. The first sign of degeneration appeared 3 to 4 days after the operation when the protecting Schwann-cover leaves free the nerve fibres in which lysosomic vacuoles make their appearance, without otherwise affecting their structure. At 6 days following operation, histiocyte processes surround the fibres and gradually dissolve the superficial membrane (axolemma). This process always starts at the vesicular (dilated) portions of the nerve fibres and leads within 8—10 days to a breaking up of the axon. The histiocytes finally devour the fibre rests. These findings show that the transmitter substances are synthesized in the nerve cells and for being fixed in the terminals they require energy consuming processes of the axon. Secondary degeneration means a predominance of proteolysis over protein synthesis under conditions when the perikaryon has ceased to supply protein for the nerve fibre.

P. Kása, B. Csillik

Institute of Anatomy, Histology and Embryology, University Medical School, Szeged)

Axo-Axonal Synapses in the Cerebellar Glomeruli

The structure of the cerebellum, although the organ is one of the most studied areas of the nervous system, is still not fully explored.

Relying on light-microscopic data, SZENTÁGOTHAI claims the existence of axo-axonal synapses in the parenchymal islets of the cerebellum, but physiological examinations by ECCLES and the CANBERRA group have been unable to prove a presynaptic inhibitory effect in these areas.

The present examinations in cats and guinea pigs have demonstrated the existence of axo-axonal synapses in the glomeruli of the cerebellar cortex at the preterminal portion of the oncoming mossy fibres, close to the site where the mossy fibre casts off its medullary sheath. According to SZENTÁGOTHAI, only the mossy fibres and Golgi-cell axons terminate close to the dendrites in the parenchymal islets. ECCLES attributes an inhibitory effect to the Golgi-cell axons.

If these two statements are valid, the axo-axonal synapse observed on the mossy fibres within the cerebellar parenchymal islet may exert a presynaptic inhibitory effect.

On the evidence of electron microscopic histochemical examinations, the nervous structures in the parenchymal islet, i.e. the mossy fibre, granule cell dendrite, Golgi axon, and Golgi dendrite display acetylcholinesterase activity not only in the synaptic areas but often over the entire membrane surface.

F. Joó, B. Csillik

(Institute of Anatomy, Histology and Embryology, University Medical School, Szeged)

Ultrastructural Changes in the Cerebellar Cortex Following Inhibition of Arylesterase

In the parenchymal islets of the granular layer of the cerebellar cortex there exists a special form of juncture between the excitatory mossy fibre system and the inhibitory Golgi-cell axon system, as both the excitatory and the inhibitory axons are forming synapses with the dendrites of the granular cells. According to previous histochemical studies of the "complex synapsis" in the archicerebellum of the rat, both the excitatory and the inhibitory synapses display an intensive acetylcholin-esterase activity. Elsewhere in the central nervous system, where histochemistry failed to demonstrate cholinesterase activity, a well-defined arylesterase activity has been observed.

The present studies of the rat neocerebellum have shown that inhibition of arylesterase by copper sulphate did not cause ultrastructural changes of any importance in either the excitatory or the inhibitory axons. The post-synaptic granular cell dendrites, however, were found to display a great number of ribosomes multiplication even at those sites (e.g. the dendritic protrusions) where ribonucleic acid particles do not occur under normal circumstances. Certain changes in the granular cell cytoplasm were indicative of intensified protein synthesis.

On the strength of the findings a significant role is attributed to synaptic arylesterase in the decomposition of stimulating transmitters.

I. Törk, B. Vigh, T. Wenger

(Institute of Histology and Embryology, University Medical School, Budapest)

Autoradiographic Examination of the Circumventricular Ependymal Organs

The ependymal organs of the rat, i.e. circumscribed areas in the wall of the cerebral ventricles, containing special ependymal, glial and nerve elements were examined by means of ^{35}S methionine. The following areas were studied: the subcommissural, paraventricular, sub-

fornical, and recessus organs, the organon vasculosum laminae terminalis, furthermore the ependymal lining of the third ventricle, medial eminence and the choroid plexus. The animals were killed at intervals of one half, one, two and four hours following administration of the ^{35}S methionine. Serial sections were cut. Their autoradiographic pictures on Kodak AR 10 stripping emulsion show a rapid accumulation of amino acids in the ependymal organs and the ependyma. In the ependimal organs showing ependymosecretory activity e. g. in the subcommissural organ, the activity was localized in sites corresponding to the secretory regions and the isotope was probably incorporated in the produced secretion. The pronounced amino acid uptake of the non-secretory ependymal organs is due to their intensive protein metabolism and absorption, respectively.

B. Vigh, Ingeborg Teichmann

(Institute of Histology and Embryology, University Medical School, Budapest)

Histologic and Histochemical Examination of the Paraventricular Organ in Various Vertebrates

The paraventricular organ, one of the ependymal organs in the 3rd ventricle, is made up of a special multi-layered ependyma and a richly vascularized hypendyma. Clusters of Herring's bodies spread from the neurosecretory cell groups towards the organ, the function of which has not been clarified.

A nerve cell group of the organ (nucleus organi paraventricularis) is described; it was found to occur in vertebrates from fish up to mammals. One process of the nerve cells innervates the ependymal portion of the organ, the other infiltrates the brain substance. The histochemical tests used were the aldehyde-fuchsin, chromium-haematoxylin (nonoxidized), DDD, Ninhydrin-Schiff and PAS reactions, toluidine blue, Sudan black B, acid and alkaline phosphatase, succinodehydrogenase and cholinesterase reactions as well as catecholamine determination. The findings pointed to a pronounced metabolic activity, especially in the ependymal portion of the organ.

Ingeborg Teichmann, B. Aros

(Institute of Histology and Embryology, University Medical School, Budapest)

Fluorescence Microscopic Demonstration of Catecholamine Containing Nerve Cells and Fibres in the Central Nervous System of Invertebrates

Earlier histochemical tests (TEICHMANN et al.) and electron-microscopic examinations (AROS et al.) revealed a positive chromaffin reaction and dense core vesicles typical for catecholamine in the central nervous systems of invertebrates (*Lumbricus* sp.). The present paper gives an account of further examinations, using the paraformaldehyde method for the demonstration of catecholamine by means of fluorescence microscopy. Intensive fluorescence was shown by numerous nerve cell groups and nerve paths in the area of the cerebral and subpharyngeal ganglia. In the cerebral ganglion no direct connection was found to exist between the neurosecretory system and the catecholamine containing cells.

Katalin Hollósi

(Department of Pathology, Municipal István Hospital, Budapest)

Neurovegetative Structures in Sialoadenopathies

Changes in the autonomous ground plexus, the chromaffin cells and the arterio-venous anastomoses have been studied in connection with 200 cases of sialoadenopathy of various aetiology.

The autonomous ground plexus revealed alterative-reparative changes in cases of common sialoadenitis, while hyperplastic phenomena were predominant in those of neurogenic sialosis. Grave lesions and consequent hyperregenerative processes were observed in allergic sialoadenopathies associated with Sjögren's syndrome.

The chromaffin (argentaffin) cells of the salivary glands (such cells presumably being the cellular representatives of the adrenergic mechanism) were found to have decreased in number and to have undergone degeneration in destructive processes and tumours.

Hyperplasia of the chromaffin cells and the appearance of peculiar forms were observed in the salivary gland of patients suffering from neurogenic sialosis or hypertension.

The arterio-venous anastomoses, juxtaposed with the system of ducts, contain also chemoreceptors and may, therefore, play a special vegetative-regulatory role in the maintenance of glandular homeostasis. Marked hyperplasia and, in one case, a tumour of the arterio-venous anastomoses was observed in cases of sialosis due to hypertension and emotional stress.

T. Kerényi, L. Haranghy, I. Hüttner, Klára Szentágothai, B. Veress

(Second Institute of Pathology, University Medical School, Budapest)

Enzymatic-Histochemical and Ultrastructural Aspects of Experimental Lipofuscin Pigment in the Nervous System

Following neurotraumatisation induced in white rats by repeated changes of stimuli accumalation nutritional and defence reflexes' senile pigment has been observed in the central nervous system, especially in the Purkinje cells. As compared to controls, the acid-phosphatase, non-specific esterase and succinic-dehydrogenase activity of the Purkinje cells showed a moderate increase during the establishment of the conditioned reflexes. Repeated changes of the conditioning stimuli caused a considerable decrease of the enzymatic activities. Enzymatic reactions became once more intensive after a rest of six weeks, but their localization was different from that observed in the control animals.

Prolonged dinitrophenol treatment induced vigorous enzymatic activity. Accumulation of pigments was significant in this group also.

While the respective ultramicroscopic structures of the pigment granules observed in the course of normal senescence and after the neurotraumatism were essentially similar, the structure of pigment granules observed after dinitrophenol treatment revealed different features. It is suggested that pigmentation following neurotraumatism is due to the absolute, that following dinitrophenol treatment to the relative insufficiency of the cellular enzyme systems.

Mária Éder, B. Horányi

(Department of Neurology, University Medical School, Budapest)

Histopathology of Chronic Nodular Panencephalitis

Ten, 5½- and 4-year old cases of panencephalitis nodosa have been studied histopathologically in order to find answers to the following problems. 1. Pathogenesis and course of demyelinization which — in a 10-year old case — destroyed a considerable portion of the hemispheral white matter. 2. Cytological analysis of meningeal and intraparenchymal inflammatory infiltrations which are encountered in chronic cases also; their immune-pathological relationships. 3. At different points of the cerebral cortex, characteristic Alzheimer type fibrillary changes were enocuntered in one of the cases, — a phenomenon hitherto not registered in literature, their histochemical structure and pathogenesis has been studied. 4. The vessels have also been studied since signs pointing to ischaemic necrosis were observed. 5. Cytological structure and origin has been studied of the glial nodules usually encountered in panencephalitis nodosa.

I. Kiss, K. Máté, T. Major, J. Horváth, G. Pados, L. Fónyad

(First, Second and Third Departments of Medicine, and Department of Pathology, Tétényi Hospital, Budapest)

Clinico-Pathological Evaluation of 1000 Cases of Death of Cardiac Origin

A clinico-pathological survey is given of 1000 patients who died of heart diseases between January 1st, 1960, and December 3rd, 1965. The cases have been tabulated according to diagnosis; they included a variety of conditions from congenital abnormalities to vascular diseases. The immediate cause of death, the course of the disease, and the problem of evaluating the data revealed by the case history, are discussed in detail. Sex and age distribution has been studied. Special care has been taken of the evaluation of the pulmonary state of the patients, irrespective of whether the disease of the lungs was primary, or a consequence of the cardiovascular condition.

K. Máté, I. Kiss, T. Major, J. Horváth, G. Pados, L. Fónyad

(First, Second and Third Departments of Medicine, and Department of Pathology, Tétényi Hospital, Budapest)

The Role of Extracardial Factors in Death Caused by Heart Disease

The role of extracardial factors has been evaluated on the basis of a clinico-pathological survey of 1000 cases of cardiac death, to study the relationship between renal state and the compensation of cardiac failure and the data concerning electrolyte and water household. The significance of multiple embolisation is discussed.

Anna Maria Novi

(Istituto di Patologia Generale dell' Università di Pisa)

Vesicle-like Formations Observed under the Electron Microscope in the Isotropic Bands of the Rat Heart

The protomembranes which build up the intercalary discs in the papillary muscles of the rat, present numerous spindle and sack-shaped dilatations of their interstices. These spaces, delimited by the cytoplasmic membrane, contain numerous structures made up usually by two hemispherical vesicles, each measuring up to 1400 Å in diameter, up to 800 Å in radius and approximately 40 Å in wall thickness, with their flat surfaces arranged in parallel. Their appearance reminds of diplococci. The membrane in the flat portions of the vesicles appears especially rich in contrasts; less so are the granules on their inner sides which present a regular structure.

The two opposite situated flat surfaces in each diplococcus-shaped growth are separated by a 160 Å wide interspace which appears to be subdivided once more by a 20 Å thick membrane. The diplococcal structures appear, depending on the section plane, as differently osmiophilic roundish formations or bubbles containing a richly contrasting matter.

The same kinds of growth have been observed on the cell surface, outside the perimembrane. They occur in the normal rat heart, but much more numerously in the papillary muscles of rats with aortic stenosis.

The presence of these structures in the isotropic band seems to support the hypothesis of SPERELAKIS et al. (1960) in that the release of chemical substances in this region causes excitation to spread from muscle cell to muscle cell.

G. Korb, A. Krug

(Pathological Institute of the University, Marburg a. d. Lahn)

Late Findings in the Heart Muscle of Cats after Temporary Coronary Ligature

Temporary ligature for periods of 60, 120 and 180 minutes of the ramus descendens together with the accompanying vein was carried out in 32 cats. Seven more animals, subjected to permanent ligature, served as controls. The animals, unless they died spontaneously, were killed on the tenth postoperative day and their heart was subjected to histochemical and enzyme histochemical examination.

In all cases of permanent and 180-minute ligature, typical extensive transmural infarcts in the anterior wall of the left ventricle were observed. They often spread over the ventral portion of the septum.

Out of eight hearts with ligature for 2 hours, three presented transmural infarcts in the left anterior wall, and two displayed narrow stratified infarcts in the same region close to the endocardium; the remaining three showed only focal and stripe-shaped myocardial necroses.

In 11 animals with one-hour ligature, the findings were, in three cases, subendocardial stratified infarcts; in eight cases focal or stripe-shaped myocardial necroses; and in no case were transmural infarcts observed.

These findings seem to confirm the earlier assumption, that coronary ligature for 60 and 120 minutes leads to necrosis only in parts of the supplied areas, probably in those sites where ischaemia persists after removal of the ligature.

G. Waldmann

(Institute of Pathology, Friedrich Schiller University, Jena)

Submicroscopic Myocardial Changes in Rabbits Treated with Streptolysin 0

Sublethal intravenous doses of Streptolysin 0 induced changes in the myocardial fine structure, such as an early swelling of the mitochondria, oedema of the apillary endothelium and parenchyma cells, alterations of the endothelial and parenchyma cell membranes and a dilatation of the sarcoplasmic reticulum of parenchyma cells. As to the pathogenesis, a process has been assumed by which Streptolysin 0 affected the cell membrane on analogy of the mechanism operative in erythrocytolysis. Water influx and derangement of the intra and extracellular environment by the primary permeability disturbance could perhaps account for the changes.

