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Institute of Pathology, University Medical School, Debrecen

ELECTRON MICROSCOPIC MORPHOMETRY OF THE ZONA GLOMERULOSA CELLS OF THE WISTAR RAT

GY. LUSTYIK and J. SZABÓ

(Received August 6, 1974)

Results of morphometric measurements were compared on the cells of the zona glomerulosa (ZG) of the adrenal glandof the rat. The mean area covered by the cells, the nuclei, the mitochondria and the lipid droplets, as well as the surface density of the endoplasmic reticulum membrane and of internal mitochondrial membrane indicated an inhomogeneity even in the ZG cells of the same animal, with considerable deviations in the ZG of different animals. The dark and light ZG cells were compared too. The results suggest hormonal-functional differences, both among the normal animals and among the cells of the same animal.

The light and electron microscopic structure of the zona glomerulosa (ZG) cells and the function of these cells are well known. Their morphometric investigation provided important data on the functional state of the cells. The adrenal gland has repeatedly been subjected to histometric measurements with the light microscope [2, 11, 24, 34], when often contradictory results were obtained with respect to the morphological reactions of the ZG cells. This might explain why so few morphometric data obtained with the electron microscope have been published concerning the ZG. The bulk of quantitative ultrastructural studies on the adrenal gland have been carried out on the zona fasciculata [19—23]. Because of this scarcity of data we were obliged to find suitable methods of sampling and measurement. In this paper we report on our pertaining observations.

Material and method

Ten 160 to 180 g male Wistar rats, kept on a normal synthetic diet and drinking tap-water *ad libitum* were used. Under light ether anaesthesia, the abdominal cavity was exposed and the left adrenal gland of every animal was removed on the same day within half an hour in the morning. The excised tissue was fixed in 3% glutaraldehyde at room temperature for 3 hours, followed by fixation with 1% osmium tetroxide at 4°C for 1 hour. The pH of both fixing media was adjusted to 7.2 by Milolnig buffer. The samples were dehydrated in an ascending alcohol series and embedded into Durcupan ACM. For the preparation of ultrathin sections, the blocks were oriented using semi-thick sections stained with 0.5% toluidine blue. Ultrathin sections prepared with a Reichert OM U2 ultra- microtome were stained with uranyl acetate and lead citrate. A Tesla BS 513A electron micro- scope was used.

Blocks containing 5 ZG were prepared from each animal. Measurements were done on the micrographs of 10 ZG cells of every animal, the nuclei of which were situated in the plane of section. As in this way the statistical criteria were not met these data were not suited

for stereological processing, but still offered a possibility for comparisons of the ZG cells of the same animal and of different animals. This comparison of the cells provides information on the homogeneity of the ZG which again might help in the choice of the method of sampling. From the results of different animals we tried to find an answer to the question whether the ZG cells of animals kept under identical conditions were characterized by identical quantities of cell organelles.

The area of cells, nuclei, mitochondria, lipid droplets and the cytoplasm containing endoplasmic reticulum was measured on a total of 250 micrographs at 18,750 magnification. The areas were measured by means of the planimeter and the point counting method [27, 38, 40]. The surface density of membrane-structures in the perinuclear space (the surface of endoplasmic reticulum + Golgi membrane per unit cytoplasmic volume) was determined on 200 micrographs at 48,750 magnification together with the area of the cross section of the endoplasmic reticulum and the surface density of free ribosomes (this latter was referred to the cytoplasmic area containing the endoplasmic reticulum) as well as with the density of the internal membranes of the mitochondria (membrane surface per unit mitochondrial volume).

The methods of measurement and calculation were described by WEIBEL [36—40], CHAYES [4] and LOUD [16, 17).

The validity of these results is in fact limited to the perinuclear space of the ZG cells. This restriction was introduced for the sake of comparison of individual cells. We measured organelles which — taking account the ultrastructural changes of stimulated ZG [6, 7, 12, 14, 26, 28, 29, 30, 31, 41] and the cellular path of mineralocorticoid formation [8, 14, 25, 35] might play a role in aldosterone synthesis.

Results

The ultrastructure of ZG cells showed the pattern known from the literature [3, 9, 13, 18, 26, 27, 29, 41—43]. Table I shows the mean area of cells, the number of mitochondria and their mean area per animal. The absolute values for these data offer no direct information, but their comparison means

Table I

Animal	Area of cells	Number of mitochondria per section of cells	Average area of mitochondria
1.	61.0 ± 5.9	46 ± 8	0.31 ± 0.04
2.	57.3 ± 5.8	39 ± 5	0.26 ± 0.02
3.	55.2 ± 5.6	38 ± 4	0.17 ± 0.02
4.	40.7 ± 4.6	27 ± 6	0.21 ± 0.04
5.	66.4 ± 7.8	44 ± 4	0.27 ± 0.03
6.	77.2 ± 7.5	43 ± 5	0.32 ± 0.05
7.	103.7 ± 16.1	37 ± 4	0.45 ± 0.07
8.	69.5 ± 8.0	36 ± 7	0.24 ± 0.02
9.	81.1 ± 12.1	39 ± 6	0.28 ± 0.06
10.	86.5 ± 17.9	44 ± 8	0.29 ± 0.04

The Table shows the mean area (μ^2) of 10 cells for each animal, the number of mitochondria in the cell and the mean area of the mitochondria (μ^2) for each animal. Comparison of the area of cells and mitochondria means a comparison of their volumes. The value for the calculated mean error provides information on the deviations in the cells of the same animal

Table II

Animal	Percentage of the area of				
	nuclei	mitochondria	lipid droplets	membrane space	ER sacs
	in the area of the cell				
1.	16.4 ± 1.9	23.8 ± 2.0	1.9 ± 0.8	56.4 ± 2.8	3.2 ± 1.1
2.	28.1 ± 2.6	18.9 ± 2.1	4.0 ± 2.1	47.6 ± 2.2	3.2 ± 0.5
3.	28.8 ± 3.1	12.1 ± 1.9	6.8 ± 2.4	51.1 ± 1.8	5.4 ± 0.7
4.	36.8 ± 5.0	12.8 ± 2.2	5.5 ± 2.6	42.1 ± 1.8	2.1 ± 0.8
5.	23.7 ± 2.4	18.7 ± 2.5	7.3 ± 2.7	50.0 ± 2.6	3.0 ± 0.6
6.	17.3 ± 1.8	16.1 ± 2.0	3.2 ± 1.8	61.4 ± 3.3	6.1 ± 1.2
7.	21.3 ± 3.2	16.9 ± 2.2	8.7 ± 3.5	52.3 ± 2.2	5.8 ± 0.8
8.	27.8 ± 4.3	11.9 ± 2.2	10.0 ± 4.6	50.2 ± 3.6	3.1 ± 0.8
9.	23.6 ± 3.7	11.7 ± 2.2	7.0 ± 3.2	55.7 ± 3.3	4.2 ± 0.6
10.	27.5 ± 5.6	16.0 ± 3.6	24.0 ± 6.9	34.2 ± 4.1	2.4 ± 0.5

The percentage ratio of the area of the nucleus, the mitochondria the lipid fraction, and the membrane space as well as the area enclosed by the endoplasmic reticulum membrane in the cell is shown. The devirations in the ratios of the lipid droplets per cell and per animal are the greatest, in the case of the other data the differences are significant between the different animals. The high relative error for the endoplasmic reticulum in due to the great experimental error

Table III

Animal	Membrane density in cytoplasm (μ^{-1})	Density of mitochondrial membranes (μ^{-1})	Density of free ribosomes (μ^{-2})	Percentage ratio of dark cells (%)
1.	5.1 ± 0.3	1.97 ± 0.10	122 ± 20	18
2.	4.7 ± 0.3	2.08 ± 0.08	96 ± 12	22
3.	5.8 ± 0.4	1.72 ± 0.18	103 ± 15	14
4.	4.8 ± 0.4	1.85 ± 0.09	84 ± 9	24
5.	6.2 ± 0.3	1.90 ± 0.11	128 ± 22	20
6.	5.0 ± 0.4	2.10 ± 0.12	76 ± 8	16
7.	7.2 ± 0.5	1.88 ± 0.09	131 ± 16	12
8.	5.4 ± 0.3	1.75 ± 0.16	99 ± 10	15
9.	4.0 ± 0.4	2.05 ± 0.14	103 ± 12	14
10.	3.8 ± 0.4	2.40 ± 0.19	112 ± 12	13

Table III shows the density of the endoplasmic reticulum membranes (the membrane surface) per unit cytoplasmic volume (μ^{-1}) and the density of the mitochondrial membrane (surface of the cristae plus the internal membrane in unit mitochondrial volume) (μ^{-1}), the number of free ribosomes in the unit area of the cytoplasm containing the endoplasmic reticulum (μ^{-2}) and the ratio of dark cells per 100 cells. There is a significant difference in the density of free ribosomes per cell and per animal and there are remarkable deviations in the density of the endoplasmic reticulum membrane in the different animals

a comparison of the volume of cells and mitochondria and of the number of mitochondria per cell. This showed less discrepancy, while the differences in the volume of cells and mitochondria were remarkable. The calculated mean error will provide information about the deviation from the average of the different cells of the same animal. Thus, the data for the same animal show significantly less deviation than those found among different animals.