J. Juhász, Éva Magyar

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Myocardial Changes in Uremia

The heart of 30 patients who had died with uremia has been subjected to morphological examination. In the wall of the left ventricle characteristic, usually focal, changes were revealed, consisting in a swelling of the fibres, vacuolization of the sarcoplasm, fibrillolysis and interfascicular edema. In the hypertrophized muscle cells nuclear anomalies such as duplication and giant nuclei, etc. were frequent. Hyaline changes of the arterioles, eventually signs of inflammation, were also common.

A double relationship is assumed between the renal failure and the metabolic disturbance of cardiac muscle, *viz.* 1. hypertension accompanying the renal disease leads to cardiac hypertrophy and thus to relative coronary insufficiency; 2. toxic effects of uremia contribute significantly to the myocardial lesions.

A. Bajtai, Klára Németh, F. Alföldy

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Myocardial Changes Caused by Pacemaker Electrodes

Artificial pacemakers of different types are increasingly employed for the therapy of conduction disturbances, especially in Adams-Stokes'syndrome. The electrodes are implanted into the myocardium or introduced through one of the large veins into the right heart ventricle.

The present paper reports on 7 cases with implanted pacemakers. Two of the patients had a congenital septal defect and the surgical intervention had caused a conduction disturbance. In the elderly patients typical Adams-Stokes attacks made it necessary to implant a pacemaker.

One patient died 42 hours after the operation; the myocardium showed signs of necrosis. In two other cases, death occurred 9 days after implantation of the electrodes; there was necrosis associated with a marked inflammatory reaction in the myocardium. One patient lived for 6 weeks after the intervention; the electrodes were surrounded by fibrotic tissue showing signs of chronic inflammation, with foreign body giant cells.

F. Alföldy

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Histological Study of the Cardiac Conduction System in Disturbances of Conduction and in Resuscitated Patients

Surgical complications become more frequent with the increase in the number of heart operations. One of the most serious complications is a disturbance of atrioventricular conduction, the cause of which has been thoroughly studied by numerous investigators (LENÈGRE, BLONDEAU, etc.).

The present paper deals with 5 cases of atrioventricular block caused by heart operation. In all cases histological examination revealed haemorrhages within the conduction system.

In further two cases a pacemaker was implanted for the control of Adams-Stokes' syndrome. Excessive fibrosis of the His bundles sclerosis of the vessel supplying the bundle underlay the attack, as revealed by histology.

In two patients subjected to resuscitation attempt excessive haemorrhages could be observed at various sites of the conduction system; they apparently contributed to the failure of resuscitation.

The present observations allow the conclusion that improvement of the technique of heart operations is of great importance concerning the further reduction of mortality in this field of surgery.

Éva Magyar

(First Institute of Pathology and Experimental Cancer Research, University Medical School, Budapest)

Genesis of Focal Cardiomyocytolysis

Focal cardiomyocytolysis is a patchy malacia of the myocardium, with complete lack of the myofibrils within the apparently normal sarcolemma, and of an inflammatory reaction.

In part of the cases the pathologic changes were due to a relative coronary insufficiency (hypoxia) resulting from myocardial hypertrophy. In all the 5 examined cases, cardiomyocytolysis occurred in subjects with aortic and pulmonary artery hypoplasia. Changes were always most severe in the hypertrophic cardiac muscle lying before the hypoplastic vessel.

Myocytolysis accompanying hypoplasia of the large vessels occurred in young individuals, and caused symptoms similar to those of myocardial infarction.

Angela Gyévai, I. Ökrös, D. Szabó

(Department of Morphology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest)

Cholinesterase Activity in the Myocardial Fibres of Mammals

Earlier histochemical examinations showed that the myocardium and the pacemaker differ in behaviour in mammals. The enzymes participating in the oxidative metabolism displayed a more intense activity in the myocardium than in the pacemaker. Light microscopic and electron microscopic studies now revealed that acetyl- and butirylcholinesterase activities are more pronounced in conducting than in contracting fibres. This finding may be related to the specific physiological role of the conducting system.

I. Oláh, P. Röhlich

(Institute of Histology and Embryology, University Medical School, Budapest)

Myofibril-like Structures in the Endothelial Cells of the Myometrial Arterioles of the Rat

The vascular network (stratum vasculosum) between the two smooth muscle layers in the myometrium of the rat consists of arteries mainly of arteriolic structure. Their endothelial cells are elongated lengthwise and present masses of cytoplasm containing numerous endoplasmic reticulum tubules, free and bound ribosomes, extensive Golgi apparatus, mitochondria, centrioles and a multitude of cytoplasmic microtubules. Characteristic of the endothelial cells is the bundle located near the basal membrane, consisting of filaments 60—70 Å in diameter and presenting a peculiar longitudinal periodicity. This periodicity appears to be produced by dark transversal bands, arranged in intervals of 0.5 to 0.75 μ, simulating the "Z" structure of striated muscle. The function of these fibrils is unknown but their morphology and their resemblance to the filaments of smooth muscle and myo-epithelial cells suggests the idea that they operate as contractile elements.

Anna Kádár, B. Veress, H. Jellinek

(Second Institute of Pathology, University Medical School, Budapest)

Fibre Formation in Experimentally Induced Intima Proliferation

Following partial destruction of the aortic wall of albino rats there appear newly formed fibres in the intimal proliferation which according to our observations consist of smooth muscular elements.

Microscopically, elastic fibre fragments can be found in the ground substance; ultrastructural analysis of these elements revealed that part of them is composed of vesicles and part shows a filamentous structure. Under the endothelial cells these elastic elements tend to form a wavy internal elastic membrane. Under this formation the elastic elements are closely linked with the basement membrane of the smooth muscle.

B. Veress, Anna Kádár, H. Jellinek

(Second Institute of Pathology, University Medical School, Budapest)

Ultrastructure of the Cells of Experimentally Induced Intimal Proliferation

An electron microscopic study was made of the intimal proliferation following partial destruction of the aortic wall in albino rats.

As the first sign of regeneration there appeared a layer of endothelial cells above the thickened and straightened internal elastic membrane. Later, beneath this layer cells usually provided with processes appeared, at the beginning in one, then in more layers. In agreement with previous light and polarization microscopic observations, the electron microscope revealed in these cells an ultrastructure closely similar to that of smooth muscle cells. These cells migrated into the proliferation from the media of the vessel, through the interstices or widened stroma of the broken internal elastic membrane.

G. Gyurkó, M. Szabó

(Institute of Surgical Anatomy, and Institute of Forensic Medicine, University Medical School, Debrecen)

The Role of Mechanical Circulatory Factors in the Localization of Arteriosclerotic Plaques

Localization and extension of arteriosclerotic plaques in the aorta and large vessels of 100 cadavers selected arbitrarily from a material including subjects of either sex and above 45 years of age have been studied with respect to their relationship with haemodynamic factors. The material has been analyzed by mathematical methods. A study of the hydromechanical aspects of the sites where arteriosclerotic plaques tend to occur most frequently has revealed the influence of mechanical factors, particularly turbulence on the localization of the plaques.

Although arteriosclerosis constitutes a disease of the entire organism, and biochemical disturbances are of primary importance in its pathogenesis, circulatory factors have their part in determining the site of appearance and progression of plaques.

M. Németh-Csóka, I. Földi

(First Department of Surgery, University Medical School, Pécs)

Wound Healing in Rats of Different Ages

The healing of surgical wounds has been studied in rats of different ages; in part of the animals lathyrism was previously induced. An incision was made in the midline of the back and the force needed to tear the scar was determined at various points of time after surgery and the scar was subjected to microscopic examination. Breaking resistance of the scar, as determined on the 10th, 20th, and 30th day, increased gradually from 10 to 58 per cent of that of intact skin. The increase was greatest in the young animals, while the resistance was reduced in the animals with lathyrism, especially in the old ones. Resistance of the scar was not related to the microscopic finding.

A. Nagy, Sarolta Petrás

(Department of Pathology, County Hospital, Szolnok)

Morphology of Myocardial Metastatic Tumours

The autopsy material of five years included 43 cases (2.4 per cent) of myocardial metastasis. The morphological picture showed two forms, 1. myocardial carcinosis in which the entire heart muscle was infiltrated by the tumorous tissue, and the myogenic elements showed

atrophy; 2. cancerous myocarditis in which the neoplasm formed islets surrounded by inflammatory reaction. Melanosarcoma and certain differentiated carcinomas belonged to the first category, while haematogenic metastases and inflammatory phenomena were seen in anaplastic, rapidly disintegrating cancers.

Zsuzsa Halmai, Á. Csontai, Anna Árkai

(Second Institute of Pathology, University Medical School, Budapest)

Comparative Histological Study of Recurrent Tumours of the Urinary Bladder

In the autopsy material of 16 years those relapsing cases of vesical tumour have been surveyed regarding which a sufficient amount of surgical and autopsy data was available. The study extended to the types of the tumours and their surroundings. The observed changes were compared with histological findings made in non-tumorous cases or in non-tumorous parts of the tumorous bladder.

S. Woyke, K. Marlicz

(Institute of Pathology and Second Department of Medicine, Pomeranian Medical Academy, Szczecin)

Cytodiagnosis of Gastric Cancer. Evaluation of the Results of Gastric Lavage with Chymotrypsin and Physiological Salt Solution

Cytological investigations have been carried out in patients with clinically and radiologcally suspected gastric cancer. Cytological diagnosis was made on the basis of smears of gastric lavage.

Lavage with chymotrypsin solution alloved to demonstrate cancer cells in about 90 per cent of patients with gastric cancer, without misleading erroneously positive results.

In the second group of patients gastric lavage was performed with physiological salt solution. It has been concluded, that the technique of the examination and the cytologist's experience are of profound importance in the cytodiagnosis of gastric cancer, but the composition of the fluid used for lavage does not influence the result.

Due to the simplicity of the examination and the high percentage of positive and no falsely positive results, the cytological study should be considered as a valuable supplement to the hitherto applied diagnostic methods.

S. Fritsch

(German Academy of Sciences, Berlin and Institute of Microbiology and Experimental Therapy, Jena)

Light and Electron Microscopic Studies of a Transplantable Mouse Chloroleukaemia

Transplantable chloroleukaemia has been induced in mice of the Agnes Bluhm/Jena strain by the cell-free filtrate of (RAB I) murine. On the basis of electron and light microscopical and histochemical findings of the observed chloroleukaemia and other experimental leukoses, the biological significance and biomorphosis of the tumour have been discussed.

J. Tóth, J. Sugár

(Institute for Oncopathological Research, Budapest)

Histology and Ultrastructure of Muco-Epidermoid Tumours

Stewart, Foote and Becker drew attention to the mucoepidermoid tumours of the salivary glands.

Eleven cases of mucoepidermoid tumours originating from the salivary glands, skin, breast, and uterine cervix were examined by the authors. A metastasis formed in the epiglottis of mucoepidermoid carcinoma originated from the submandibular gland served for light- and electron microscopic investigations. In some tumour cells mucin production was observed by light microscopy.

In the ultrastructure of the tumour of transitional cell type ergastoplasm, secretional vacuoles, extensive Golgi zones and annulate lamellae caracteristic for serous cells of salivary glands can be observed. Intracellular tonofilaments and desmosomes were lacking. It was concluded that the tumour cells, as for their ultrastructure, may be regarded as having the character of serous glandular epithel.

L. Döbrössy, J. Sugár, Anna Gulbert

(Institute for Oncopathological Research, Budapest)

Light and Electron Microscopic Study of Melanoblastoma Cells Isolated from Blood

During the growth of melanoblastoma, both amelanotic and melanin containing cells are detached into the blood stream; these cells are easily identified by light microscopy, according to SEAL. After excision of the tumour the cells appear in blood in increased numbers. Melanosomes of the melanocytes can be identified also in the cells isolated from blood. The ultrastructure of these cells has been studied for damages suffered in the blood stream.

I. Pályi, B. Kellner

(Institute for Oncopathological Research, Budapest)

Formation and Fate of Multinucleated Cells in Tumour Cell Cultures

Observations were mostly made on HeLa cells, but also on several types of tumour cell of mice and rats (NK mouse lymphoma, Yoshida's rat sarcoma, Crocker's mouse sarcoma). The cells were cultivated in Rose's chamber and their growth was recorded by phase contrast microcinematography.

The formation of multinucleated cells occurred 1. by fusion of mononucleated cells; 2. by fusion of the daughter cells after multipolar division of large mononuclear polyploid cells; 3. by fusion of the offsprings of multinucleated cells following multipolar division.

Concerning the further growth and behaviour of multinucleated cells, the following phenomena were observed. 1. Multinucleated cells tend of persist longer without division or any sign of degeneration. 2. From a binuclear cell a mononuclear cell tends to separate by fission, and both cells then persist for long periods. 3. In giant multinucleated cells the nuclei become arranged in two distinct groups, which then migrate in opposite directions, producing a long cytoplasmic bridge between them; this cytoplasmic thread often fails to break, or takes a considerable time to do so. 4. Multinucleated cells are rounded off and undergo destruction. 5. Binuclear cells undergo multipolar division, and both mono- and binuclear cells are formed; the latter cells then repeat the process. 6. Binuclear cells often divide by mitosis, the two mononuclear offsprings each then fuse, forming a binuclear cell again.

Amitotic divisions have not been observed in cells under study.

G. Baradnay, Z. Mónus

(First Department of Surgery, and Institute of Pathology, University Medical School, Szeged)

Sex Chromatin Tests in Human Tumours

After a survey of recent advances in the research of Barr's bodies, a study of sex chromatin in more than 200 different human neoplasms is reported. Attempts have been made to establish a relationship between prognosis, hormone dependency and the sex chromatin, in cases of carcinoma of the breast, prostatic hypertrophy and cancer, pulmonary cancer in women, gynaecomastia, and other tumours and hormone dependent tissue abnormalities. It is concluded that, although several important results have been achieved in this field, sex chromatin tests are not suitable for definite conclusions to be drawn concerning prognosis and therapeutical results in the case of human neoplasms.