The area-percentage of the nuclei, mitochondria, lipid droplets and the membrane space are shown in Table II. The nature of the deviations was similar as in the differences in cell areas. Particularly high relative deviations were found in the quantity of the lipid fraction and these deviations per cell were also remarkable.

The surface density of membranes (endoplasmic reticulum + Golgi membranes) and that of the internal membrane of the mitochondria, the numerical density of free ribosomes as well as the percentage of dark cells are shown in Table III. There were marked differences between the animals and also among the densities of free ribosomes per cell.

The morphometric characteristics of the dark and light cells of ZG were calculated separately (Table IV). The discrepancies between the two types of cell might have been due to the shrinking of the dark cells during fixation, since there were slight deviations between the percentage of the areas of the various organelles.

Table IV

	Dark cells	Light cells
Cell area (μ^2)	50.1 ± 1.5	70.2 ± 2.2
Mean area of mitochondria (μ^2)	0.22 ± 0.01	0.31 ± 0.01
Number of mitochondria in the cell area	33 ± 3	40 ± 3
Percentage ratio of the area occupied by the nucleus in the cell	29.4 ± 1.6	24.2 ± 1.1
Percentage ratio of the area occupied by the mitochondria in the cell	14.4 ± 0.5	14.3 ± 0.5
Percentage ratio of the area occupied by lipid droplets in the cell	6.2 ± 1.3	4.8 ± 1.1
Percentage ratio of the area occupied by the membrane space in the cell	49.6 ± 1.5	55.9 ± 1.7
Percentage ratio of the area occupied by the membrane sacs in the cell	4.0 ± 0.4	4.6 ± 0.4
Density of free ribosomes in the membrane space (μ^{-2})	114 ± 8	88 ± 6
Density of the endoplasmic reticulum membrane in the cytoplasm (μ^{-1})	6.3 ± 0.2	5.1 ± 0.2
Membrane density of the mitochondria (μ^{-1})	2.15 ± 0.1	1.89 ± 0.1

The morphometric indices of the dark and light cells of the ZG are shown separately in Table IV. There is a significant difference in the mean cell and mitochondrial areas, as well as in membrane density and in the density of free ribosomes. The difference in the percentage ratios of the various organelles in the two types of cell is less significant

Discussion

With respect to the ZG, there are few morphometric data available [18]. To the best of our knowledge no similar measurements have been reported in the literature, so that we were unable to compare our results with those of other authors.

We found remarkably great differences mainly among the ZG cells of different animals, though — primarily on ground of the density of lipid droplets and free ribosomes — the ZG of the same animal does not seem to be homogeneous either. The role of lipids in the synthesis of mineralocorticoids is not yet fully known, although it is beyond doubt that they have a role in the production of adrenal hormones [26]. Thus, the quantity of lipid droplets in a single cell might be the function of cellular activity. Similarly, as supposed by several authors [7, 10, 13], the dark and light ZG cells might be the expression of two different functional states. In connection with these cells we must bear in mind that — as supposed by RHODIN [26] — the appearance of cells different in density might be the result of different degrees of shrinking during fixation. This assumption was supported by the slight difference between the percentage of the areas of the organelles in the two types of cell while membrane density and the density of ribosomes was greater in the dark cells. On this basis no unequivocal answer can, however, be given to the question, and the numerical ratio between dark and light cells, the separately measured morphometric indices of these cell varieties, might contribute to the assessment of the structural-functional relationship.

The quantitative morphological differences among of the different animals may have been due to the different functional states of the ZG in individual normal animals even under identical conditions. Changes in the functional state from the morphological aspect are probably reflected in the simultaneous changes in the number of the various cell varieties (cells with high and low lipid content, dark and light cells) and in the quantity of cell organelles in the various cells.

Hence, the quantitative morphological characterization of the ZG cells is only possible by a number of simultaneously obtained parameters. Since changes in function are accompanied by macroscopic and light microscopic signs, planning of experiments for the study of the structural-functional relationship demands some caution. The method of fixation might also be of importance and only results obtained on tissues which have been fixed and processed by identical methods are suitable for comparison. A sufficiently large number of blocks obtained from different areas of the ZG must be examined. The number of blocks and the number of electron microscopic pictures necessary for the measurements can be estimated from the relationship suggested by DE HOFF (cit. [36], p. 269) and important help might be obtained from light

microscopic measurements too. In addition, parallel biochemical and morphometric tests are necessary for the study of the structural-functional relationship. Only such investigations might answer the question whether there exists a quantitatively assessable relationship between mineralocortocoid production and the structure of the ZG cells.

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REFERENCES

1. AHERNE, W.: (1970) Quantitative methods in histology. J. med. Lab. Technol. **27**, 160—170. — 2. BAHN, R. C., STORINO, H. E., SCHMIT, R. W.: (1960) The mass of the zona glomerulosa following complete anterior and posterior hypophysectomy and subtotal removal of the pars tuberalis in the dog. Endocrinology **66**, 403—408. — 3. BENEDECZKY, I.: (1966) A mellékvese submikroszkópos szerkezete és sejtélettani jelentősége. Morph. Ig. Orv. Szle. **6**, 248—253. — 4. CHAYES, F.: (1965) Determination of relative volume by sectional analysis. Lab. Invest. **14**, 249—257. — 5. DORFMAN, R. I., UNGAR, F.: Metabolism of Steroid Hormones. Academic Press, New York–London 1965. — 6. FISHER, E. R., HORVAT, B.: (1971) Ultrastructural, features of aldosterone production. Arch. Path. **92**, 172—179. — 7. GIACOMELLI, F., WIENER J., SPIRO, D.: (1965) Cytological alterations related to stimulation of the zona glomerulosa of the adrenal gland. J. Cell. Biol. **26**, 499—516. — 8. GLÁZ, E., VECSEI, P.: Aldosterone. Akadémiai Kiadó, Budapest 1971. — 9. KAHRI, A.: (1966) Histochemical and electron microscopic studies on the cells of the rat adrenal cortex in tissue culture. Acta endocr. (Kbh.) **52**, Suppl. 108. 1—96. — 10. KENDREI, G., SZENDE, B.: (1969) Elektronmikroszkópos megfigyelések „világos" és „sötét" sejtek előfordulásáról és szerkezetéről patkány mellékvese zona glomerulosájában. Morph. Ig. Orv. Szemle **9**, 271—280. — 11. KOVÁCS, K., ZÁMBÓ, E., LÁSZLÓ, F.: (1969) A mellékvesék histometriai vizsgálata patkányban. Morph. Ig. Orv. Szemle **9**, 81—90. — 12. LEVER, J. D.: (1956) Cytological studies of hypophysectomized rat adrenal cortex: alterations of its fine structure following ACTH administration and on lowering the Na/K ratio. Endocrinology **58**, 163—180. — 13. LEVER, J. D.: (1955) Electron microscopic observations on adrenal cortex. Amer. J. Anat. **97**, 409—429. — 14. LONG, J. A., JONES, A. L.: (1970) Alterations in fine structure of the opossum adrenal cortex following sodium deprivation. Anat. Rec. **166**, 1—26. — 15. LONG, J.: A., JONES, A. L.: (1967) Alterations in fine structure of zona glomerulosa in the opossum adrenal following sodium deprivation. Anat. Rec. **157**, 280. — 16. LOUD, A. V.: (1962): A method for quantitative estimation of cytoplasmic structures. J. Cell Biol. **15**, 481—487. — 17. LOUD, A. V., BARANY, W. C., PACK, B. A.: (1965) Quantitative evaluation of cytoplasmic structures in electron micrographs. Lab. Invest. **14**, 258—270. — 18. NUSSDORFER, G. G.: (1968) The fine structure of the newborn rat adrenal cortex. I. Zona glomerulosa and zona fasciculata. Z. Zellforsch. **103**, 382—397. — 19. NUSSDORFER, G. G., MAZZOCCHI, G.: (1973) Effects of 3'5' cyclic nucleotides in adrenocortical cells of hypophysectomized rats: a stereologic and autoradiographic study. Lab. Invest. **28**, 332—342. — 20. NUSSDORFER, G. G., MAZZOCCHI, G.: (1972) A stereologic study of the effects of ACTH and cyclic 3'5'-AMP on adrenocortical cells of intact and hypophysectomized rats. Lab. Invest. **26**, 45—52. — 21. NUSSDORFER, G. G., MAZZOCCHI, G.: (1970) Correlated morphometric and autoradiographic studies of the effects of corticosterone on adrenocortical cells of intact and hypophysectomized ACTH-treated rats. Z. Zellforsch. **111**, 90—105. — 22. NUSSDORFER, G. G., MAZZOCCHI, G., REBONATO, I.: (1971) Long-term trophic effect of ACTH on rat adrenocortical cells. An ultrastructural, morphometric and autoradiographic study. Z. Zellforsch. **115**, 30—45. — 23. MAGALHAES, M. C., MAGALHAES: (1969) A stereologic study of the effects of metopiron on the rat adrenal. Lab. Invest. **21**, 491—496. — 24. MÜLLER, R. A.: (1965) Effect of hypophysectomy and a low sodium diet on the volumes of the glomerular zone, fascicular and reticular zones and medulla of rats. Acta endocr. (Kbh.) **50**, 486—492. — 25. PSYCHOYOS, S., TALLAN, H. H., GREENGARD, P.: (1966) Aldosterone synthesis by adrenal mitochondria. J.

biol. Chem. **241,** 2949—2956. — 26. RHODIN, J. A. G.: (1971) The ultrastructure of the adrenal cortex of the rat under normal and experimental conditions. J. Ultrastruct. Res. **34,** 23—71. — 27. SABATINI, D. D., DERROBERTIS, E. D. P.: (1961) Ultrastructural zonation of adrenocortex in the rat. J. Biophys. Biochem. Cytol. **9,** 105—119. — 28. SARASON, E. L.: (1943) Morphologic changes in the rat's adrenal cortex under various experimental conditions. Arch. Path. **35,** 373—390. — 29. SATO, T.: (1962) Electron microscopic studies on the ultrastructure of the adrenal cortex. Correlation of its structure and function. Folia endocr. Jap. **38,** 881—889. — 30. SEELING, L. L., RENNELS, E. G.: (1969) Light and electron microscopic studies on adrenal cortical hypertrophy in the rat. Texas Rep. Biol. Med. **27,** 822—838. — 31. SHELTON, J. A., JONES, A. L.: (1971) The fine structure of the mouse adrenal cortex and the ultrastructural changes in the zona glomerulosa with low and high sodium diets. Anat. Rec. **170,** 147—181. — 32. SHEPPARD, H., SWENSON, R., MOWLES, T. F.: (1963) Steroid biosynthesis by rat adrenal functional zonation. Endocrinology **73,** 819—824. — 33. SMICIKLAS, H. A., PIKE, R. L., SCHRAER, H.: (1971) Ultrastructure of adrenal glands in sodium-deficient pregnant rats. J. Nutr. **101,** 1045—1055. — 34. STARK, E., PALKOVITS, M., FACHET, J., HAJMAN, B.: (1965) Adrenocortical volume and adrenocortical function. Acta med. Acad. Sci. hung. **21,** 263—269. — 35. TCHEN, T. T.: In: Functions of the Adrenal Cortex (Ed.: K. W., McKerns). North Holland Publ. Co.. Amsterdam 1968, pp. 3—26. — 36. WEIBEL, E. R.: (1969) Stereological principles for morphometry in electron microscopic cytology. Int. Rev. Cytol. **26,** 235—302. — 37. WEIBEL, E. R.: (1963) Principles and Methods for the Morphometric study of the lung and other organs. Lab. Invest. **12,** 131—155. — 38. WEIBEL, E. R., ELIAS, H.: (1967) Introduction to stereologic principles. In: Quantitative Methods in Morphology (Ed.: E. R. Weibel, H. Elias). Springer, Berlin—Heidelberg—New York pp. 89—98. — 39. WEIBEL, E. R., GOMEZ, D. M.: (1962): A principle for counting tissue structures on random sections. J. appl. Physiol. **17,** 343—348. — 40. WEIBEL, E. R., KISTLER, G. S, SCHERLE, W. F.: (1966) Practical stereological methods for morphometric cytology. J. Cell Biol. **30,** 23—38. — 41. YAMORI, T., MATSUURA, S., SAKAMOTO, S.: (1961) An electron-microscopic study of the normal stimulated adrenal cortex in the rat. Z. Zellforsch. **55,** 179—199. — 42. YOSHIMURA, F., HARUMIYA, K., SUZUKI, N., TOTSUKA, S.: (1968) Light and electron microscopic studies on the zonation of the adrenal cortex in albino rats. Endocr. Jap. **15,** 20—52. — 43. YOSHIMURA, F., HARUMIYA, K., WATANABE, M., ONOTO, T., SEKIGUCHI, T.: (1968) Functional significance of tubular agranular endoplasmic reticulum in the adrenocortical cells of albino rats. Endocr. Jap. **15,** 145—169.

ELEKTRONENMIKROSKOPISCHE QUANTITATIVE MORPHOLOGISCHE UNTERSUCHUNGEN AN DEN ZONA-GLOMERULOSA-ZELLEN VON INTAKTEN WISTAR-RATTEN

GY. LUSTYIK und J. SZABÓ

Mittels elektronenmikroskopischer morphologischer Messungen wurden die Zellen der Zona glomerulosa (ZG) von Rattennebennieren verglichen. Aufgrund der Meßergebnisse der Fläche der Zellen, der Zellkerne, der Mitochondrien sowie der lipid Droplets, ferner der Dichte der Membran des endoplasmatischen Retikulums (ER) und der Innenmembran der Mitochondrien erwies sich, daß sogar die Zona glomerulosa der einzelnen Tiere inhomogen ist, jedoch wurden weit größere Abweichungen zwischen den ZG-Zellen verschiedener Tiere erhalten. Die »dunklen« und die »hellen« Zellen der ZG wurden verglichen. Die Unterschiede wiesen darauf hin, daß bei den Tieren auch in der Norm hormonelle und funktionelle Abweichungen bestehen bzw., daß Zellen in unterschiedlichem hormonellem Zustand vorhanden sind.

ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ КОЛИЧЕСТВЕННОЕ МОРФОЛОГИЧЕСКОЕ ИЗУЧЕНИЕ КЛЕТОК КЛУБОЧКОВОЙ ЗОНЫ У НОРМАЛЬНЫХ КРЫС ПОРОДЫ ВИСТАР

Д. ЛУШТЬИК и И. САБО

Авторами было проведено сравнение клеток клубочковой зоны (ZG) надпочечников крыс с помощью электронномикроскопических морфометрических измерений. На основе измерения поверхности клеток, клеточных ядер, митохондрий, липоидных капелек, а также плотности мембраны эндоплазматической сеточки и внутренней мембраны митохондрий

было установлено, что клубочковая зона даже у отдельных животных неоднородна, но еще более значительные отклонения были обнаружены между клетками клубочковой зоны различных животных. Авторы сравнивали «темные» и «светлые» клетки клубочковой зоны. Обнаруженные отклонения указывают на гормональные и функциональные различия, наблюдаемые у животных также в нормальном состоянии, или на одновременное наличие клеток, находящихся в различном функциональном состоянии.

György Lustyik
Dr. Jenő Szabó
DOTE Kórbonctani Intézet,
4012 Debrecen, Pf. 22., Hungary

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Institute of Anatomy, Histology and Embryology, University Medical School, Debrecen

EFFECT OF TRIIODOTHYRONINE TREATMENT, THYROPARATHYROIDECTOMY AND MERCAPTOIMINAZOLE TREATMENT ON ENCHONDRAL BONE GROWTH

CHANGES IN THE HISTOLOGICAL STRUCTURE OF THE GROWTH ORGAN

G. Lévai, S. Varga,* J. Gyarmati, Jr. and J. Laczkó

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Histological changes caused by triiodothyronine (T_3) and mercaptoiminazole treatment as well as by thyroidectomy have been studied in the proximal growth organ of the tibia of growing rats. On triiodothyronine treatment morphometric examinations revealed an increased proliferation and resorption of cartilage associated with a transitory acceleration of linear bone growth. Administration of mercaptoiminazole and thyroidectomy inhibited cartilage proliferation and resorption resulting in a slowing down of bone growth.

Anomalies due to changes in thyroid represent a considerable part of bone growth disturbances of endocrine origin. According to Riekstnice and Asling [31] thyroid hormones influence both the growth and the differentiation of bone, and the effect of thyroxine on skeletal growth and development is the one easiest to follow. There are numerous data to show the favourable influence on bone growth of the thyroid hormones [12, 27, 30, 33, 38], although some contradictory ones have also been published [5, 10]. Our knowledge on the ossification disturbances due to reduced thyroid function uniformly refers to a slowing down of growth [8, 17, 18, 32]. Though the role of the thyroid in controlling ossification is indisputable, the mechanism of action of its hormones is not clear. In earlier papers [23, 24] we described the histological changes observed in the growth organ of the tibia of developing male rats after thyroxine and thyroid stimulating hormone (TSH) treatment. In the present work the changes taking place in the proximal growth organ of the tibia of male rats were subjected to morphometric analysis.

Material and methods

Male rats 29—30 days of age of our own breed were used. The animals were divided into four groups; three consisted of 40 and one of 60 animals. From each group five animals were sacrificed at the end of every week throughout 5 weeks from the beginning of treatment.

* Present address: Central Research Laboratory, University Medical School, Debrecen

The first group was treated of liothyromine orally with daily 0.01 mg/100 g body weight. The animals in the second group were untreated controls. The third group was treated orally with daily 10 mg/100 g body weight of thiamazole.

The 60 animals of the fourth group were subjected to thyroparathyroidectomy according to GRIESBACH and PURVES [11]. Five animals being successfully operated on both in anatomical and histological, respect were selected for the experiment; 8 to 12 animals were sacrificed every week. The death rate ranged from 0 to 30%.