L. Józsa, G. Szederkényi

(Department of Pathology, County Hospital, Kecskemét)

Histochemical and Cytochemical Observations in Erythromyelosis (Di Guglielmo's Disease)

A cytochemical study has been made of the bone marrow and the formed elements of blood in four cases of erythromyelosis verified at autopsy. Three types of PAS positive granules were found in the erythroblasts. One kind of granule proved to be sensitive to diastase, the other displayed characteristics of neutral mucoprotein, contained hexosamine and sialinic acid, but showed no metachromasia. The third type of granule had properties of acid mucoprotein, showed metachromasia, proved to be alcianophilic, and tended to bind colloidal iron.

The granules present in the erythroblasts are thought to result from the altered saccharide metabolism.

I. Fodor

(Department of Pathology, National Institute of Rheumatology and Balneology, Budapest)

Experimentally Induced Osseous Tumour

Osteoma has been induced in rabbits by the intravenous administration of beryllium oxide, a compound poorly soluble in water, which accumulates in the reticulo-endothelial system. In more than 70 per cent of the animals the tumour had become apparent after 10 to 15 months of treatment. Most frequently it arose from the rapidly growing metaphyses of the long bones, i.e. at the lower end of the femur and the upper end of the tibia and humerus, a localization similar to that observed in the human. Not infrequently, multiple osteomas developed and metastases, especially in the lungs, were common. Histologically, the tumour proved to be an osteosarcoma which started as a proliferation of the mesenchyma of the medullary cavity. The metaplastic sarcomatous tissue formed irregular osteoid and bone trabecules, which invaded the cortex and surrounded the bone with neoplastic tissue.

Histochemical observations of the tumour have been presented.

L. Takácsi-Nagy

(Second Department of Medicine, University Medical School, Budapest)

Myelofibrosis Induced by Lead Acetate

Myelofibrosis was induced in rats by the repeated intravenous injection of lead acetate. Connective tissue metaplasia of the bone marrow occurred in the vertebrae and the femur. The erythropoietic elements were replaced by reticulum cells and fibroblasts, showing marked

fibre formation. This bone marrow fibrosis was associated with hepato-splenomegaly. Most of the animals developed anaemia. The thrombocyte count was often reduced, the white cell count usually increased.

Myelofibrosis induced by lead acetate in the rat seems to be a suitable model for studying the pathomechanism of myelofibrosis in the human.

Éva Gáti

(Institute for Oncopathological Research, Budapest)

Concurrent Malignant Neoplasms

According to the general view, malignant tumours rarely occur concomitantly. In recent years, however, several reports have appeared on the occurrence of two, or even more, independent malignant tumours in the same patient. Other patients have recovered from one neoplasm and then developed an other tumour independent of the former in a different area. In some cases necropsy reveals that the original tumour has been cured, and that the other one, thought to be a metastasis of the first one, is a different neoplasm.

The results of treatment and surgical intervention considerably differ if there are two or more independent tumours, or metastases of one neoplasm. Detection of the second tumour is particularly important if it is one with a favourable surgical prognosis. Thus, clinically it is of paramount significance that concurrent neoplasms should be diagnosed as early as possible.

I. Szőnyi, I. Kiss, S. Ferkó

(Departments of Obstetrics and Pathology, Tétényi Hospital, Budapest)

Effect of Cyclophosphamide on Uterine Cancer

The effect of cyclophosphamide has been studied in 59 cases of carcinoma of the uterus of which 37 were on the cervix and 22 on the corpus.

After histological verification of carcinoma, the patients were treated with 200 mg of cyclophosphamide daily, up to a total of 8000 mg. Biopsies obtained prior to, and after treatment were compared; the differences in the structure of tumour cells were attributed to the treatment. A marked cytostatic effect could be observed in all cases; this manifested itself in swelling and vacuolization of the cytoplasm and diminution of its pyroninophilia, or in swelling and vacuolization of the nuclei and often karyolysis. Proliferation of the stroma at the expense of the parenchyma was a common finding. Giant cells with large vacuoles or vacuolized nuclei were occasionally observed as a result of pathological ripening; especially in cases of adenocarcinoma of the corpus, considerable dissolution of the chromatin occurred, resulting in the appearance of balloon-shaped nuclei.

The results have been interpreted as sufficient proof of the cytostatic effect of cyclophosphamide in the case of uterine cancer.

J. T. Kelemen, L. Mándi, S. Bacsa

(Institute of Pathology, and Department of Tuberculosis, University Medical School, Debrecen)

Postlymphographic Reactions in Lymph Nodes

Reactions elicited in human inguinal lymph nodes by contrast material in oil have been studied by histological and histochemical methods. The contrast medium, stored in the sinuses, elicited a macrophage histiocytic and foreign body giant cell reaction of varying intensity. The cells revealed signs of lipid phagocytosis with strong acid phosphatase activity. The introduced contrast medium persisted in the sinuses and the cells. The reaction is well distinguishable from tuberculous and sarcoid foci. In some cases the contrast material elicited a hyperergic reaction.

Sarolta Petrás, A. Nagy

(Department of Pathology, County Hospital, Szolnok)

Bronchial Papillomata

Bronchial papilloma is, according to SPENCER, one of the rarest tumours of the lung, and so far no such case has been reported in Hungary. In the authors' biopsy material of 33946 cases there were three cases of pulmonary papilloma. The morphology of the tumour is discussed and it is shown that in one of the cases the benign bronchial tumour had degenerated into carcinoma.

R. Jankovics, K. Szepesházy, J. Manninger, G. Kazár, L. Zolczer

(National Institute of Traumatology, Budapest)

Necrosis of the Head of the Femur After Fracture of the Femoral Neck

(Comparative Phlebographic and Histological Examinations *in vivo*)

The blood supply of the caput femoris suffers a severe disturbance after medial femoral neck fracture. It takes 1 to 3 years until the circulatory disorders have become clinically and radiographically manifest. They often give rise to grave complaints. Determination of the disturbance to be expected is of prime importance for both the prognosis and the treatment of the fracture. It was for this reason that the circulation of the femoral head has been studied by means of arteriograms, venograms and isotopes. Comparison of vasograms with the anatomical picture is important for the correct evaluation of circulatory investigations. Phlebograms made in cases of femoral neck fracture have been compared with the results of histological examinations concerning the necrosed caput femoris and the teres ligament with a view to gaining prognostic clues.

A. Glauber, T. Vizkelety, K. Szepesi

(Department of Orthopaedics, University Medical School, Budapest)

The Role of the Iliopsoas Muscle in Dislocation of the Hip

In the development of congenital dislocation of the hip the imperfect growth of the proximal end of the femur and the impairment of antetorsion are important factors. In the reduction of antetorsion the iliopsoas muscle was found to play a prominent part. X-ray studies have revealed that in the normal anatomical situation the iliopsoas muscle complements the anterior segment of the acetabulum and, as its active anterior wall, supports the head of the femur. If the hip is dysplasic or dislocated, the muscle is incapable of accomplishing this function.

It has been shown that the iliopsoas muscle both in itself and concurrently with the outward-rotators, is capable of causing a tension in the neck of the femur which, when acting continuously, remodels the tone and thus reduces antetorsion.

Tenso-optical measurements have shown on the frontal plane model of the proximal end of the femur that the tension exerted by the iliopsoas muscle is the greatest at the basis of the neck of the femur.

The present results indicate that the iliopsoas muscle is involved in the pathomechanism of congenital luxation of the hip not only by the previously known mechanism of isthmus-formation, but also by affecting antetorsion of the femur in the early stage of dislocation.

K. Szepesi, A. Glauber

(Department of Orthopaedics, University Medical School, Budapest)

Function of the Iliopsoas Muscle

Clinical observations have shown the iliopsoas muscle, owing to its particular position and function, to play a part in the development of certain diseases of the hip joint (congenital luxation, Perthes' disease, coxarthrosis).

In order to analyze the force effects, it is required to know their size and direction. Owing to the special anatomic conditions of the iliopsoas muscle, the forces arising on contraction of the muscle may greatly differ in size and direction, in dependence on the position of the hip joint.

A mathematical method has been elaborated for the precise examination of these effects. On the basis of X-ray pictures of anatomic preparations and of measurements performed during operation, the exact sites of the points required for calculation, as determined in relation to the femoral head (e.g. the exit point of the iliopsoas muscle from the pelvis) have been established. On the basis of these the hip joint together with the determined points was drawn in a coordinate system.

Direction and size of the forces exerted by the iliopsoas muscle was determined according to the vectors in three dimensions. Thus it was possible to determine the flexional, adductional and rotational moments in every position of the hip joint. In the ground position, for instance, the proportion of these components was 1 : 4 : 1. Establishing the muscle function in any given case, thus allows useful practical inferences.

L. Kéry, A. Glauber, T. Farkas

(Department of Orthopaedics, University Medical School, Budapest)

On the Pathology of Osteolathyrism

Osteolathyrism was induced in rabbits by β-amino-propiolnitrile and the changes developed on the bones have been studied.

Bones of the extremities showed hyperostosis and torsion deformities. Epiphyses were widened, tubercles and tuberosities became pronounced. Scoliosis was usually absent, and if present, of mild degree. Epiphyseolysis was noted in numerous cases on the head of both the femur and the humerus.

Histological examination revealed degenerative and proliferative changes, especially in the growth cartilage, joint cartilage, and at the fixation points of muscles and tendons. The main signs of degeneration in the costochondral junction were desintegration of the columnal structure of chondrocytes in the zone of mature cartilage and the presence of fissures pushing the chondral columns aside. The joint cartilages became thin and cystic cavities appeared in them. Signs of proliferation in the hyperostotic areas were a periosteal thickening and the subperiosteal formation of immature osseus tissue.

Histochemical observations showed metachromasia of the sites of proliferation, and disappearance of metachromasia at the sites where degeneration prevailed; the latter was associated with PAS positivity by Ritter-Oleson's technique. Protein and amino acid content, as estimated by the tetrazotized benzidine reaction, and the ninhydrine reaction, respectively, showed a diminution in the lathyritic bones; these findings agreed well with the paper electrophoretic results.

The simultaneous administration of β-aminopropiolnitrile and low dose total body X-ray irradiation enhanced the proliferative changes, particularly in the cartilaginous tissue.

G. Lőrincz, K. Farkas, D. Tanka, Mária Keller

(Department of Pathology, National Institute for Rheumatism and Balneology, Budapest)

New Experimental Models of Arthritis

Numerous experimental and clinical data are in support of the immunological theory of rheumatoid arthritis.

In the present study complete adjuvant was injected into the skin of the neck and the sole of rats. Arthritis was more marked in the animals receiving the adjuvant into the skin of the sole.

In other experiments arthritis was induced by inflammatory exudate. It has been reported by MIEHLKE and EGER that the injection of the exudate of Selye's granuloma pouch into the tail vein of rats induces chronic polyarthritis.

Exudate obtained from a granuloma pouch on the 6th day was injected under the skin of the sole. The exudate was passaged in another granuloma pouch; with the passaged exudate thus obtained further rats were treated. The passaged exudate induced increased swelling of the ankle and paw. Inflammation was starting from the periarticular tissues and spreading towards the synovia. Synovitis was observed in several cases.

D. Tanka, K. Farkas, G. Lőrincz, Mária Keller

Department of Pathology, National Institute for Rheumatology and Balneology, Budapest

Experimental Amyloidosis and Experimental Arthritis

Rheumatoid disease is characterized by well-defined histological changes of the connective tissue. Rheumatoid arthritis as well as other rheumatoid diseases are frequently associated with amyloidosis.

The aim of the present investigation was to establish the changes in enzymatic activity detected by histochemical methods that are likely to develop prior to amyloidosis in the spleen, liver and kidney of animals treated with nutros, and on the other hand, to study the course of experimental arthritis induced in such animals prior to the appearance of amyloidosis.

In the animals treated with nutros and treated with Freund's adjuvant or granuloma pouch exudate, arthritis developed earlier and the changes were more severe than in the controls.

In the spleen of animals treated with nutros the activity of hydrolytic enzymes was considerably reduced, while in the kidney a focal diminution of activity was revealed; in the liver, Kupffer's cells showed a considerable increase of activity. The activity of non-specific esterase decreased in the spleen, and exhibited a somewhat altered localization pattern in the liver and kidney. Enzyme activity was reduced to a significantly higher extent in the animals treated simultaneously with nutros and either Freund's adjuvant or granuloma pouch exudate.

Histochemical changes were detected also in the arthritic tissues of the animals treated with nutros and Freund's adjuvant. In the connective tissue surrounding the joints, the activity of lysosomal enzymes increased considerably, and occasional pannus formation occurred with increased acid phosphatase and non-specific esterase activity. The increase in enzyme activity was less marked in the arthritic tissues of animals treated with granuloma pouch exudate.

The present results indicate that 1. amyloidosis is preceded by characteristic changes in enzyme activity in the organs in which later amlyoidosis develops; 2. amyloidosis enhances the development of experimental arthritis; 3. the simultaneous occurrence of amyloidosis and arthritis results in a considerable increase in the activity of lysosomal enzymes.

A. Géhl, I. Földes, L. Tasnády, L. Módis, L. Csedreky

(Institute of Anatomy, University Medical School, Debrecen)

Regenerative Chondral Osteogenesis (Callus Formation)

Callus formation depends upon numerous factors such as vascularization, hormonal, physical, chemical, mechanical factors, etc. Among these, the mechanical factors affecting chondral osteogenesis have been studied in the dog. A specially constructed spring-mechanism was applied on the previously broken tibia and the effect of compression on callus formation was observed by histological and histochemical methods, between the first and sixth weeks. Different areas of the callus were examined separately, and the interference of pressure at each site was established. Some aspects of the stimulating effect of compression on chondrogenesis have been elucidated.