The bones of the most radically operated 5 animals were processed weekly. The drugs were mixed to the food and conditions for feeding, environment, temperature, humidity and illumination were identical for each group. The animals were given tap water ad libitum. They were sacrificed in ether anaesthesia by heart transection. Their left tibia was fixed and decalcinated in Susa's mixture and after orienting the proximal end, embedded in paraffin. Sections 7 μ thick were cut and stained with haematoxylin-eosin, azan, and alcian green–picrofuchsin. Before sacrifice the body weight and distance between the caudal base and the tip of the nose were measured. For quantitative estimation, the sections were projected on graph paper by a Leitz microprojector at 1 : 1500 magnification and the contours of the epiphyseal cartilage were drawn. The thickness of the cartilage was measured, and at 1, 3 and 5 weeks an area of 500×750 μ^2 was subjected to analysis of the epiphyseal growth organ from 3 animals of each group. With the modified method of GLAGOLYEV [9] a point-network with a density of $d = 15$ μ was used and positions of 1836 points were examined within the mentioned region. The percentage distribution of the cartilage ground-substance, chondrocytes, capillary loops, trabecules and bone marrow of the growth organ was determined after calculating their projected area.

Results

Table I demonstrates the body weight and length of the experimental animals. Body weight of the treated animals was at every point of time less than that of the controls and this difference (except for the first week) was the smallest in the case of T_3. Body length of T_3 treated rats was approximately the same as that of the controls, while the thyroidectomized and thiamazole treated animals were significantly smaller. The thickness of the epiphyseal cartilage of the growth organ showed significant changes (Table II) with deviations in both positive and negative directions.

After T_3 *treatment* the epiphyseal cartilage was always significantly narrower. After the first week of *thiamazole treatment* the cartilage was significantly

Table I

Mean body weight and tip of nose — caudal distance in experimental animals at the end of the 1st, 3rd and 5th weeks

Period of treatment	Control		Triiodothyronine		Thiamazole		Thyroparathyroidectomy	
	b. weight (g)	b. length (cm)	b. weight (g)	b. length (cm)	b. weight (g)	b. length (cm)	b. weight (g)	b. length (cm)
1 week (7 days)	59.70	12.95	52.00	13.40	58.40	12.20	53.00	11.42
3 weeks (21 days)	91.00	14.20	77.00	14.00	74.00	13.50	73.00	13.54
5 weeks (35 days)	127.00	17.78	105.00	17.85	98.00	14.95	98.00	16.32

Table II

Thickness (μ) of the epiphyseal cartilage at the end of 1st, 3rd and 5th weeks

	1 week	3 weeks	5 weeks
Control	455±37	336±39	243±15
Triiodothyronine treatment	351±23 [1]	291±41 [1]	219±16 [1]
Thiamazole treatment	500±51 [1]	223±28 [1]	223±17 [1]
Thyroparathyroidectomy	374±16 [1]	306±15 [1]	313±10 [1]

[1] $P < 0.001$

thicker, while after the third and fifth weeks significantly thinner, than in the controls. At the end of the first and third weeks after *thyroparathyroidectomy*, thickness of the cartilage did not reach the control values, surpassing them, however, by the end of the fifth week.

The thickness of the epiphyseal cartilage depends on two factors: the rates of proliferation and decomposition. The extension of the proliferative and hypertrophic chondrocyte zone refers to the activity of these processes. In this respect, we have adopted HAM's [15] designations of proliferative zone

Table III

Total thickness of epiphyseal cartilage (μ), thickness of the proliferative and hypertrophic cell zones (μ) and their ossification rate at the end of the 1st, 3rd and 5th weeks

		1 week	3 weeks	5 weeks
Control	Total thickness of epiphyseal cartilage	455±37	336±39	243±15
	Proliferative zone	184±37	171±16	133±10
	Hypertrophic zone	166±19	102±16	74±25
	Prolif./hypertroph. ratio	1.11	1.67	1.79
Triiodothyroine treatment	Total thickness of epiphyseal cartilage	351±23	291±41	219±16
	Proliferative zone	189±12 [7]	158±12 [2]	110±13 [1]
	Hypertrophic zone	91±15 [1]	59±12 [1]	40±15 [1]
	Prolif./hypertroph. ratio	2.07	2.67	2.75
Thiamazole treatment	Total thickness of epiphyseal cartilage	500±51	223±28	223±17
	Proliferative zone	206±22 [5]	90±13 [1]	123±23 [6]
	Hypertrophic zone	178±14[4]	90±13 [3]	78±17 [7]
	Prolif./hypertroph. ratio	1.15	1.00	1.57
Thyropara-thyroidectomy	Total thickness of epiphyseal cartilage	374±16	306±15	313±10
	Proliferative zone	156±39 [5]	144± 9 [1]	167± 9[1]
	Hypertrophic zone	120±18 [1]	116±17 [3]	103±19 [1]
	Prolif./hypertroph. ratio	1.30	1.24	1.62

[1] $P < 0.001$ [2] $P < 0.005$ [3] $P < 0.02$ [4] $P < 0.025$ [5] $P < 0.05$ [6] $P < 0.1$ [7] $P < 0.6$

and hypertrophic zone; the latter comprises both the maturation and calcification zones.

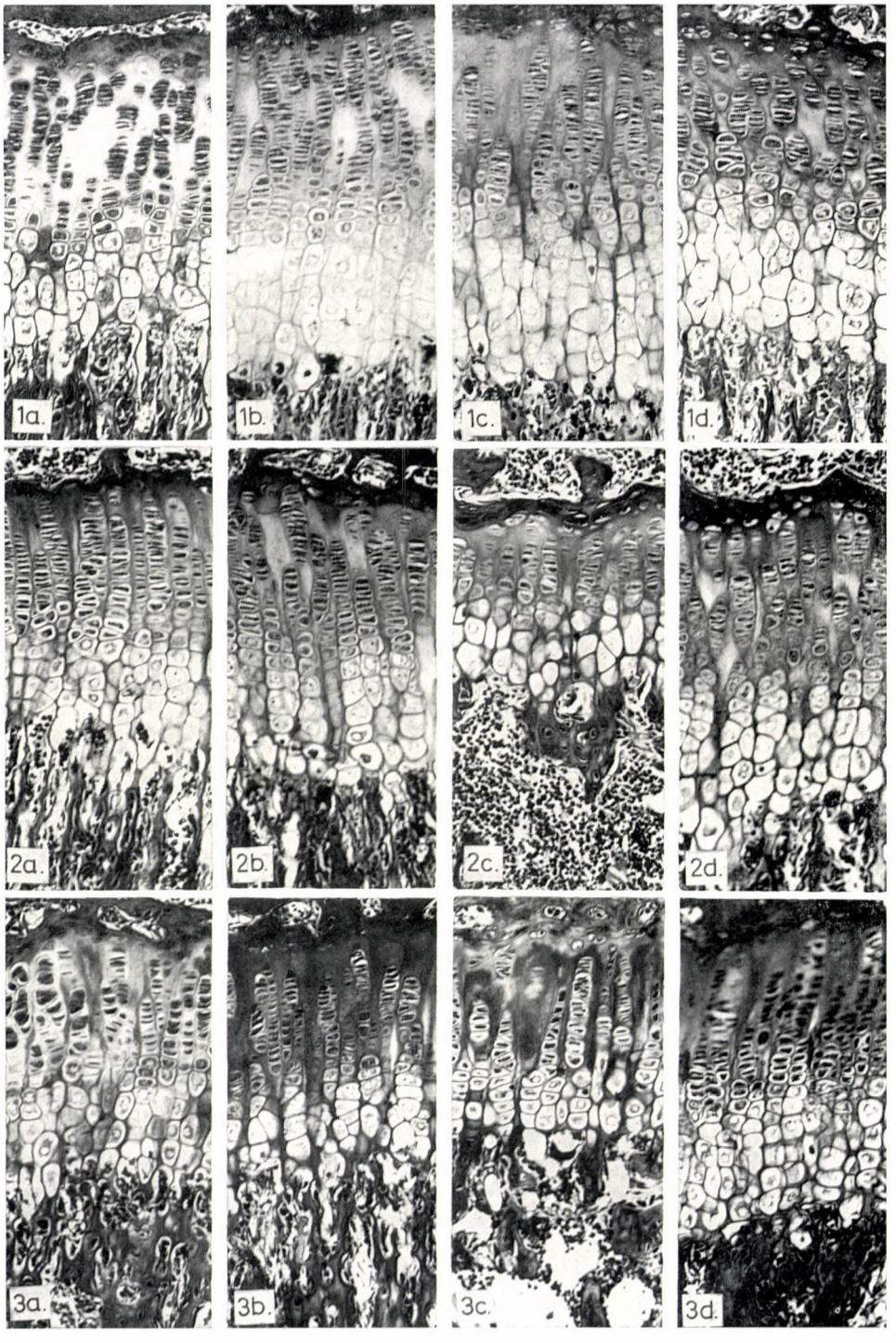
Table III demonstrates measurements of the proliferative and hypertrophic zones. The data show that the hypertrophic zone narrowed down significantly after T_3 treatment as compared to the controls. At the same time a significant narrowing of the proliferative zone was observed after thyroparathyroidectomy and thiamazole treatment. Even more interesting correlations were obtained on dividing the thickness values for the proliferative and the hypertrophic zone. This value of the rate of ossification showed an upward tendency in the control animals with progressing age, as the hypertrophic zone diminished more than the proliferative zone. The effect of T_3 treatment was similar, but the ratio was considerably higher than that for the controls. At the third and fifth weeks after thyroidectomy and thiamazole treatment the value for the ratio was lower than the control, due to the widening of the hypertrophic and narrowing of the proliferative zone.