In addition to the effects exerted by mechanical factors, the combined effect of mechanical and locally applied chemical factors on the formation of regenerative callus has been studied.

B. Somogyi

(Institute of Anatomy, University Medical School, Budapest)

Model Experiments to Study Ligament Injuries in Connection with Flexional Spine Fracture

Osseous-ligamentous spine preparations comprising the vertebrae L_1 to L_3 were subjected to flexional compression. Flexion could be increased without vertebral fracture after division of the supraspinous and intraspinous ligaments. The same degree of flexion could not be achieved after dividing the arcuate ligament. The model experiments supported the clinical assumption that in connection with flexional fractures a rupture of the ligaments between spinous processes is almost inevitable.

Injuries of preparations associated with hyperflexional fracture lacking the vertebral arcs and processes are accompanied by ruptures of the postero-lateral part of the intervertebral disk, later also of the short fibres located on the broad part of the posterior longitudinal ligament adhering to the disk. Injuries of this kind have by all means to be reckoned with in the case of fractures reducing the height of the vertebra by more than one third.

T. Vízkelety, L. Kéry

(Department of Orthopaedics, University Medical School, Budapest)

Perivascular Lymph Spaces in Bone

Perivascular lymph sheaths of bone have been described repeatedly in the past century, nevertheless their existence has remained doubtful and left unmentioned.

Relationships between vessels and bone channels have been examined in the rabbit by arterial silver impregnation. Fluid circulation in the bone was studied by introducing into the cortex a stained gelatin plomb and by injections into calf bone.

A fibrous connection was found to exist between vessel wall and bone channel wall, but no separate endothelium sheath could be discovered around the vessels. Occasional spaces between vessel and bone channel must be considered artefacts. Sheaths lined with endothelium independent of the vessel wall were not found at these sites. The vessel diameter may vary in comparison to the constant size of the bone channel.

Fluid accumulation in the perivascular connective tissue took place in the interfibrous spaces, but no in endothelial recesses.

Dye absorption in the cortex permitted the statement that the lacunae, canaliculi and channels make up an integral space system in the bone.

K. Szepesházy, R. Jankovics, G. Szabó

(National Institute of Traumatology, Budapest)

Postmortem Quantitative Study of Fat Emboli

Postmortem examination reveals pulmonary fat embolism in the majority of fatally injured persons. Experiments have shown that the amount of fat entering into the circulation must reach a certain limit to cause clinical symptoms. The degree of embolism is rarely determined at autopsy; therefore, the present study was undertaken to compare the gravity of fat embolism in persons who had been fatally injured with the fat embolism in persons who had died of some acute or chronic disease. Comparisons have been based on the number of emboli per unit of pulmonary, cerebral and renal tissues. Pulmonary embolism was present in all persons who had died within a week following the fracture of long bones, and it was heavy (more than 180 emboli per cu. mm) in about 80 per cent of the examined cases. Fat embolism was of medium degree in those who had died after a week. Systemic embolism was comparatively rare and not invariably associated with the gravest form of pulmonary embolism. Pulmonary fat embolism was encountered in about 50 per cent of those who had died of some internal disease; its degree was slight (less than 60 emboli) or medium (60 to 200 emboli per cu. mm). Systemic embolism was exceptional in this group.

R. Jankovics, K. Szepesházy, Zsuzsa Magyar, G. Szabó

(National Institute of Traumatology, Budapest)

Human and Experimental Fat Embolism

Autopsy of patients who had died after the fracture of long bones invariably revealed the presence of mostly grave fat embolism. This notwithstanding, cases of fat embolism giving rise to clinical manifestations or leading to death are extremely rare. The intravenous DL 100 of triolein for the rabbit has been found to be 1.25 ml/kg, and the DL 50, 1.02 ml/kg. The number of emboli in lungs of human subjects who had suffered fatal injury was less than what is found in the rabbit after the intravenous injection of 0.25 ml/kg of triolein.

After the intravenous injection of 0.1 to 1.0 ml per kg of ^{131}I triolein the activity was determined in different organs and the results were compared with those of histological analyses. There seemed to be no correlation between the histological picture and the amount of fat contained in the lungs. Activity per unit weight of lung is limited so that the lungs are able to retain just a certain amount of fat. On increasing the dose of fat, the pulmonary proportion of fat became lower and the proportion of fat in other organs (brain, kidney, liver, gastrointestinal tract, etc.) correspondingly higher.

M. Kellermayer, T. Heim

(Institute of Pathology and Department of Paediatrics, University Medical School, Pécs)

Brown Adipose Tissue of Newborns in Pathologic States

Experiments were performed on newborn rabbits in order to study the changes induced by fasting in the brown and white adipose tissues. Neither the brown nor the white fat cells contained fat droplets, when the fasting animals had been kept at room temperature (20—23° C); but in thermoneutral surroundings (35° C), numerous fat droplets were found in the brown and none in the white adipose tissue. The results seemed to indicate that brown adipose tissue, as a special effector organ, played an important role in chemical heat regulation. On the basis of observations of the brown fat of newborns and prematures some relationship is assumed to exist between brown fat and the disturbances of heat regulation in infants.

Anna Horváth-Kovács

(Institute of Pathology, University Medical School, Pécs)

Surface Effects of Basophilic Crystalline Silicogenic Powders

The crystalline quartz powders in the lungs of patients and experimental animals with silicosis showed intense staining with basic dyes (toluidine blue, pyronin) and in the polarization microscope their staining proved to be anisotropic in nature. Amorphous inactive powders did not reveal any degree of basophilia. These findings showed a new aspect in the pathogenesis of silicosis, the importance of the negative surface charges of the quartz particles and prompted us to study the silicogenic effect of crystalline powders combined before application with basic dyes. It was found that in animals treated wuth crystalline quartz powder-basic dye complex, a considerably slighter tissue reaction developed than in the controls which received the crystalline quartz powder only. The stained powders have been found to retain the basic dyes on their surfaces in the lung tissues for several months.

A. Kardeván, F. Vetési

(Department of Pathology, University Veterinary School, Budapest)

Generalized Equine Aspergillosis

Generalized aspergillosis has been revealed as the cause of death of about 50 per cent of a stock of foals. The primary lesions were in the intestinal tract, but spread of the pathogen by the blood and lymph gave rise to metastases in various organs. Part of the metastatic foci consisted of colonies of fungi, while another part represented infarcts due to mycotic thrombosis. The pathogen proved to be *Aspergillus fumigatus*, and the vector was mouldy fodder. Spread and development of the disease were promoted by the youth of the animals, their previous infection by *Bact. pyosepticum* and its prolonged treatment with antibiotics.

T. Wenger, I. Törk

(Institute of Histology and Embryology, University Medical School, Budapest)

Comparative Morphology of the Vascular Organ of the Terminal Lamina in Various Vertebrates

The vascular organ of the terminal lamina (OVLT) is one of the ependymal organs. It extends from the anterior commissura to the optic chiasma and closes the lumen of the third ventricle ventrally.

In fish, caudate and non-caudate amphibia, reptiles, birds and mammals the OVLT has been found to occur as a clearly distinct organ of the ependyma. Marked morphologic differences within one and the same species were not observed. The OVLT presents three distinct parts, an external portion abounding in vascular and connective tissue; an intermediate one with a multitude of glia cells and nerve fibres; and an internal part made up of special ependyma. In fish, a fibrous structure dominates, birds present a rich capillary network; mammals are characterized by a cell-rich intermediate layer.

The OVLT presents a similar degree of morphologic development in the various vertebrates, offering the suggestion to be one of the phylogenetically ancient ependymal organs of vertebrates.

A. Korényi-Both, K. Lapis, Margit Gallai

(Department of Pathology, and Department of Neurology, Postgraduate Medical School, Budapest)

The Microscopic Structure of Muscles in Juvenile Progressive Dystrophy

Biopsy specimens obtained from the muscles of two siblings (15 and 17 years old, respectively), suffering from juvenile progressive dystrophy, have been examined under the light and the electron microscope. The observed changes were identical in both patients. The nuclei

showed partial chromatolysis, pycnosis and marked dentation of the nuclear membrane. The sarcomeres were distorted, the Z-disk showed nodular disintegration, and the interfibrillar space was widened. These changes extended to single sarcomeres or to larger areas. Destroyed myofibrils were replaced by groups of disintegrated, homogeneous myofilaments and basal sarcoplasm. The sarcoplasmic reticulum was distended even in the comparatively unimpaired areas; the amount of sarcoplasmic glycogen was increased, the mitochondria were swollen, their crests fragmented. Some areas contained condensed, moderately electron-dense, amorphous substance in which scattered myofilaments, rests of intermediate disks and damaged mitochondria were found, the latter phenomenon being presumably equivalent to Zenker's hyaline degeneration as seen under the light microscope.

Zs. Kocsis

(Department of Pathology, Second Municipal Hospital, Miskolc)

Pathology of the Distress Syndrome in Premature Infants

The pathologic changes observed in 116 newborns with hyaline membrane are discussed. Conspicuous features were the marked oedema of the organs and the dilated lymph vessels, especially in the lungs, kidneys, myocardium, liver, lymph nodes and the adrenal glands. The ganglion cells in the brain and cerebellum were partially disintegrated. The kidneys were remarkably immature.

The changes are partly attributed to anoxic or metabolic causes and partly to a grave disorder in water household, the development of which seems to be closely related with the immature state of the kidneys.

I. Mészáros, L. Wallacher, J. Balogh

(Department of Pathology, County Hospital, Szekszárd)

Morphological Signs in Respiratory Distress Syndrome

The morphological characteristics have been studied in infants who had died with espiratory distress, with or without hyaline membrane formation.

Characteristic features of the lung parenchyma and of the pulmonary vessels are compared with the clinical findings, and the primary and supposedly secondary phenomena have been discussed.

J. Horgász

(Department of Pathology, County Hospital, Szekszárd)

Senile Alterations of the Bronchial Epithelium

The material of the present study consisted of the bronchial mucosa of human subjects who were older than 65 years, had not been suffering from bronchial tumour or a specific inflammatory condition of the bronchi, and whose death had not been caused by a primary disease of the pulmonary parenchyma. After filling the lungs with 8 per cent formalin solution the principal and segmental bronchi and the peripheral bronchioles were studied for senile changes in the cell nucleus and cytoplasm, in the stratification of the epithelium, in the production of mucus, in the behaviour of the connective-tissue elements and in the intercellular fibres.

Ilona Szíjj, K. Kovács

(First Department of Medicine, University Medical School, Szeged)

Effect of Mannomustin on Changes Induced in the Rat by Hexadimethrine-Bromide

The intravenous administration of 5 mg hexadimethrine-bromide induces focal infarcts of varying size in the adenohypophysis, the inner layers of the adrenal cortex and — less frequently — in the liver of rats. The zona glomerulosa is destroyed in all cases, and extensive necrosis occurs in Henle's loops as well. Pretreatment with mannomustin (10 mg per 100 g body weight intravenously 24 hrs before the administration of hexadimethrine-bromide) reduced the susceptibility to infarction in the adenohypophysis, the adrenal cortex and the liver but failed to prevent necrosis in the zona glomerulosa and Henle's loops.

Considering that the applied dose of mannomustin induces pronounced leukopenia, the development of necroses of vascular origin in the various organs may be assumed to require the presence of an adequate number of leucocytes.

A. Jakobovits*

(Department of Gynaecology, University Medical School, Szeged, and University of Hamburg)

Aetiology and Pathology of Uterine Myo-Fibrohypertrophy

Hypertrophy of uterine muscle and connective tissue was found to produce benign, non-tumorous, uniform enlargement of the uterus and the disease should therefore be termed uterine myo-fibrohypertrophy; to apply the term "chronic metritis" to the observed cases is incorrect in view of the absence of any sign that would refer to an inflammatory process in the uterine wall.

Owing to cell enlargement, visual fields of the same size presented less muscle and connective tissue cells in the hypertrophied uteri than in the controls.

In the enlarged uteri of multiparae and of subjects past 40, the degree of hypertrophy was not greater than in younger women but the increased weight of their uteri seemed to indicate that hypertrophy had associated with hyperplasia, i. e. with an increase in the number of muscle and connective tissue cells.

Menstrual disorders and pain predominated among the clinical symptoms.

Clinical observation as well as animal experiments allowed the assumption that endocrine factors have a significant part in producing clinical patterns with oestrogenic or androgenic preponderance. Attention is called to the presumed aetiological significance of the prolonged oestrogenic effect associated with pregnancy. The results of animal experiments suggest that progesterone does not essentially influence the development of uterine hypertrophy. The fact that uterine hypertrophy often appears in the menopause, seems to indicate that it takes long time to develop.

Mária Gajó, A. Gellért

(Institute of Anatomy, Histology and Embryology, University Medical School, Szeged)

Comparative Histology of the Vocal Cord and the Vocal Muscle

The morphology and physiology of phonation has been studied by numerous authors. It is, however, still undecided whether or not the fibres of the vocal muscle are inserted into the vocal cord; neither is it clear, what part the vocal muscle is playing in the finer modulations. This is why there are two different theories concerning the mechanism of phonation. One is the myo-elastic (aerodynamic) theory, proposed by Johannes MÜLLER more than hundred years ago, according to which subglottic air pressed from the lungs up the respiratory ducts represents the active force that sets the vocal cords into vibration; the second, so-called neuro-

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muscular (neurochronaxic) theory states that the vibration of vocal cords is under central nervous controls.

Considering the extremely varied data concerning the arrangement of the vocal muscle fibres, and their relationship to the vocal cord, in serial sections of the larynx of humans (mainly newborns), dogs and rats the vocal muscle fibres have been studied for their relationship to the collagen and elastic fibres of the vocal cord and the elastic conus.

The arrangement of the vocal muscle fibres is much simpler in animals than in man. In the dog and rat larynx many parallel fibres were found to run sagittally. The human fibres presented an intricate picture hardly definable in the histologic sections. Nevertheless, the relationship of muscle fibres to the surrounding collagen and elastic fibres is essentially the same. The fibres of connective tissue enmesh those of the muscle which appear as if embedded in the collagen-elastic tissue. Thus the connective tissue elements are involved in the muscle contractions, and the different structures form an integral functional unit.