Table IV demonstrates the results obtained with the GLAGOLYEV' s method [9]. The table shows the percentage distribution of the 1836 points within the examined region concerning cartilage ground substances, chondrocytes, capillary loops of the metaphyseal surface trabecules and bone marrow. It is seen that under the effect of T_3 the area of capillaries on the metaphyseal

Table IV

Percentage distribution of measuring points corresponding to the constituents of the proximal growth organ in the tibia as determined with Glagolyev's point network method

	Period of treatment, weeks	Percentage distribution of points within the total examined area Total area = 100% = (750×500) μ^2 = 1836 measuring points				
		Chondrocyte	Cartilage ground substance	Capillary loop	Trabecule	Bone marrow
Control	1	24.78	27.45	5.66	11.55	30.56
	3	17.10	25.49	5.01	15.85	36.55
	5	11.17	22.22	2.72	20.37	43.52
Triiodothyronine treatment	1	20.92	25.65	11.60	15.20	26.23
	3	17.59	18.79	7.24	19.72	36.66
	5	13.34	15.25	6.21	10.46	54.74
Thiamazole treatment	1	24.40	38.89	4.25	9.04	23.42
	3	11.00	27.70	1.20	5.01	65.09
	5	8.17	23.20	1.74	15.36	51.53
Thyroparathyroidectomy	1	19.01	24.95	3.10	14.27	38.67
	3	12.64	26.74	4.36	13.83	42.43
	5	11.66	29.47	2.56	12.15	44.16



Figs 1–3. Histological changes in the growth organof the tibia folowing interventions affecting the thyroid gland. H.E. × 120. 1a—d: 1-week treatment; 2a—d: 3-week treatment; 3a—d: 5-week treatment; a = triiodothyronine treated; b = control; c = thiamazole treated; d = thyroidectomized

surface of the epiphyseal cartilage grew significantly as compared to controls as soon as in the first week, and this remained unchanged till the end of the experiment. This phenomenon may be considered a sign of the acceleration of cartilage decay. The number of chondrocytes increased similarly upon hormone treatment in the fifth experimental week, at the same time the amount of ground substance decreased. Thus the relative cell content of the epiphyseal cartilage increased on T_3 treatment. A similar increasing tendency was observed in the first and third experimental weeks in the number of points situated on the trabecules, while by the end of the fifth week an opposite tendency prevailed. The accelerated bone formation in the first and third weeks, associated with increased bone decomposition in the fifth week, may have been responsible for the change. Under the effect of *thiamazole treatment* and thyroparathyroidectomy the number of capillaries was less than in the controls at every point of time. A similar phenomenon was observed in the distribution of trabecules. The higher number of points corresponding to the cartilage ground substance and the lower number corresponding to the chondrocytes suggested a relative decrease of cell content of the epiphyseal cartilage.

Figure 1 shows the proximal growth organ of the tibia in rats *treated with* T_3 (1a, 2a, 3a), in the controls (1b, 2b, 3b), in *thiamazole treated* (1c, 2c, 3c), and *thyroparathyroidectomized* (1d, 2d, 3d) animals, 1, 3, and 5 weeks after treatment. The photos were taken of areas subjected to morphometric analysis and they show the above-described extension of the proliferative and hypertrophic zones, the thickness and cell richness of the epiphyseal cartilage, the distribution of the metaphyseal capillary loops, the number of trabecules and the extension of the bone marrow cavity.

Discussion

The changes developing in the so-called growth organ [25] of young male rats subjected to different interventions during the period of enchondral bone formation can be summarized as follows. T_3 *treatment* accelerated the resorption of epiphyseal cartilage, the hypertrophic cell zone was narrowed down. Almost simultaneously, proliferation of the chondrocytes increased, the proliferative zone became wider, the relative cell content of the cartilage increased. The ratio proliferative/hypertrophic zone thickness increased under the effect of thiamazole and thyroparathyroidectomy; this ratio was smaller than in the controls after 3 and 5 weeks, as a result of a relative narrowing of the proliferative zone and a widening of the hypertrophic zone. The results indicated a slower proliferation and resorption of cartilage. The reduced number of the metaphyseal capillary loops and the relative cell content of the cartilage were further proofs of the above statement.

According to KROMPECHER [22], normal enchondral bone growth is characterized by the balanced process between cartilage formation and resorption as well as bone formation and resorption. An increase in the activity of either of these processes results in pathological bone growth. In our studies experimentally produced thyroid hormone disorders induced a disturbance of linear bone growth, by altering the afore-mentioned proportions. The changes occurring in the process of bone growth can be characterized by the ratio proliferative/hypertrophic zone. The numerical value of this fraction increases with age in the control animals. Thus the hypertrophied, decaying chondrocyte zone, which is at the stage preceeding resorption, narrows down more rapidly than does the proliferative zone, which produces the supply of chondrocytes. In other words with age the proportions in the narrowing epiphyseal cartilage shift towards the proliferative zone. Under normal conditions chondral absorption reaches the proliferative tone which then is also resorbed. The epiphyseal cartilage is resorbed completely, and growth comes to an end. The values for the treated groups well characterized the process of ossification under pathological conditions.

T_3 compensates the growth disturbance of thyroidectomized rats 3—5 times more than does thyroxine [13] and is twice more effective in enhancing appearance of the ossification centre [45]. These data are in good agreement with our results according to which T_3 treatment enhances chondral proliferation and resorption, and also with the observations that upon thyroxine treatment the epiphyseal cartilage of the third metacarpal bone is absorbed earlier, and that the chondroclasts, being present in high number, play a role in the absorption besides the capillary loops [23, 24]. The acceleration of bone formation upon T_3 treatment has also been recorded, in agreement with earlier observations [29, 31, 35, 36]. The findings of HALL [14], NOBACK et al. [29] and WALKER [45] refer indirectly to an enhanced cartilage decomposition and bone formation, and show that the ossification centres appear earlier under the effect of thyroxine.

THORNGREEN and HANSSON [43, 44] reported that thyroxine in a 20 μg/kg dose increased the proliferation of epiphyseal chondrocytes of young hypophysectomized rats. Longitudinal bone growth is the result of chondral proliferation occurring in the epiphyseal cartilage [15, 16, 39, 40]. Linear growth is accelerated by T_3 in growing rats [3, 4, 30], the process is, however, temporary and the transient acceleration leads to premature resorption of the epiphyseal cartilage [23], thus animals treated with thyroid hormone remain shorter than the controls. MCLEAN and URIST [25] observed a delay of growth in pubertal hyperthyroidism. We have, however, failed to observe signs referring to osteoclast accumulation and osteoporosis, despite the presence of numerous chondroclasts. This might be explained by the fact that our experiments were performed on growing young animals and, according to human observations [20, 26], thyrogenous osteoporosis is a rare phenomenon in puberty. The rate

of ossification of T_3 treated animals was significantly higher than that of the controls at every point of time. This points to changes in the growth organ where the increased proliferation and absorption of the cartilage indicate that enchondral ossification in the treated animals proceeds much faster than in the controls.

Hypothyroidism due to *thiamazole treatment* and *thyroparathyroidectomy* delays *cartilage resorption* as well as cartilage proliferation [2, 18, 19, 23, 32, 37]. The pathological stage can be followed well in the skeletal alterations in cretinism, the corresponding human condition: linear growth is retarded, persisting cartilaginous islands may be observed even at the age of 40—50 years at sites that normally have long before been replaced by bone [46]. Our measurements corresponded with the above-mentioned observations. Thiamazole treated and thyroidectomized animals display certain differences in the mechanism of dysostosis, due probably to differences in the loss of thyroid hormone production. Under the effect of thiamazole, hypothyroidism develops gradually, the blood TSH level is increased by a feed-back mechanism, the acidophilic cells which produce the growth hormone degenerate gradually [17, 21, 34]. The minimum quantity of thyroid hormone essential for TSH production persists for a long time after thyroparathyroidectomy[42]. Thiamazole inhibits peripheral metabolism and hormone activity and this effect cannot be neglected. It is, however, difficult to estimate its rate as not even a simultaneous substitution treatment with thyroid hormone helps to draw conclusions since the peripheral inhibitory effect of the drug cannot be eliminated [41]. The behaviour of the C cells of the thyroid gland, which produce thyrocalcitonine (TCT) as well as the effect of TCT may represent further problems. According to WUTTKE et al. [47] with the increased TSH level, the activity of the C cells increases. Parathyroidectomy following the thyroidectomy is further complicating the aspect. Parathyroid hormone plays a role in enchondral ossification by influencing the calcium level. Parathyreoprival tetany, as a sign of hormone loss, did not occur in our operated animals. This may have been due to the parathyroid islands always demonstrable in the thymus of rats [7], and these islands become hypertrophic after parathyroidectomy [6]. All these endocrine changes resulted in changes of the histological structure of the growth organ of thyroidectomized and thiamazole treated animals. This was manifest also in our measurements. In the case of thyroid hypofunction, the calculated ossification rate was lower than in the controls at the end of the third and fifth weeks of treatment. This indicated that, owing to the slowing down of chondrocyte proliferation and cartilage resorption, bone growth was delayed as compared with the controls.

Considering KROMPECHER's [22] above mentioned thesis the conclusion has been drawn that T_3 treatment increased cell proliferation in the epiphyseal cartilage and resorption of the latter, and enhanced to a lesser extent the proc-

ess of bone formation. The contrary was found in the case of decreased thyroid activity, although bone formation was less affected than chondrification and chondral destruction.

REFERENCES

1. Asling, C. W., Evans, H. M.: (1956) Anterior pituitary regulation of skeletal development. In: The biochemistry and physiology of bone. Ed.: Bourne, G. H. Academic Press, New York, pp. 671—704. — 2. Becks, H., Kirbick, E. A., Evans, H. M.: (1942) The bone histology of adult male rats thyroparathyroidectomized when one month of age. J. exp. Zool. **89,** 297. — 3. Becks, H., Simpson, M. E., Scow, R. O., Asling, C. W., Evans, H. M.: (1948a) Skeletal changes in rats thyroidectomized on the day of birth and the effects of growth hormone in such animals' tibia, metacarpal and caudal vertebrae. Anat. Rec. **100,** 561. — 4. Becks, H., Asling, C. W., Collins, D. A., Simpson, M. E., Li, C. H., Evans, H. M.: (1948b) Response to pituitary growth hormone and thyroxin of the third metacarpal in the hypophysectomized female rat. Anat. Rec. **101,** 17. — 5. Bielschowsky, F., Bielschowsky, M., Fletcher, E. K.: (1962) Investigations on the role of thyroxine in the development of hepatomas in hypophysectomized rats and pituitary dwarf mice. Brit. J. Cancer **16,** 267. — 6. Casewell, M. V., Fennel, R. H.: (1970) Supernumerary parathyroid structures in the neck and thymus of parathyroidectomized rats and their relationship to recovery from hypocalcaemia. Brit. J. exp. Pathol. **51,** 197. — 7. Dunai, Cs., Oláh, I., Kiss, J.: (1969) Accessory parathyroid tissue in the rat thymus. Acta biol. Acad. Sci. hung. **20,** 193. — 8. Evans, H. M., Simpson, M. E., Pencharz, R. J.: (1939) Relation between the growth promoting effect of the pituitary and thyroid hormone. Endocrinology **25,** 175. — 9. ГЛАГОЛЕВ, А. А. (1933): О геометрических методах количественного минералогического анализа горных пород. Труды Института Прикладной Минералогии No **59,** 1—47. — 10. Goodall, C. M., Gavin, J. B.: (1966) Absence of growth in thyroparathyroidectomized rats treated with thyroid hormones. Acta endocr. (Kbh.) **51,** 315. — 11. Griesbach, W. E., Purves, H. D.: (1945) The significance of the basophil changes in the pituitary accompanying various forms of thyroxin deficiency. Brit. J. exp. Path. **26,** 13. — 12. Groot, C. A., de: (1963) Tail growth in the thyroxine-treated hypophysectomized rats as a sensitive criterion for growth hormone activity. Acta endocr. (Kbh.) **42,** 423. — 13. Gross, J., Pitt-Rivers, R.: (1952) Physiological activity of 3,5,3'-l-triiodothyronine. Lancet **1,** 593. — 14. Hall, B. K.: (1973) Thyroxine and the development of the tibia in the embryonic chick. Anat. Rec. **176,** 49. — 15. Ham, A. W.: (1969) Histology. Blackwell, Oxford. — 16. Hansson, L. I.: (1967) Daily growth in length of diaphysis measured by oxytetracycline in rabbit normally and after medullary plugging. Acta orthop. scand., Suppl., 101. — 17. Herlant, M.: (1964) The cells of the adenohypophysis and their functional significance. Int. Rev. Cytol., **17,** 299. — 18. Hulth, A., Nylander, G.: (1962) Effect of thyroxin and propylthiouracil on the epiphyseal cartilage in growing rats. A histologic and autoradiographic study. Acta Soc. Med. Uppsal. **67,** 219. — 19. Hulth, A., Nylander, G.: (1963) The effect of thiouracil on growing cartilage in the rat. Virchows Arch. path. Anat. **336,** 580. — 20. Klein, E.: (1963) Die thyreogenen Osteopathien. Dtsch. med. Wschr. **88,** 1087. — 21. Koneff, A. A., Scow, R. O., Simpson, M. E., Li, C. H., Evans, H. M.: (1949) Response by the rat thyroparathyroidectomized at birth to growth hormone and thyroxin given separately or in combination. II. Histological changes in the pituitary. Anat. Rec. **104,** 465. — 22. Krompecher, St.: (1958) Die enchondralen Ossifikationsstörungen. Beitr. ges. Arbeitsber. Orthop. **5,** 87. — 23. Lévai, G., Nagy, I. Zs.: (1966) Effect of prolonged thyroxin and methylthiouracil treatment on the epiphyseal cartilage of young rats. Acta morph. Acad. Sci. hung. **14,** 39. — 24. Lévai, G., Móricz, F., Szerze, P., Petrányi, Gy., Jr., Laczkó, J.: (1969) The effect of thyrotropic hormone treatment on the epiphyseal cartilage of the white rat. Acta morph. Acad. Sci. hung. **17,** 7. — 25. McLean, F. C., Urist, M. R.: Bone. Fundamentals of the Physiology of Skeletal Tissue. Univ. of Chicago Press, Chicago 1968. — 26. Meglioli, G. T.: (1966) Osteopathie bei hyperthyreose. Schweiz. Med. Wschr. **96,** 647. — 27. Meites, J., Kragt, C. L.: (1964) Effects of a pituitary homotransplantate and thyroxine on body and mammary growth in immature hypophysectomized rats. Endocrinology **75,** 565. — 28. Módis, L., Földes, I., Nagy, Z.: (1970) Combined staining of alcian green picrofuchsin for the investigation of regenerative enchondral ossification. Acta histochem. **35,** 411. — 29. Noback, C. R., Barnett, J. C., Kuppermann, H. S.: (1949) The time of appearance of ossification centers in the rat is influenced by injections of thyroxin, thiouracil, estradiol and testosterone propionate. Anat. Rec. **103,** 49. — 30. Ray, R. D., Asling, C. W., Simpson, M. E., Evans, H. M.: (1950) Effect

of thyroxin injection on growth and differentiation of the skeleton of hypophysectomized female rats. Anat. Rec. **107,** 253. — 31. RIEKSTNICE, E., ASLING, C. W.: (1966) Thyroxine augmentation of growth hormone-induced enchondral osteogenesis. Proc. Soc. exp. Biol. (N.Y.) **123,** 258. — 32. SALMON, T.: (1938) The effect on the growth rate of thyroparathyroidectomized newborn rats and the subsequent administration of thyroid and anterior hypophysis. Endocrinology **23,** 446. — 33. SCOW, R. O.: (1954) Effect of thyroxine on the weight and composition of muscle, pelt and other tissues in young hypophysectomized rats. Endocrinology, **55,** 344. — 34. SCOW, U. D., SIMPSON, M. E., ASLING, C. W., LI, C. H., EVANS, H. M.: (1949) Response by the rat thyroparathyroidectomized at birth to growth hormone and to thyroxin given separately or in combination. I. General growth and organ changes. Anat. Rec. **104,** 445. — 35. SILBERBERG, M., SILBERBERG, R.: (1938) The effect of thyroid feeding on growth process and retrogressive changes in bone and cartilage of immature guinea pig. Growth **2,** 327. — 36. SILBERBERG, M., SILBERBERG, R.: (1940) Changes in the skeletal tissues of mice following the administration of thyroxin. Growth **4,** 305. — 37. SILBERBERG, R., SILBERBERG, M.: (1954) Skeletal effects of radioiodine induced thyroid deficiency in mice as influenced by sex, age and strain. Amer. J. Anat. **95,** 263. — 38. SIMPSON, M. E., ASLING, C. W., EVANS, H. M.: (1950) Some endocrine influence on skeletal growth and differentiation. Yale J. Biol. Med. **23,** 1. — 39. SISSONS, H. A.: The Growth of Bone. In: The Biochemistry and Physiology of Bone. Ed.: Bourne, G. H. Academic Press, New York 1956. — 40. SISSONS, H. A.: The Growth of Bone. In: The Biochemistry and Physiology of Bone. Ed.: Bourne, G. H. Academic Press, New York 1971. — 41. STURKIE, P. D.: Avian Physiology. Cornell University Press, Ithaca, N. Y. 1965. — 42. TAUROG, A., EVANS, E. S., POTTER, G. D., CHAIKOFF, I. L.: (1960) Plasma I^{131}-thyroxine in hypophysectomized rats injected with radioiodide. Endocrinology **67,** 609. — 43. THORNGREN, K. G., HANSSON, L. I.: (1973a) Cell kinetics of the growth plate in hypophysectomized rats treated with growth hormone and thyroxine. Z. Zellforsch. **142,** 431. — 44. THORNGREN, K. G., HANSSON, I. L.: (1973b) Effect of thyroxine and growth hormone on longitudinal bone growth in the hypophysectomized rat. Acta endocr. (Kbh.) **74,** 24. — 45. WALKER, D. G.: (1957) An assay of the skeletogenic effect of triiodothyronine and its acetic acid analogue in immature rats. Bull. Johns Hopkins Hosp. **101,** 101. — 46. WEINMANN, J., SICHER, H.: Bone and Bones. Fundamentals of Bone Biology. Mosby Co. St Louis, Mo. 1947. — 47. WUTTKE, H., KESSLER, F. J., JUNGER, A.: (1971) Mikrokinetischer Einfluss des thyrotropen Hormons auf die C-Zellen der Schilddrüse. Z. ges. exp. Med. **155,** 20.