R. Poche

(Institute of Pathology, University of Düsseldorf)

Electron Microscopic Examination of the Human Myocardium

Electron microscopic examinations of the myocardium have usually been carried out on experimental animals. Human postmortem material namely undergoes such an autolytic change that it is exceptional to obtain reliable finding while biopsy of the myocardium cannot be carried out in the way performed in the liver and other organs. Cardiac surgery, however, offers advantageous possibilities in this respect if the muscle removed during heart operations is immediately subjected to proper treatment. Results of such investigations in the case of chronic rheumatism, tetralogy of Fallot, idiopathic hypertrophic subaortic stenosis, have been presented.

FORENSIC MEDICINE

RELATOR

B. Rex-Kiss

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MEDICO-LEGAL ASPECTS OF SEROLOGICAL GENETICS

In general, human blood groups are understood to mean hereditary traits linked to the red corpuscles. On the other hand, the serum of human blood, too, contains substances (the so-called blood group antibodies) which are determined by blood-group properties. The reason why, originally, blood groups were set up according to the genetic properties of the erythrocytes and not according to the antibodies contained in the serum, was that the group specificity of the latter was variable (especially in systems other than the classic AB0 system). Besides, the principle that the serum contains the homologous antibodies whenever the erythrocytes fail to reveal a certain specific character, is but rarely practicable with the currently available procedures. The situation has now changed inasmuch as the discovery of genetic properties in the various protein fractions of the serum makes it reasonable to speak not only of blood groups (in the original sense of the term) but of "serum groups" as well. It was in the wake of JAYL (1947), SMITHIES (1955) and their associates that investigations were started with a view to proving that — like erythrocytes — serum proteins too possessed genetic properties. Discoveries in this field were greatly facilitated by the adoption of biochemical research methods. A thorough analysis of protein components would have been impossible without the facilities offered by electrophoresis. The inheritance patterns of the "serum groups" (that are perfectly independent of the blood groups) are fairly clear and have become a highly interesting subject of recent serological research work. They are of prime significance for anthropology, genetics, even for clinical practice, and it is safe to expect numerous new discoveries in this field. The reliability and simplicity of determinations concerning heraditary transmission allow their forensic employment in cases of doubtful parentage.

From a serological angle, up-to-date "inheritance biology" may be divided into two branches.

(a) *Blood groups* which comprise both the classic and the newly discovered systems.

(b) Hereditarily determined proteins, i.e. *systems of serum groups* of which eight are known at present: heptoglobins; transferrins; postalbumins; group Gc; group Gm; group Ag; group Lp; pseudocholinesterase-group.

The rapid progress of research work resulted not only in the discovery of new blood groups but has modified existing conceptions also. Of course, the practical significance of new discoveries cannot always be estimated at once. Let us refer in this connection to the discovery of the sexually determined blood group Xg. It meant the long expected discovery of the first blood-group gene linked to a special sexual chromosome and may shortly acquire great importance.

Satisfactory as the steady progress is, it becomes increasingly difficult to fit the new findings into the frame of known facts. Since the discovery of the factor Rh by LANDSTEINER and WIENER not less than 21 antibodies have been detected which antagonize the known components or the partial antigens of this system.

The possibility of determining nonpaternity (i.e. to rule out the paternity of an incorrectly accused man) by serological methods depends on

(a) the distribution of the examined character in the population to which the putative father belongs;

(b) whether one or more hereditary characters can be ascertained in the examined system;

(c) whether only the phenotype or both the phenotype and the genotype can be directly determined. After HIRSZFELD, one speaks of a fully utilizable system in the latter, and of a partially utilizable one in the former, case. It follows that only blood groups and serum groups satisfying these requirements are useful for medico-legal practice. Systems of such groups will be discussed in the following.

AB0 system. Reliability of the determination of the subgroups A, of prime importance for forensic medicine, has been greatly promoted by the employment of phytagglutinins in recent years. A great number of substances specifically reacting with A_1 and A_2(H) of vegetable origin are now available.

MNSs system. The combination of the groups MN with the system Ss was of considerable significance. The conclusive force of the exclusion of paternity by MN analyses is now incontestable, whereas there still exist doubts in respect of the Ss system, evidently owing to the fact that the number of family studies concerning the characters S and s is as yet insufficient.

P system. Up to 1955 it seemed that one was dealing with a one-factor system, but the discovery of the antibody anti-Tja^a and the factor P^k has complicated matters so much that, at present, there are only conjectures regarding the phenotypes and genotypes of this system. Besides, its usefulness for the purposes of forensic medicine is greatly impaired by the fact that the intensity of the P receptors is subject to fluctuations (especially influenced by storage) and also by the fact that these receptors are immature in the newborn.

Rh system. That this system has achieved a top position in human genetics is mainly due to its practicableness. Yet, nowadays there are not many persons even among specialists who have a full command of all problems connected with the application of the system. One of the principal difficulties consists in the nomenclatorial discrepancies which present a true mirror of the contradictory conceptions regarding the genetic correlations between factors that can be determined by test sera. Notwithstanding differences in the matter of nomenclature and in that of the genetic principles regarding the transmission of Rh groups (WIENER's one-gene theory and the multiple-gene theory of FISHER—RACE), practice is now uniform as regards the performance of Rh examinations because both FISHER and RACE postulate a linkage of genes which is so close that always "triple" Rh genes are inherited that produce but a one-gene effect. LAURER, the eminent Hamburg serologist, recently advanced a new nomenclature with the following six genes: Ce, cE, ce, CE, C^we and C^wE.

Of the variants of Rh antigens, discovered so far, C^w and Du are important for legal medicine. The first is an allelomorph (C^w — or, according to WIENER's terminology, R^{1w}) on the locus C, and the second is a weak D factor. Both can be demonstrated with adequate methods and suitable sera.

Also Rh antibodies have been found which react with known Rh antigens provided their genes are closely connected. The antigen G(rh^G), for example, yields positive results with anti-CD sera but fails to do so if brought together with pure anti-C, -D and -E sera. Anti-G(CD) is no mixture of antibodies but a specific antibody against the antigen G. The discovery of various antibodies of a similar nature (CcEe = Hr_o; ce(f) = hr; Ce = rh_i; cE = hr_i) has raised fresh genetic problems. Bloods lacking genes (e.g. -D- or -d- and ---, as also numerous new variants) are of especial interest in this connection. These discoveries do not fit the inheritance patterns of FISHER-RACE and seem to require the postulation of new allelomorphs.

Hp system. By the employment of vertical (instead of the usual horizontal) starch gel-electrophoresis (after preliminary treatment) it was recently ascertained that the gene product H_p 1 is no homogeneous substance but consists of three separate parts, each with its own autonomous genes which would mean that there exist six phenotypes H_p 1. If the technique involved can be simplified, the armentarium of medicol-egal serology will have gained a new valuable tool.

Gm system. This system has become very useful during recent years and, — as regards genetic composition — is almost as complicated as the Rh system. Factors (a) and (x) of the system mean no problem; difficulties exist, however, in respect of factor (b) which is not homogeneous. — InV, that was a one-factor system a few years ago, has developed into a complete four-gene one since then (Inv^1, Inv^a, Inv^b and Inv).

The serum-group Gc, a recent addition to the armentarium of medico-legal

serology, is now gaining ground in serological research work, although its technique is complex and laborious.

The full exploitation of all new possibilities in the field of serological genetics encounters certain limitations. (a) The acquisition of the required test sera may be difficult. (b) Shortcomings, be they of a personal or a technical nature, may impede the use of new methods. (c) There may be a disproportion between the sums to be invested and the results to be reasonably expected.

As regards particular conditions in Hungary, it must be borne in mind that the economicalness of the adoption of new procedures depends to a great extent on whether the necessary test sera can be obtained on the home market or have to be imported. Only the sera required for AB0 and MN examinations are manufactured in Hungary, neither do Hp examinations require imported material. Since these three tests are recognized in Hungary as sufficient for the reliable exclusion of paternity, examination of parentage is based on them in our judicial practice. As regards the question as to how many systems should be included in the examinations, i.e. how many kinds of tests should be made in parternity cases, we have to remember that — once paternity has been exclused out by even a single absolutely reliable test — no further tests are really needed. As far as chronological order is concerned, examinations should always start with the classic blood groups and continue with the blood groups MN. The next steps depend on the special conditions of each given case. If, for instance, paternity has been exclused on the evidence of the AB0-test (or possibly on that of the MN or Hp tests), no further tests are necessary. As regards subgroups A1/A2, the AB0-test should, in our opinion, be accompanied by the determination of these subgroups as it is very useful in the establishment of paternity and, besides, its omission means the disregard of a one per cent possibility of exclusion.

Still further tests are necessary (a) if the preceding ones have still left the possibility of paternity open; (b) if there are several putative fathers and the paternity of at least one is still possible; (c) if the object of the test is not merely the exclusion but also the possible establishment of paternity.

Examination of the serum-group system Hp should be the third step, in the first line on account of the simplicity of the technique involved, and also because of the strong conclusive force of its evidence. Examination of the Rh group is recommended as the fourth measure, and it should extend to the antigens D, C, c, E, e. In order to economize test sera, the antigen C^w should only be examined if the test is expected to yield a clearly positive or negative result. If necessary, the so-called genotype sera (anti-C^e, anti-f) should be applied. If the available technical equipment prevents the performance of the Hp-test, its place in the sequence should be occupied by the examination of the Rh group. As fifth examination, we suggest that of serum-group Gm [first the factor Gm(a) and then the factor Gm(x)].

The performance of these examinations affords a 67 per cent chance of excluding paternity (i.e., a non-father has a 67% chance of proving his nonpaternity). In other words, the above tests do not suffice for excluding the paternity of about one third of the non-fathers.

As regards now the efficiency of blood-group serology in the matter of *establishing parentage*, the maximum we can attain with the present methods is, on an average, a 90 per cent chance of exclusion. To reach a percentage of this height would involve much superfluous work because, after all, genuine fathers are involved in about two thirds of the cases whose paternity cannot be excluded anyhow. Since it happens in some 33—34 per cent of the cases that non-genuine fathers are accused, further, since the five blood-group systems enumerated above allow the exclusion of paternity in 27—28 per cent, there remain only 6 to 7 per cent where still further blood-group tests may present the chance of ecluding paternity. Of course, this comparatively low percentage does not justify the neglect of efforts to exonerate non-fathers from the charge of paternity. At even such low percentage the decision of Hungarian courts is unfavourable to about 90 to 100 defendants per annum, mostly on the evidence of blood-group tests which do not definitely rule out the possibility of paternity.

It seems best to resort to the calculus of probabilities in the matter of possible further measures. Additional examinations should only be made if the results of all preceding tests point to the improbability of paternity. At this stage the *method of the indirect exclusion of paternity* may be employed. As is known, phenotype is not always identical with genotype, and one phenotype may include several genotypes. For instance, a person of group A may have the phenotype AA or A0; again, if subgroups A_1/A_2 are taken into account, an individual of group A_1 may have the genotypes A_1A_1; A_1A_2 and A_10. The same principle applies to the blood-group system Rh: an individual of phenotype C + D + E — c + e + may have the phenotypes CDe/cde, CDe/cDe or cDe/Cde (abbreviated, R_1r, R_1R_o, R_oR'). It means that the possibilities of excluding paternity are limited in these blood-group systems, since present serological methods do not allow a direct determination of the genotype in such cases. The determination of the actual one among the possible genotypes is only possible in an indirect way, i.e. by extending the range of persons to be examined to the accused person's (or persons') consanguineous kinspeople, in the first place to his (their) parents and possibly even to the collateral relatives and descendants. The fact that, by knowing the gene frequency of the various blood-group systems, it is possible to compute the percentage of possible genotypes within each phenotype means a great facility. It is, for instance, known that in Hungary the incidence of A0 amounts to about 80 per cent and that of AA to about 20 per cent in group A. Again, in the case of Rh, the frequency of genotype R_1r within the phenotype is 93 per cent and that of R_oR' about 0.2 per cent. That such indirect methods are still not sufficiently employed is due to that the search for

and the presentation of the necessary persons are laborious and expensive, and also because the tests are not always successful.

Another method is the determination of ESSEN-MÖLLER's so-called paternity index, i.e., the degree of the probability of paternity. It is obvious that an improbability index exceeding 90 per cent will have much weight with the courts.

The following additional examinations might be carried out.

Examination of the other factors of the Gm system. Test sera for the demonstration of factors Inv (1) and Gm(b) are available. Examinations concerning the system MN may be combined with those of the *system Ss.* Test sera are available also in this respect. Also the examination of the *system Gc* is now possible in Hungary.

Tests in respect of other blood and serum groups not mentioned in the foregoing, while feasible in exceptional cases, are still at the experimental stage and their significance is more scientific than practical. Besides, the courts are unlikely to base their decisions on the evidence of these tests.

To sum up: when making use of serological genetics in paternity cases, (a) we should apply the results of scientific research work in a manner that is suited to existing conditions; (b) the results of serological tests should be mathematically evaluated; (c) medico-legal expert opinion should be based also on the mathematically evaluated results of anthropologico-genetic analyses.

M. Fehér

Blood Group Incidence and the Probability of Exclusion in Paternity Suits

In the question of paternity the blood group systems will decide the issue in part of the cases only. The probability to exclude paternity greatly depends on the incidence of the given blood group; it is obviously greater, if the child's blood shows some rare quality not contained in the maternal line. Usually, however, common hereditary qualities are only found and exclusion of paternity depends on the incidence of the blood groups, in the population. The maximum number of non-fathers equals the fifth power of the gene frequency. Therefore the probability of paternity exclusion has to be calculated separately for each population area. The results of blood group tests performed in 43,000 persons have been worked up by statistical methods to establish the incidence of the blood groups and the probability of paternity exclusion in Hungary.