DIE WIRKUNG VON TRIJODTHYRONIN- (T_3) UND MERKAPTOIMIDAZOL-BEHANDLUNG SOWIE VON THYREO-PARATHYREOIDEKTOMIE AUF DAS ENCHONDRALE KNOCHENWACHSTUM

G. LÉVAI, S. VARGA, J. GYARMATI Jr. und J. LACZKÓ

Im proximalen Wachstumsorgen der Tibia wachsender Ratten wurden die nach Trijodthyronin- und Merkaptoimidazol-Behandlung sowie nach chirurgischer Thyreoidektomie entstehenden histologischen Veränderungen untersucht. An den histologischen Präparaten wurde mit verschiedenen morphometrischen Messungen festgestellt, daß die Trijodthyronin-Verabreichung das Tempo der Knorpelproliferation und -resorption steigert, was mit der vorübergehenden Beschleunigung des linearen Knochenwachstums einhergeht. Unter der Wirkung von Merkaptoimidazol und nach Tyreoidektomie erfährt hingegen sowohl die Knorpelproliferation als auch die Knorpelresorption eine Hemmung, was zur Verlangsamung des Knochenwachstums führt. Die Ergebnisse werden mit den Literaturangaben verglichen.

ДЕЙСТВИЕ ЛЕЧЕНИЯ ТРИЙОДТИРОНИНОМ И МЕРКАПТОИМИДАЗОЛОМ, А ТАКЖЕ ТИРЕО-ПАРАТИРЕОИДЭКТОМИИ НА ЭНХОНДРАЛЬНЫЙ РОСТ КОСТЕЙ

Г. ЛЕВАИ, Ш. ВАРГА, Й. ДЬАРМАТИ, МЛ., и Й. ЛАЦКО

Авторы изучали в проксимальном органе роста большеберцовой кости растущих крыс гистологические изменения, наблюдаемые под влиянием лечения трийодтиронином и меркаптоимидазолом, а также под влиянием хирургической тиреоидэктомии. При проведении различных морфометрических измерений на гистологических препаратах было установлено, что под влиянием дачи трийодтиронина повышается темп пролиферации всасывания хряща, что сопровождается преходящим ускорением линейного роста костей. Под влиянием лечения меркаптоимидазолом и тиреоидэктомии как пролиферация так и всасывание хряща претерпевают торможение, и это приводит к замедлению роста костей. Полученные результаты сопоставляются с литературными данными.

Dr. Géza Lévai
Dr. Sándor Varga
Dr. János Gyarmaty, Jr.
Dr. Jenő Laczkó

DOTE Anatómiai, Szövet- és Fejlődéstani Intézet,
4012 Debrecen, Hungary

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First Institute of Anatomy, Histology and Embryology, Semmelweis University Medical School, Budapest

BLOOD SUPPLY OF THE RAT HYPOTHALAMUS. III. ANTERIOR REGION OF THE HYPOTHALAMUS

(NUCLEUS SUPRACHIASMATIS, NUCLEUS HYPOTHALAMICUS ANTERIOR, NUCLEUS PERIVENTRICULARIS)

G. AMBACH and M. PALKOVITS

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The arterial and venous blood supply of the anterior hypothalamus was studied by means of double perfusion technique. The angiotopography and cytoarchitecture of the hypothalamus were compared on serial sections by accounting for the three-dimensional coordinates. A detailed description is given of the arteries and veins contributing to the blood supply of the suprachiasmatic, the anterior hypothalamic and the periventricular nuclei. The topography of the arterial and venous trunk on the base surface of the rat diencephalon is described, as well as that of the larger branches which enter from below and pass through the anterior hypothalamus.

Introduction

There are few data on the topography of the arteries and veins of the hypothalamus in experimental animals, particularly in the rat [4, 8, 9]. The majority of earlier works concentrated on the clarification of the structure of portal veins which are of primary importance from the aspect of neuroendocrine regulation, but no data have been published on the vascularity of the individual hypothalamic nuclei.

The vascularity of the hypothalamus — investigated in the rat [1] and in other species — is more or less the same as that of the other regions of the brain, except for the outstanding vascularity of the two magnocellular nuclei: the supraoptic and the paraventricular nuclei. The extremely rich vascularity of these nuclei is no doubt related to their functional role, hence their angiotopography was studied separately from the other vessels of the medial hypothalamus [2, 3].

The method of double perfusion technique allowed to show the arteries and veins separately [2]. By using our detailed map (PALKOVITS, in preparation) of the rat hypothalamus, showing the three-dimensional coordinates of the various nuclei and subdivisions, this method can be applied to the preparation of a survery of the distribution of the hypothalamic arteries and veins. In addition we wished to study:

1. any possible correlation between blood supply and cytologic topography (whether any of the nuclei possesses an isolated blood supply, or the

vascularization of the subdivisions within the various nuclei are identical or different);

2. the relationship between the portal veins and the vessels supplying the other parts of the hypothalamus;

3. the vascular link between the hypothalamus and the neighbouring regions of the brain;

4. how and where the veins of the various hypothalamic nuclei meet and what kind of topographic regularity exists between the arterial blood supply and venous system of the various regions.

In this paper we shall deal with the vascular structure of the anterior part of the medial hypothalamus and, within it, of the suprachiasmatic nucleus, the anterior hypothalamic nucleus and the periventricular nucleus. Vascularization of the other hypothalamic regions will be discussed in another paper (AMBACH and PALKOVITS, in preparation).

Materials and methods

Bicoloured Rotring ink perfusion preparations were made from 35 rats of 150 to 200 g body weight by means of our method described earlier [2]. The highly viscous red ink filled the arteries, the less viscous blue ink the veins. Serial sections of 100 to 400 μ thickness were cut from various planes and projected, by accounting for the space coordinates, on the coordinates of similar dimensions of the cytoarchitecture of the hypothalamus (PALKOVITS, in preparation). Black Indian inks different in viscosity were also used for investigation of the arteries only or of the entire vasculature.

Results

Since several classifications of the hypothalamus are known from the literature, it seemed necessary to determine the spatial extension of the area investigated. The medial hypothalamus extends laterally to the theoretical perpendicular line passing through the fornix, up to the fibres of the medial forebrain bundle, forming the lateral hypothalamus, i.e. up to the group of cells embedded in these fibres. Its upper boundary is the thalamus which practically corresponds to the horizontal passing on top of the 3rd ventricle. Rostrally it is calculated to the preoptic region which corresponds to a distance of 400 to 500 μ behind the bregma. (It has not been settled whether cytologically the nuclei of the preoptic area can be regarded as part of the hypothalamus; in this paper they will be discussed separately.) Caudally, the limit of the medial hypothalamus is the mamillary body which is not a sharp vertical line, but can be drawn dorsally 3.8 mm behind the bregma and at 4.6 on mm the base.

Didactically, the medial hypothalamus can be divided into 3 parts (PALKOVITS, in preparation): 1. *anterior part* of the hypothalamus which contains the suprachiasmatic nucleus (NSC), the anterior hypothalamic nucleus (NHA) consisting of four subdivisions and the

periventricular nucleus (NPE) which is divided from the next region by the retrochiasmatic area (AR); 2. the *middle* (tuberal) *part* whose nuclei are the first three subdivisions of the arcuate nucleus (NA I—III), the ventromedial nucleus (NVM) with seven and the dorsomedial nucleus (NDM) with three subdivisions. The medial eminence (EM) with the portal veins also belongs to this part; 3. the *posterior* (premamillary) *part* is the area between the mamillary body and the infundibulum. Its nuclei are the two posterior subdivisions of the arcuate nucleus (NA IV—V), the dorsal and ventral premamillary nuclei (NPMD and NPMV, respectively), the posterior hypothalamic nucleus (NHP), and the supramamillary nucleus (NSM).*

ARTERIES

The arteries of the medial hypothalamus

The arteries of the hypothalamus originate directly from the basal vascular trunks or branches forming the circulus arteriosus Willisii (see Figs 1A, B). At the anterior boundary of the bridge the *a. basilaris* splits into two *aa. cerebellares superiores* and two *aa. communicantes posteriores*. The latter are S-shaped and join on two sides, 2.5 and 3.0 mm from the midline and 6.0 mm behind the bregma, the *aa. cerebri posteriores* coming from the *a. carotis interna*. The internal carotic artery runs 1.6 to 2.2 mm laterally from the midline and 3.5 mm behind the bregma branches into the posterior cerebral artery which runs backwards on the boundary of the cortex and the cerebral peduncle, taking up the posterior communicating artery.