E. Horváth, B. Rex-Kiss

(National Blood Bank and Institute of Forensic Medicine, University Medical School, Budapest)

Gc Serum Group in Paternity Cases

Classification and incidence in Hungary of the Gc serum group types have been studied. Among 1000 persons subjected to examination the distribution was Gc 1—1, 48 per cent; 2—1, 44 per cent; 2—2, 8 per cent. Their value in respect of the probability to exclude paternity is approximately 16 per cent, nearly as high as that of the MN or Hp systems. In 100 legal cases, in which they were examined together with the A_1A_2B0, MN, Rh, Hp and Gm groups, the Gc finding allowed to exclude paternity in 12 cases. In 9 cases the results coincided with those yielded by blood groups but 3 were established on the basis of Gc examination alone.

P. Guth, R. Budvári

(Institute of Forensic Medicine, University Medical School, Budapest)

Serology of Beta-Lipoprotein Type Isoprecipitins

Routine tests have been carried out with anti-Lp(a) serum. The results of these examinations are described with special regard to the distribution of Lp(a) properties in the population and the maximum frequency of exclusion achieved by their use in paternity proceedings.

The discovery of precipitating iso-antibodies in the serum of a four-month old infant previously subjected to blood transfusion, calls attention to the prevention of transfusion complications in infants and to the possibility of foeto-maternal diaplacental immunization.

Edit Birtha, B. Rex-Kiss

(Institute of Forensic Medicine, University Medical School, Budapest)

Significance in InV Gamma-Globulin Serum Groups in Paternity Cases

The incidence in Hungary of serum InV gamma-globulin system have been studied in 3300 persons, regardless of sex and age. An incidence of 12.1 per cent has been established. In 21 of 820 lawsuits paternity was excluded on the basis of the InV(1) factor; in 10 cases it was solely this test which decided the issue. The probability of paternity exclusion by the method in question amounts to approximately 1.3 per cent.

L. Fésűs, B. Rex-Kiss

(University Veterinary School, and Institute of Forensic Medicine, University Medical School, Budapest)

Incidence of Serum-Transferrin Types and their Significance in Paternity Cases

Kristjansson's modified method (1963) of Smithies's starch gel electrophoresis (1955) has been used for the determination of serum transferrin types in 1000 individuals of various age, sex and nationalities. Two stripes corresponding to the Tf^C allele have invariably been present in every case; but Tf^B bundles occurring together with Tf^C types in the heterozygote form were identifiable in 5 persons. Thus incidence of the Tf^B allele in Hungary can be estimated at 0.5 per cent. The significance of serum transferrin types in paternity proceedings is discussed.

B. Rex-Kiss

(Institute of Forensic Medicine, University Medical School, Budapest)

Evaluation of Rh-Tests in Paternity Suits

Rh-tests performed in 1683 lawsuits over three years allowed the exclusion of paternity in 171 cases (16.1 per cent), among which 156 (9.3 per cent) were established alone on the basis of this test. In 222 cases out of the 271 they furnished absolute evidence, in the remaining 49 only a high degree of improbability. Each of the latter fell under the C+c+D+E+e+Rh phenotype which does not make it possible to establish the genotype by any direct method. A great advantage in these cases is the use of chromosome sera, particularly of anti-C^e, which allow to determine the presence or absence of the most important genotypes. Equally advantageous is the C^w-factor test which has been employed in 50 cases and which alone furnished sufficient evidence for paternity exclusion in 5 cases.

E. Somogyi, B. Rex-Kiss

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Lethal Hemolytic Complications of Blood Transfusions

Postmortem serologic examinations carried out in cases of blood transfusion leading to lethal complications are surveyed. The blood was subjected to AB0, MN and Rh blood group tests and antibody determinations. AB0 or Rh-incompatible blood had been transfused in each case in consequence of an error in the blood group determination and in the compatibility test, or the neglect to perform them, and to take the history duly into consideration. Attention is called to the importance of studying the patient's history and the documentation of the blood group tests prior to transfusion.

L. Szabó, B. Rex-Kiss

(Institute of Forensic Medicine, University Medical School, Budapest)

Blood Group Test Evidence in Disputed Paternity without Examination of the Mother

The possibility of paternity determination on the basis of blood group tests in cases when the mother cannot be subjected to examination is discussed. The probability of exclusion of paternity in such cases is 64 per cent of that when the examination can be extended to the mother.

In 60 paternity proceedings without maternal blood tests, paternity could be excluded in 14 cases.

B. Rengei

(Institute of Forensic Medicine, University Medical School, Szeged)

Application of Native Protein Immunized Antiserum in a Case of Irregular Death

A blind and deaf-mute man, aged 73, suffered a grave traffic accident and was brought to hospital where, after 6 days of unconsciousness, he succumbed to cerebral changes and pneumonia. The postmortem examination identified in the laryngeal port two inwedged pieces of meat, as the direct cause of suffocation death. Attempts at discovering their origin by means of native protein immunized antiserum were made by the immune precipitation method of Ochterlony' agar gel diffusion. None of the observed reactions indicated that any protein of animal origin was present and the reaction characteristic of human origin was the only one to furnish positive result. In spite of this finding, the human origin of the test samples remained questionable, partly because the necropsy failed to discover any injury in the mouth, throat or pharynx, and partly on the consideration that obviously some specific proteins of human origin may have left their traces on the test samples. Evidence in favour of animal origin, on the other hand, was doubtful because of the very obvious probability that cooking heat had denaturized proteins while it turned raw meat into eatable food. Model experiments, to study the effect of this heat factor on specific proteins, have been carried out with the meat of various animals fresh from the abbatoir. In physiologic salt solutions of equal proportion, these samples were exposed for 0—72 and 0—8 hours to the action of heat at 37, 56 and 100 °C and then subjected to immune precipitation tests of agar gel diffusion. Each sample of the 37 and 56 °C test series yielded precipitation even after 72 hours of exposure, while those of the 100 °C series were found negative already after half an hour of cooking.

Applying these results to the case under review, it was impossible to find out the origin of the meat samples because of protein denaturization in the course of cooking. The suggestion is made to use also heat-denaturized antiserums for medico-forensic purposes, as that type of agent is able to precipitate proteins both in native and in heat-denaturized state.

Gy. Gerencsér

(Criminal Laboratory, Police Headquarters, Budapest)

Mixed Cell Agglutination, a New Method of Blood Stain Examination

The knowledge about blood stain diagnostics dates back nearly to the discovery of blood groups and has developed parallel with blood group serology. Its criminalistic significance has been realized as early as 1903 and blood stain identification methods have been in use since 1923; nevertheless it was only in the past decade that they have furnished valuable criminalistic procedures.

More recent demands have led to the elaboration of new methods, ranging from receptor identification over inhibited agglutination (or absorption) to elution; but lastly the elution (or extraction) method proved to be the only one that really met the requirements of medicolegal practice. The mixed cell agglutination method worked out by COOMBS et al. in 1956, has become by now an efficient procedure in criminalistic practice.

S. Ökrös

(Institute of Forensic Medicine, University Medical School, Budapest)

Electron Microscopic Examination of Thrombocyte Thrombus

The thrombocyte thrombus signals a vital damage of the heart and arteries. Following histologic location a sample of the substance has been subjected to electron microscopic examination. In collaboration with Dr. D. SZABÓ and I. ÖKRÖS, the structure of the thrombocyte sack was studied. It was found to consist of aggregated necrosed thrombocytes, surrounded by a massive, reticular fibrin capsule which emitted fine filaments towards the inside of the sack in between the thrombocytes. In the alpha, beta and gamma granulomeres of the thrombocytes, in the hyaloplasm and in the membrane covering the thrombocytes, there were conspicuous changes which might furnish some basis for clarifying the fibre mechanism of fibre development.

E. Somogyi, Gy. Rózsa, P. Sótonyi, T. Varga, Á. Nevelős

(Institute of Forensic Medicine, University Medical School, Budapest)

Changes in the Fine Tissue Structures after Electric Shock

The effects of electric shock have been studied in human corpses, skin and muscle parts obtained from surgery, as well as animals exposed to electric shock of predetermined character. The results have called the attention to some hitherto unnoticed phenomena.

Gy. Rózsa, E. Somogyi, P. Sótonyi, T. Varga, Á. Nevelős

(Institute of Forensic Medicine, University Medical School, Budapest)

Nuclear Changes Following Electric Shock

The cell nuclei of various tissues have been subjected to morphological, histochemical, polarization and fluorescent optical electron microscopic and cytophotometric studies. They presented significant alterations of a similar nature, the most pronounced having been an elongation of the nuclei with specific lesions of the membrane, derangement of the chromatin structure, dehydration of the nuclear substance and depolymerization of DNA.

P. Sótonyi, E. Somogyi, Gy. Rózsa, Á. Nevelős, T. Varga

(Institute of Forensic Medicine, University Medical School, Budapest)

Myocardial Changes due to Electric Shock

The effect on the myocardium of direct exposure to electric shock has been studied by routine histological, histochemical, phase contrast, fluorescent, polarization microscopic and electron microscopic methods.

Attention is called to the phase in which the histochemical reactions became weak or ceased, injury of the membranes was shown by polarization microscopy and various mitochondrial lesions by electron microscopy, while light microscopy failed to reveal any kind of alteration.

T. Varga, E. Somogyi, P. Sótonyi, Gy. Rózsa

(Institute of Forensic Medicine, University Medical School, Budapest)

Skeleton Muscle Changes due to Electric Shock

The effect on the skeletal muscles of electric shock and the resulting marks have been studied in the rat and the guinea pig by routine histological, enzyme histochemical, polarization optical, phase contrast and electron microscopic methods.

The observed changes consisted in a gradual disappearance of the enzyme reactions parallel with the structural modifications, furthermore in membrane damage. The gravity of the lesions was in close relationship with the intensity of electric shock.

Á. Nevelős, E. Somogyi, Gy. Rózsa, P. Sótonyi, T. Varga

(Institute of Forensic Medicine, University Medical School, Budapest)

Epithelial and Connective Tissue Changes Following Electric Shock

Epithelial and connectiv tissue changes caused by electric shock have been examined by routine histological, enzyme histochemical, polarization, phase contrast and fluorescent microscopic as well as electron microscopic methods in human material obtained at surgery and in specimens of rats and guinea pigs exposed to electric shock. The gravity of the changes, ranging from characteristic structural alterations to complete homogenization, was closely connected with the intensity of the shock.

L. Buris, M. Szabó, Klára Zsigmond

(Institute of Forensic Medicine, University Medical School, Debrecen)

Histochemical Examination of Current Marks

The marks inflicted by electric current have the same gross and microscopic appearance as those arising under thermal effect. An apparently suitable method for their differentiation is to demonstrate the imprint of the metal conductor in the damaged area. In specimens obtained from animals subjected to electric shock from postmortem material gross chemical demonstration of the most frequent three types of metal conductor has been performed, using Prussian blue for iron, a saturated aqueous solution of alpha benzoinoxim for copper, and 0.2 per cent alizarin S for aluminium.

Microscopic examination has been carried out with Berlin blue reaction for iron, Na diethyl-dithiocarbamate and rubeonic acid for copper, finally an 0.2 per cent aqueous solution of morin and alizarin S for aluminium. The method has successfully been employed in doubtful cases.

L. Harsányi, Margit Kovács

(Institute of Forensic Medicin, University Medical School, Budapest, and Criminal Laboratory, Police Headquarters, Budapest)

Vital Reactions of Bones Tested by Histochemical Methods

The vital reactions of rat bones have been studied.

Leontin Jegesi

(Institute of Forensic Medicine, University Medical School, Budapest)

Traumatic and Non-Traumatic Lesions of Skeletal Muscles

The morphological and structural changes conditioned by age and morbid state of various skeletal muscles have been studied and compared with traumatic lesions of the same sites. On the basis of characteristic changes in the working and supporting muscle elements, some views are offered concerning the mechanism of injury in various types of muscle.

F. Kósa, I. Gy. Fazekas, A. Basch

(Institute of Forensic Medicine, University Medical School, Szeged)

Elongation and Tensile Strength of Human Skin from Various Body Regions

The expert examining an injury on a living subject or a corpse is able within wide limits only to determine the size of the force (as weak, medium or strong) that had caused the wound. Neither does literature offer such data for the strength of an impact required to tear the human skin.

Specific elongation and tensile strength of skins from 121 human corpses have been examined by means of an electric tensile strength apparatus with automatic recording device.

The specimens taken from the hairy scalp, anterior part of the neck, middle chest, heart region, abdomen, back, buttocks, upper arm, thigh, forearm, tibial area, were divided according to age into groups of ten years (from 3 months to 83 years of age) and according to sex (71 males, 50 females), and were tested 6 to 72 hours after death.

The test revealed that the tensile strength varies according to sex, age and body region, owing probably to the unequal proportion of collagen fibres to elastic fibres. Skins of children and females are on the whole less tenacious and tear under the impact of a smaller force than of males, up to 60 years of age; but past 60 the sex difference vanishes, owing probably to gradual atrophy with advancing age.

As to actual values, the dorsal skin presented the highest tensile strength (30—40 kg per 1 cm width) and the lower specific elongation (0.20—0.25 $\sim$ 20—25 per cent). Lower was the tensile strength (15—25 kg per 1 cm width) and much higher the specific elongation (0.40—0.60 $\sim$ 40—60 per cent) on the frontal surface of the body.

L. Takácsy, Gy. Gorácz, Erzsébet Bellus, I. Nagy

(Institute of Forensic Medicine, University Medical School, Budapest)

Myocardial Electrolyte Levels in Cases of Sudden Cardiac Death

Various parts of the myocardium (auricle, atrium, ventricle) from subjects who had died of cardiac arrest were studied and the K, Na and Ca contents were estimated in homogenized samples, in order to clear eventual connections between the two findings.

The material consisted of cases where the gross and microscopic findings did not sufficiently reveal the cause of cardiac arrest.

Explanation was mainly sought for the question why the sudden death had occurred just at the given point of time.

N. Kapusz

(Institute of Forensic Medicine, University Medical School, Debrecen)

Traumatization and Lipase Activity

The development of fat embolism and the daily changes in serum lipase activity have been studied in patients who had suffered general concussion, fractures of long bones, and decollement in traffic accidents. Patients suffering from diseases which involve an increase of lipase activity were excluded.

Disturbances in the lesser circulation were associated with an increase in lipase activity, the normal level of which is between 0.5 and 1.5 ml n/50 NaOH.

On the 3rd to 5th day following the injury the lipase value was 2.8—4.5 ml n/50 NaOH in cases of long bone, tibial and thigh fractures, and 1.9—3.7 ml n/50 NaOH in cases of general concussion, decollement and flat bone fracture.

The study of lipase activity, when diseases involving an increase of such activity are excluded, facilitates the clinical diagnosis of fat embolism, and accordingly facilitates the prescription of the treatment.

M. Szabó, L. Buris, Klára Zsigmond

(Institute of Forensic Medicine, University Medical School, Debrecen)

Hexosamine Level in Pulmonary Tissue

Pneumonia is a frequent cause of sudden death; it is, however, difficult to diagnose it, especially from lungs exhumed or in the state of decay. In such cases also the histological findings are often of questionable value.

The purpose of the present study was to elaborate an examination method which would facilitate the diagnosis of pneumonia in such cases. Data from the earlier literature indicate that the hexosamine level rises in inflammatory processes. In the present experiments the hexosamine level has been estimated in pulmonary tissue of subjects who had died with bronchial or lobar pneumonia, and of survivors of traffic accidents, as the controls. Histological studies were carried out in parallel and also after four days' putrefaction at room temperature.

The modified Elson-Morgan method yielded in average hexosamine value of 150 to 250 mg per 100 g in the normal and 350 to 500 mg per 100 g in the pneumonic lungs. The results were not influenced by the process of decay.

T. Krompecher, Éva Kiss, N. Kapusz

(Institute of Forensic Medicine, and Department of Anatomy, University Medical School, Debrecen)

Bone Regeneration under Chronic Alcohol Treatment

After fracturing the femur of rats and uniting it by transcutaneous medullary nailing, the animals were treated with 0.16 to 0.426 g per 100 g body weight of ethyl alcohol daily, while the controls were treated with egg-shell powder and the bones were examined after the 1st, 3rd and 5th weeks.

Young cartilaginous cells in the newly formed bone indicated that the ossification process advanced farther in the alcohol treated animals than in the controls.

The action mechanism may be conditioned by the peripheral vasodilator effect of alcohol as well as by the by-products of the alcohol decomposition process which increase tissue respiration.

I. Gy. Fazekas, Erzsébet Virágos Kiss

(Institute of Forensic Medicine, University Medical School, Szeged)

Free and Total Histamine in Various Skin Areas

In an earlier study it was shown that the cervical groove contains more free histamine than intact cervical skin if the person had died of hanging, but the same or nearly the same amount of free histamine if he had been hanged after death. Hence the quantity of free histamine in cervical skin may be considered a vital reaction. In view of the lack of data concerning the free and total histamine content and its postmortem changes in the various skin areas, the question has been subjected to a closer investigation.

Specimens of the hairy scalp and skin from the neck, upper arm and thigh, taken from 100 human corpses, 34 females and 66 males between 2 and 93 years, were studied at 1 to 72 hours after death. The highest total histamine value was found in the scalp (8—25 μg per 100 g), less in the neck (5—22 μg per 1 g), upper arm (5—20 μg per 1 g) and thigh specimens (5—20 μg per 1 g).

The free histamine content in all the four types of specimen was found to increase with the length of time elapsed since death. In the first 5 hours no free histamine was found. Later the values were as follows: 6—17 hours — scalp, neck and upper arm 0.0—0.7 μg per 1 g, thigh 0.0—0.5 μg per 1 g; 18—24 hours — scalp 0.0—0.8, neck 0.0—0.7, upper arm 0.0—0.6, thigh 0.0—0.5 μg per 1 g; 25—30 hours — scalp 0.0—2.9, neck 0.0—1.2, upper arm and thigh 0.0—0.9 μg per 1 g. After 30 hours, free histamine increased more and more rapidly in the various skin areas. *31—40 hours* — scalp 0.3—4.1, neck 0.2—3.7, upper arm 0.2—3.4, thigh 0.2—1.4 μg per 1 g; 41—48 hours — scalp 0.5—9.3, neck 0.1—6.0, upper arm 0.1—7.3, thigh 0.1—8.4 μg per 1 g; 50—72 hours — scalp 1.6—16.4, neck 1.3—16.1, upper arm 0.3—16.0, thigh 2.0—16.0 μg per 1 g.

The differences according to sex and age in free and total histamine content, in the same skin area were not significant. Neither was it of significance, whether death had been due to suffocation, heart failure or cerebral hemorrhage. High values for free histamine (5.3—7.8 μg per 1 g) were revealed in cases of scalding, and low values (0.0—0.6 μg per 1 g) when loss of blood had been the cause of death.

J. Nagy

(Institute of Forensic Medicine, University Medical School, Debrecen)

Postmortem Reaction of Skeletal Muscles to Electric Stimulus, as an Indicator of the Point of Time of Death

By means of an electric stimulator, furnishing five adjustable steps of voltage it could be shown that human skeletal muscles are able to respond to the stimulus for 6 to 8 hours after death. Persistance of reactibility varied with the different muscles and was longest with the facial muscles, especially those of the eyelids. Thus the time of death could be determined in the following 6 to 8 hours, with a half hour margin of error. It is recommended to test the electric irritability of skeletal muscles at the scene of death in every case.

Ágnes Major, P. Quittner, E. Szabó

(Criminal Laboratory, National Police Headquarters and Central Institute of Physical Research, Budapest)

Identification of Tracer Element Impurities in Hair Specimens by Means of Neutron Activation Analysis

Kind and quantity of tracer element impurities in human hair vary from person to person and are characteristic of the individual. Neutron activation analysis allows the quantitative demonstration of tracer elements without destruction of the hair.

Following irradiation of the specimen in an atomic pile, the contained tracer elements can be determined qualitatively and quantitatively by measurement of the induced radioactivity. The method has been in regular use for medico-legal purposes.

Hair introduced through pneumatic pipes into the atomic pile were exposed for 3 minutes to irradiation by a flux of 10^{13} neutron/cm^2/sec whereupon the energy spectra of the produced gamma radiating isotopes have been measured with a scintillation detector and a 256-channel amplitude analyzer. In this way it was possible to identify a number of elements in the hair.

D. Horváth

(Pharmacy, University Medical School, Pécs)

Rapid Demonstration of Phosphate Esters

A rapid chemical method has been worked out for the demonstration of organic phosphate ester type compounds, especially in poison residues. The intention was to offer a uniform method to deal with every type of compound containing this group.

The following model substances were used: ethylparathion, as well as two compounds used also in human therapy, *viz.* dyflos and paraxon.

G. Jóna, J. Nagy, I. Szabó

(Institute of Forensic Medicine, and Department of Radiology, University Medical School, Debrecen)

Medico-Legal Aspects of X-Ray Examinations with Contrast Media

Between January 1, 1963, and December 31, 1965, more than 10,000 examinations have been performed with iodine-containing contrast materials. Following a survey of the various examinations and the subsequent complications (none of them lethal), the medico-legal aspects of the problem and the means of prevention have been discussed.

Attention is called to the importance of having at hand the drugs and instruments needed in these cases.

Z. Dézsi, I. Szabó

(Department of Radiology and Institute of Forensic Medicine, University Medical School, Debrecen)

X-Ray Absorption by Some Poisoning Compounds

The X-ray absorbing capacity of various poisoning compounds has been studied by densitometry.

By means of suitable X-ray exposures it was possible to diagnose certain types of poisoning (e. g. arsenic) *in vivo*, and to demonstrate postmortem the presence of a larger amount of poison following rapid death.

I. Szabó, A. Simai, L. Deli

(Institute of Forensic Medicine, and Department of Radiology, University Medical School, Debrecen)

Radiologic Examination of Pulmonary Shock Induced by Benzine

Benzine administered intravenously induces grave pulmonary shock in the dog and leads to death in 1 to 5 minutes. Pneumoangiocardiography and bronchography revealed a spasm of pulmonary capillaries, venous reflux in the greater veins and dilatation of the right heart.

The angiocardiographic patterns of benzine poisoning and of asphyxial death have been found essentially the same. Bronchiography demonstrated bronchial spasm.

Gy. Farkas, A. Varró

(Institute of Forensic Medicine, University Medical School, Pécs)

A Few Aspects of Gas Chromatography Tests

A gas chromatograph fitted with a thermal detector of the Willy Giede GCHF 18 type has been used for the determination of the blood alcohol level, of the presence of anaesthetics in the blood of narcotized patients, furthermore of certain toxicologic substances. The influence upon the final result of certain details of the procedure such as the preparation of specimens, quality and length of the column charge, temperature, carrier gas quantity, pressure conditions, etc. has been discussed.

Klára Zsigmond, J. Nagy

(Institute of Forensic Medicine, University Medical School, Debrecen)

Some Topical Questions in Connection with Alcohol Probe Examinations

The results of practical tests with the alcohol probe performed in 1965 and experiments in connection with the tests are reported. The question is raised of the possibility to omit the blood test whenever certain specific shades of colour have made their appearance. Some pertaining problems are discussed with particular reference to the repeated use of the same probe if the first test yielded a negative result.

I. Gábor, Erzsébet Bellus, L. Tolnay

(Institute of Forensic Medicine, University Medical School, Budapest)

Comparative Examination of Alcohol Level in Blood, Urine and CSF

Postmortem estimations of the alcohol concentration in blood, urine and CSF were performed in order to get information on the absorption and excretion of alcohol at the time of death. Simultaneous blood and urine alcohol tests failed to furnish reliable information as to the ingested quantity of excreted amount of alcohol.

In cases when considerable loss of blood, lack of urine, etc., make it impossible to determine the amount of alcohol in these body fluids the CSF alcohol level may serve as an indicator of the degree of alcoholic intoxication.

S. Zalányi

(Institute of Medical Organization, University Medical School, Szeged)

A Survey of Agricultural Accidents in County Csongrád, with Reference to the Influence of Alcohol

A review of nearly 3000 records of accidents during farm work in County Csongrád in the period 1962—1964, has raised some doubt about the small percentage of alcoholic influence indicated by the reports. Comparison with the data of traffic accidents and the results of alcohol probe tests recorded in the same area suggest the assumption that alcoholic intoxication must play an important role in agricultural accidents.

Éva Bertók, I. Gy. Fazekas

(Institute of Forensic Medicine, University Medical School, Szeged)

Skin Catalase Activity of Rat under the Influence of Alcohol

The Feinstein perborate method was used to estimate catalase activity in the skin of intact male rats of 200—250 g weight, and of animals treated subcutaneously with 0.8 g per 100 g of 20 per cent alcohol every hour.

In the treated group skin catalase activity was higher by 242 per cent after 1 hour, 137 per cent after 2, 116 per cent after 3, 100 per cent after 4, 80 per cent after 5 and by 50 per cent after 6 hours, than in the untreated controls. Blood catalase activity also increased.

In earlier studies on humans and rats under the influence of alcohol, blood catalase activity was found to increase together with the blood corticosterone and hydrocortisone levels. On this basis the increased skin catalase activity following alcohol injection is ascribed to a more intensive functioning of the adrenal cortex. The finding seems to confirm the observation that chemical stimulation of adrenocortical function leads to an increase of the skin's biological function by raising its catalase activity.

L. Nagy, Klára Zsigmond

(Institute of Forensic Medicine, University Medical School, Debrecen)

The Effect of Adrenalin on the Blood Alcohol Level

Alcohol was administered to human subjects and test animals and after the diffusion balance had set in, adrenalin was administered in order to study its effect upon the course of the blood alcohol curve.

The human experiments were performed with an oral dose of 0.3 g per kg of alcohol in the form of brandy. After the diffusion balance had established itself, blood was taken at half-hour intervals over 280 minutes. At 2 hours 1 mg of adrenalin was injected subcutaneously and then blood samples were taken every 5 minutes over one half hour. The adrenalin effect observed in humans presented itself as a rapid rise of drop of the blood alcohol level, to an extent not exceeding 0.03 per cent in either direction.

In dogs, adrenalin caused unambiguous significant changes.

The rapid changes in the blood alcohol level under the influence of adrenalin are ascribed to alterations in the intravasal fluid volume.

V. Földes, I. Kenyeres, Gy. Gerencsér

(Police Headquarters, Criminal Laboratory and Institute of Forensic Medicine, University Medical School, Budapest)

Imbibition of the Eye and of the Vitreous Body as an Indicator of the Time of Death

One of the most important things for the medico-legal expert is to determine the age of the corps he has to examine, i.e. the period of time that has passed since the death. The purpose of the present study was to discover some cadaveric sign, suitable as a criterion for how long the body has been dead and simple enough for the postmortem examiner to record it.

In 100 examinations of cadaveric eye lenses and vitreous bodies, the following interrelations have been found to exist between the state of putrescent transudation of these organs and the age of the corps:

1. Increased tension, spherical shape and faint yellowish-green transudation of the eye lens, with limpid or scarcely transudated vitreous body, indicate that one week at the most has elapsed since the death.

2. Moderate tension, yellowish-green or faintly scarletish-brown transudation of the eye lens and slightly scarletish transudation of the vitreous body were usually found in the 2nd week after death.

3. Reduced tension, flattened shape, scarleti shbrown transudation of the eye lens and homogeneous transudation of the vitreous body were observed in the 3rd week after death.

A medicol-legal estimation of these changes in the eye lens and vitreous body, considered together with other cadaveric criteria, furnish a reasonably good ground for a simple and fast judging of the period of time that has passed since the death.

L. Kiss, V. Kovács

Medico-Legal Centre, and Institute of Forensic Medicine, University Medical School, Budapest)

Fingerprint Pattern in Twins

The types of ridge pattern and the secondary characteristics of fingerprints have been studied in 31 pairs of twins and their parents. Both parents were known in 22 and only the mother was known in 9 cases. Of the 22 cases, 7 appeared to be identical male twins, 6 identical female twins, while 9 were binovular male-female twins. In the 9 *sub-judice* cases (i.e. where the father was uncertain), the corresponding distribution was 3—3—3. No difference was found between twins and the controls (non-twin offspring) as regards fingerprint pattern and secondary characteristics of impression, nor did monozygotic and binovular twins differ in this respect.

Since the examined 31 families do not suffice for drawing definitive conclusions, further investigations are in progress.

M. Bobest

(Institute of Forensic Medicine, University Medical School, Pécs)

The Medico-Legal Significance of Amniotic Fluid Embolism

Amniotic fluid embolism associated with afibrinogenaemia, can sharply be differentiated from the other forms of shock during delivery.

The case of a 37-year-old multipara is reported who shortly after delivery died from amniotic fluid embolism associated with afibrinogenaemia. The conclusion is drawn that in similar cases of sudden death during or after childbirth, the clinical picture, postmortem examination and mainly the histological findings present a safe basis for the reliable determination of the cause of death.

Ö. Szedlák, I. Artner

(Department of Public Health, Ministry of the Interior, Budapest)

Medico-Legal Aspects of the Period of Recovery from Concussion of the Brain

Insufficient and erroneous recording of clinical symptoms as well as the inadequate conclusions drawn from them often give rise to difficult medico-legal problems in connection with brain concussion. Shortcomings of this kind influence expert opinion and delay the termination of judiciary proceedings. The main points arising in connection with the recovery period are discussed according to the following points.

1. Brief survey of the pathology and symptomatology of concussion of the brain.
2. Medico-legally important points concerning the origin of concussion.
3. Discrepancies and controversies concerning clinical findings in concussion of the brain; their analysis based on expert opinions of the Supreme Medical Council, on those of medical examiners, and on judicature.
4. Suggestions regarding expert opinions concerned with the duration of recovery after concussion of the brain.

L. Veress, I. Gy. Fazekas

(Institute of Forensic Medicine, University Medical School, Szeged)

Diagnosis and Medico-Legal Opinion in Connection with Concussion of the Brain

Uncertain and mostly subjective symptoms, their variability, the possibility of the diagnosis being misused, as also certain discrepancies between the attitude of the attending physician and that of the medical examiner are the main reasons why the elaboration of medico-legal expert opinion on concussions of the brain is a thorny task. The problems arising in this connection on the evidence of medico-legal reports on 300 non-fatal injuries are surveyed.

Among 300 cases, chosen at random, there were 202 head injuries (67.33 per cent); only the head had been injured in 121 cases, while head injury was accompanied by other injuries in 81 cases. The head had been injured by some blunt object in 191, and by some pointed, sharp tool in 11 cases. In 93 of the 202 cases of head injury (i.e. in 46.04 per cent) was concussion of the brain diagnosed.

Although the original clinical diagnosis had been concussion of the brain, it became doubtful or was proved to be erroneous in 38 cases (40.86 per cent) owing to the absence of objective data at the time of elaboration of the medico-legal opinion and owing to a re-estimation of all circumstances in each given case.

No diagnosis of concussion should be set without the cooperation of the internist, neurologist, ophthalmologist and without EEG, ECG and various laboratory results concerning the CSF, blood-sugar tolerance test, etc.

Conditional diagnosis would be indicated if the complaints and symptoms do not seem to be sufficient for the establishment of a perfectly reliable diagnosis of concussion cerebri.

2. The practice of regarding concussion of the brain as taking longer than eight days to recover irrespective of the gravity of the given case, is erroneous. *Automatic prescription of a long bed rest* in the lack of objective data justifying it, is likewise based on a false principle, and this the more so as unjustifiedly long bed rest may give rise to neurotic manifestations.

3. Especial care should be paid in this respect to suspiciously protracted postconcussional complaints.

Z. Pap

(Department of Neurology and Psychiatry, County Hospital, Debrecen)

Results of Compulsory Detoxication Treatment

Based on data provided by the Central Police Station of Hajdú-Bihar County a survey is given of the minor offences, misdemeanours and criminal offences committed in 1965 under the influence of alcohol (intemperate or unlawful consumption of alcoholic drinks). Medical problems are discussed regarding individuals who were transferred to the hospital by the investigating authorities, the district court or the public prosecutor, for compulsory detoxication treatment. Such treatment often failed, especially if the alcoholic had reached the stage of advanced character degeneration or alcoholic psychosis. The statutes regulating compulsory detoxication treatment are frequently misinterpreted by both the police and the courts. It is, for example, a frequently occurring false interpretation of the regulations that Courts of Law have the right to decide the matter of compulsory treatment irrespective of medical opinion. The personality of each alcoholic should carefully be analysed, for experience has shown that treatment cannot but fail in the case of utterly depraved and completely degenerate drunkards. Individuals of this nature should be transferred for at least six months to a reformatory where they are compelled to work and may benefit from group psychotherapy.

M. Vargha, L. Veress

(Department of Neurology and Psychiatry, and Institute of Forensic Medicine, University Medical School, Szeged)

Problems Regarding Psychopathy and Limited Criminal Responsibility

A short discussion concerning the forms of psychopathy occurring in forensic psychiatry is followed by a treatise on the correlation between psychopathy and the various criminal actions. Characteristic examples are presented. Questions in connection with criminal responsibility in the various forms of psychopathy are investigated from the point of view of the medico-legal expert.

J. Hofsang, G. Marton, M. Vargha

(Public Prosecutor's Office, Szeged, Department of Neurology and Psychiatry, University Medical School, Szeged)

Freedom of Consent of Cases of Immoral Offences

It has become necessary to determine how far the mentally deranged adult party can be regarded as having the freedom of will in cases of rape, indecent assault and sexual perversion because in recent years criminal proceedings were instituted before the County Court of Szeged in which the injured party was mentally deficient. The question has important legal, medico-legal and social aspects.

V. Földes

(Criminological Laboratory, National Police Centre, Budapest)

Binding Decision No. 4 of the Supreme Court in Medical Practice

Decision No. 4 of the Supreme Court concerning increased protection of human life in judiciary practice defines the functions of the medico-legal expert. When presenting expert opinion in cases of criminal actions against the life and health of human subjects, the expert has to pay due regard to the principles laid down in the binding decision of the Supreme Court. Judicial practice cannot dispense with correct medico-legal expertise in proceedings of the said nature.

Expert medical opinion regarding the suitability for killing of the tool employed in the perpetration of the felony, the manner in which it was committed, the site and nature of the injury is indispensable for the Court to arrive at the required decision.

Medico-legal opinion is likewise indispensable in most cases when judgement has to be passed on the particularly cruel manner of manslaughter and its premeditation.

An especially important role falls to the expert in cases of infanticide.

D. Horváth, R. Budvári

(University Pharmacy, and Institute of Forensic Medicine, University Medical School, Pécs)

Responsibility for Side Effects of Drugs

The so-called iatrogenic diseases constitute a widely discussed problem. Interest in the subject is natural in consideration of the great number of current drugs and the incidence of harm to the organism due to their employment. Untoward episodes occurring in this connection raise the question of responsibility as also theoretical and practical problems concerning the attitude of the medico-legal expert.

It is important that, when investigating the cause of sudden death, the forensic medical expert should not content himself with throwing light on the possibly harmful side effect

of the drugs involved, and ascertaining the possibility of individual hypersensitivity or drug intoxication, etc., but should also investigate the matter of drug consumption.

Taking a few fashionable groups of current drugs such as the phenothiazines, anticoagulants, etc., as examples, the fact is pointed out, that although the side effects of many current drugs may sometimes be fatal, death need not be caused exclusively by the drug involved. The prescription of biologically active compounds requires experience and careful deliberation. The physician is expected to know their complex effects and is responsible for their use, a fact which the medico-legal expert cannot ignore.

G. Szuchovszky

(Institute of Forensic Medicine, University Medical School, Budapest)

Problems of Procedural Law Regarding Laboratory Analyses in Connection with Medico-Legal Obductions

Recent legal regulations concerning the organization and function of medico-legal experts include a number of contradictory and impracticable rules as regards serological, toxicological, histological, criminalistical and biological examinations to be made in connection with medico-legal obductions. These rules are analysed and problems of the competence and the unsettled procedural aspects of the expert opinion are pointed out. New rules are necessary to eliminate the present shortcomings and contradictions in respect of laboratory analyses connected with medico-legal obductions.

L. Tolnai, V. Csernohorszky

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Medico-Legal and Anaesthesiologic Aspects of Intraoperative Death

A total of 387 cases of death occurring during surgical intervention in the 40-month period from January 1, 1963, to April 30, 1966 have been surveyed from the medico-legal and anaesthesiological points of view. Evaluation was based on autopsy findings, toxicological analyses and clinical data. In 81 cases could some causal connection be established between anaesthesia and the fatal outcome.

The primary disease alone was the cause of death in 253 cases. In addition to the primary disease in 26 cases there had occurred some surgical error and in 19 cases diagnosis had been established late. Incompatible blood transfusion occurred in 8 cases

The distribution according to anaesthesiologic technique of the 81 cases in which death appears to have been due to anaesthesia is as follows.

Open drop chlorethyl-ether	32
Endotracheal anaesthesia	18
Local ,,	11
Intravenous ,,	10
Local combined with open drop chlorethyl-ether	4
Spinal anaesthesia	3
Lytic cocktail	2
Neurolept. analgesia II.	1
Total	81

L. Harsányi, I. Kenyeres, G. Szuchovszky

(Institute of Forensic Medicine, University Medical School, Budapest)

Analysis of 2000 Suicides Committed by Poisoning

The number of felones-de-se autopsied in the institute in the period 1960 to 1965 totalled 3793 of whom 2073 individuals (54.6 per cent) had committed suicide by means of poisoning. Within the general rise in the number and ratio of suicides the frequency of self-poisonings is steadily increasing. The ratio of suicides committed by narcotics increased nearly twofold in the examined period, that of suicides by coal gas remained practically unchanged, while suicide by other means — caustic agents and heavy metal salts in particular — showed a marked decrease. Distribution according to sex, age and other criteria have also been analyzed. Relying on the evidence of literary and other data such as statistics regarding the use of drugs, etc., suggestions are advanced with a view to reducing the number of suicides, especially those committed by means of narcotics.

Emese Fazekas, I. Gy. Fazekas

(Institute of Forensic Medicine, University Medical School, Szeged)

Development of the Umbilical Ring as an Indicator of Live Birth and Life Span

Although all phenomena developing after birth can be used for determining the age of the newborn, there are few signs indicating the span of life within the first 24 hours of extrauterine life.

The umbilical ring develops, according to textbook data (KENYERES, MILLER, HABERDA, HOFFMANN, etc.), after the 24th hour of extrauterine life. Since the material under study contained numerous cases in which the umbilical ring had developed earlier, the said rule laid down in the textbooks cannot be accepted without reservation. The number of autopsied infants totalled 91; a well-developed umbilical ring was observed in 47 babies who had died within an hour, in 17 who had died within two hours, and in 9 who had died within twelve hours after birth.

Death had occurred amidst signs of suffocation in 44 cases (43.85 per cent) which shows that it is mostly in cases of asphyxia (aspiration, inspiration of blood, strangulation, drowning) that the umbilical ring is formed earlier than after 24 hours.

With a view to checking the conclusions drawn from postmortem observations, the time of formation of the umbilical ring has been studied in a hundred mature newborns. A well-defined umbilical ring was present in 82 cases before the 24th hour of extrauterine life. The ring was perceptible within 6 hrs in 4, between the 6th and 12th hours in 18, between the 12th and 18th hours in 26, between the 18th and 24th hours in 34, and between the 25th and 30th hours in 18 cases.

L. Veress, I. Gy. Fazekas

(Institute of Forensic Medicine, University Medical School, Szeged)

Cerebral Haemorrhage in Younger Aged Persons

A survey of literary data concerning spontaneous cerebral haemorrhage in juvenile patients is followed by a brief summary of autopsy data of young and middle-aged persons who had died of cerebral haemorrhage without a previous history of head injury.

1. Cranial and brain haemorrhage occurred in 19.1 per cent of the examined cases before the age of 50 years, a figure in good agreement with literary data. Distribution according to age was, 10 to 20 years, 0.56 per cent; 20 to 30 years, 2.25 per cent; 30 to 40 years, 6.17 per cent; 40 to 60 years, 10.12 per cent. The examined material showed an increasing tendency of cerebral haemorrhages.

2. All subdural and subarachnoid haemorrhages (2.81 per cent) were due to aneurysmal rupture. Haemorrhage in the brain substance (16.29 per cent) was caused partly by hypertensive arteriosclerosis and — to a greater extent — by narrow vessels and other vascular anomalies. In some cases the underlying cause could not be detected.

3. While haemorrhages of the hypertensive-arteriosclerotic type were usually of striolenticular localization in patients of advanced age, in the examined group they occurred frequently (37.9 per cent) in the cerebellum and the pons.

4. Haemorrhage occurred without demonstrable antecedents in some cases, while factors known to give rise to sudden death were at play in others, e.g. consumption of alcohol, menorrhagia, pregnancy (eclampsia), etc.

Examples are presented to demonstrate the medico-legal aspects of cerebral haemorrhage suffered by young individuals (confusion with poisoning, malpractice, suspicion of manslaughter, etc.).

Erzsébet Virágos-Kiss

(Institute of Forensic Medicine, University Medical School, Szeged)

Fatal Uterine Rupture Due to Malpractice

A case of uterine rupture in a multipara is reported. Although the physician assisting at the delivery was aware of a narrow pelvic inlet and the deflected position of the dead foetus, he administered an oxytocic drug and qualified the woman's wailing as imbecility. He failed to recognize the signs of threatening uterine rupture and sent the patient to hospital only after the rupture had supervened. It was not possible to save the woman. The physician, held criminally responsible, was condemned.

F. Fodor

(State Police Commissioner's Office, Szeged)

A Rare Case of Combined Suicide

A case is described in which the felo-de-se cut his throat, hanged himself and ingested poison. This complex suicide was of especial interest on account of its association with a simultaneous case of manslaughter.

It was necessary to disprove killing and prove suicide. The proof was based on the evidence of coloured photographs, histological and chemical analyses.

There is hardly any record in literature of a similarly complex suicide.

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