About 1000—1200 μ behind the bregma the *a. carotis interna* is divided into the *a. cerebri media* and the *a. cerebri anterior*. The *a. cerebri media* runs from its origin 1800 to 2000 μ from the midline forwards and upwards along the boundary of the piriform and parietal cortex. Near its origin the anterior cerebral artery lies below the exterior border of the optic chiasm, then 900 to 1100 μ before the bregma line it turns gradually above the optic nerve and continues its path in the longitudinal fissure of the cerebrum. It branches into the *a. communicans anterior* 800—1000 μ before the bregma line. The bilateral communicating arteries anastomose in the midline into a trunk above the optic nerve. After division the branches of this trunk (*a. hemispherica*) run parallel to the anterior cerebral artery. The branches proceeding towards the hypothalamus are shown in Table I.

Arteries of the suprachiasmatic nucleus

The nucleus is situated at a distance between 500 and 1200 μ behind the bregma, directly on the two sides of the 3rd ventricle above the optic chiasm. Both laterally and upwards its maximum extension is 500 to 550 μ.

* Of the three parts of the medial hypothalamus the present work is concerned with the blood supply of the anterior part; the vessels of the tuberal and premamillary parts will be the subject of forthcoming papers of the same series (AMBACH and PALKOVITS, in preparation).

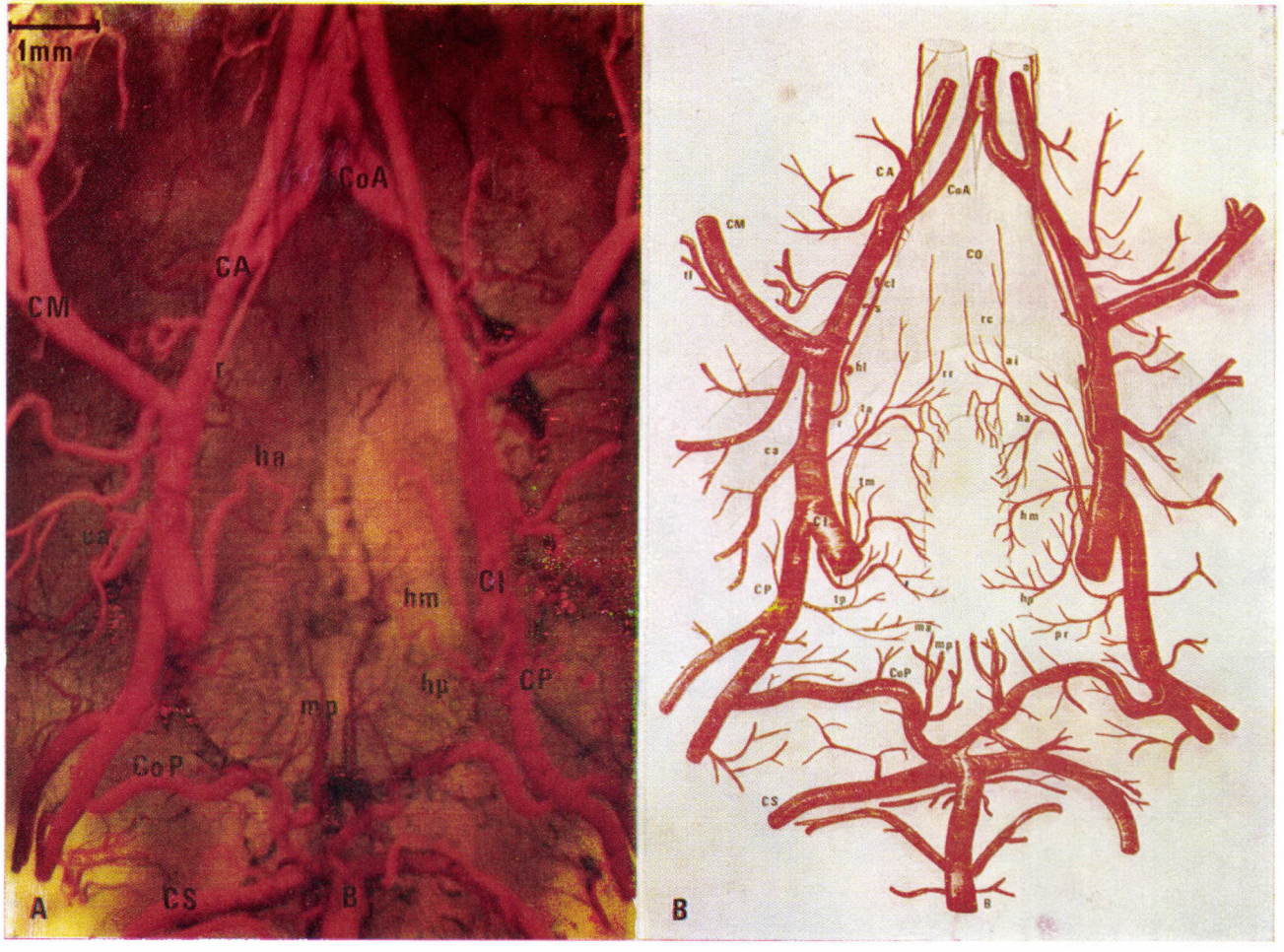
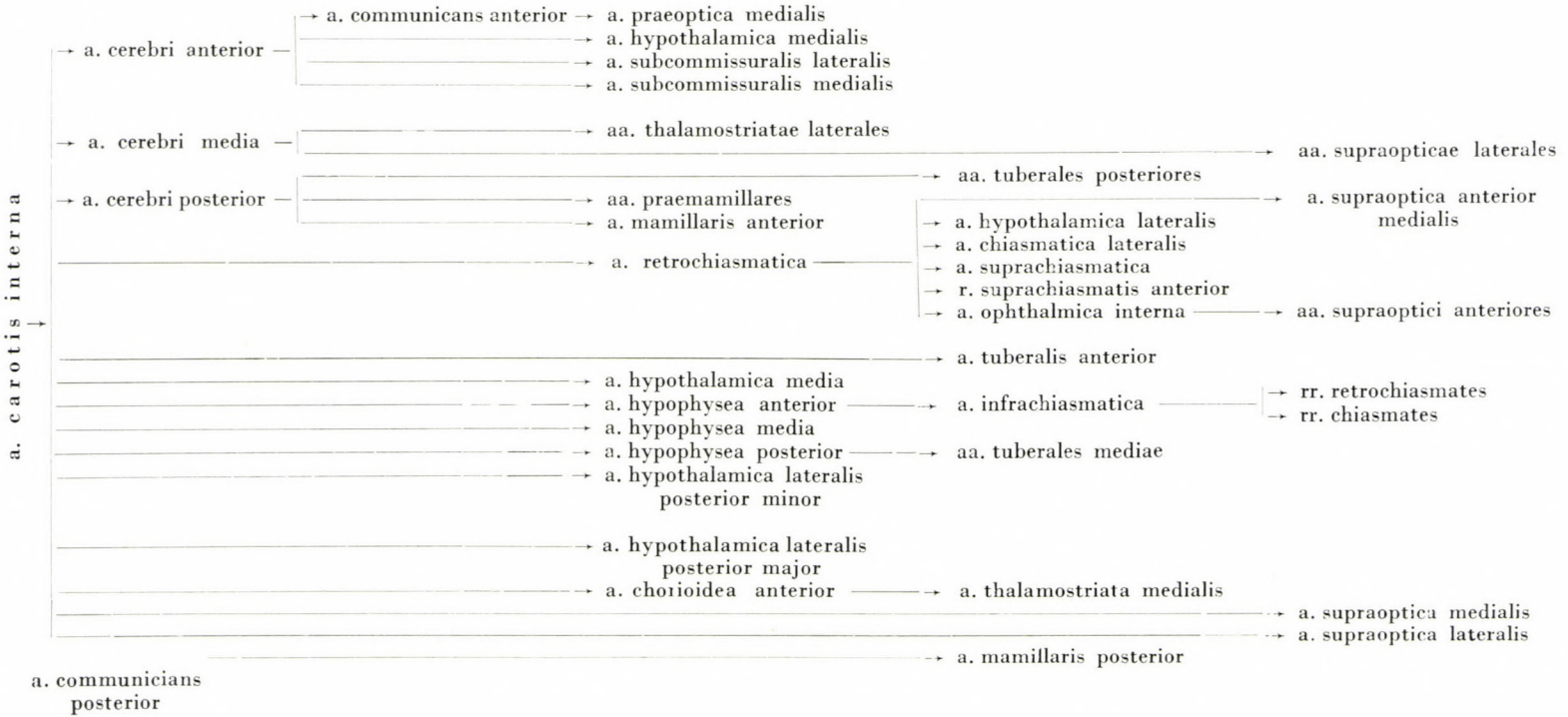


Fig. 1. Arteries of the medial hypothalamus. A. Filled with Rotring ink. B. Arteries on the basal surface of the diencephalon. Abbreviations see in text

Table I

Hypothalamic arteries



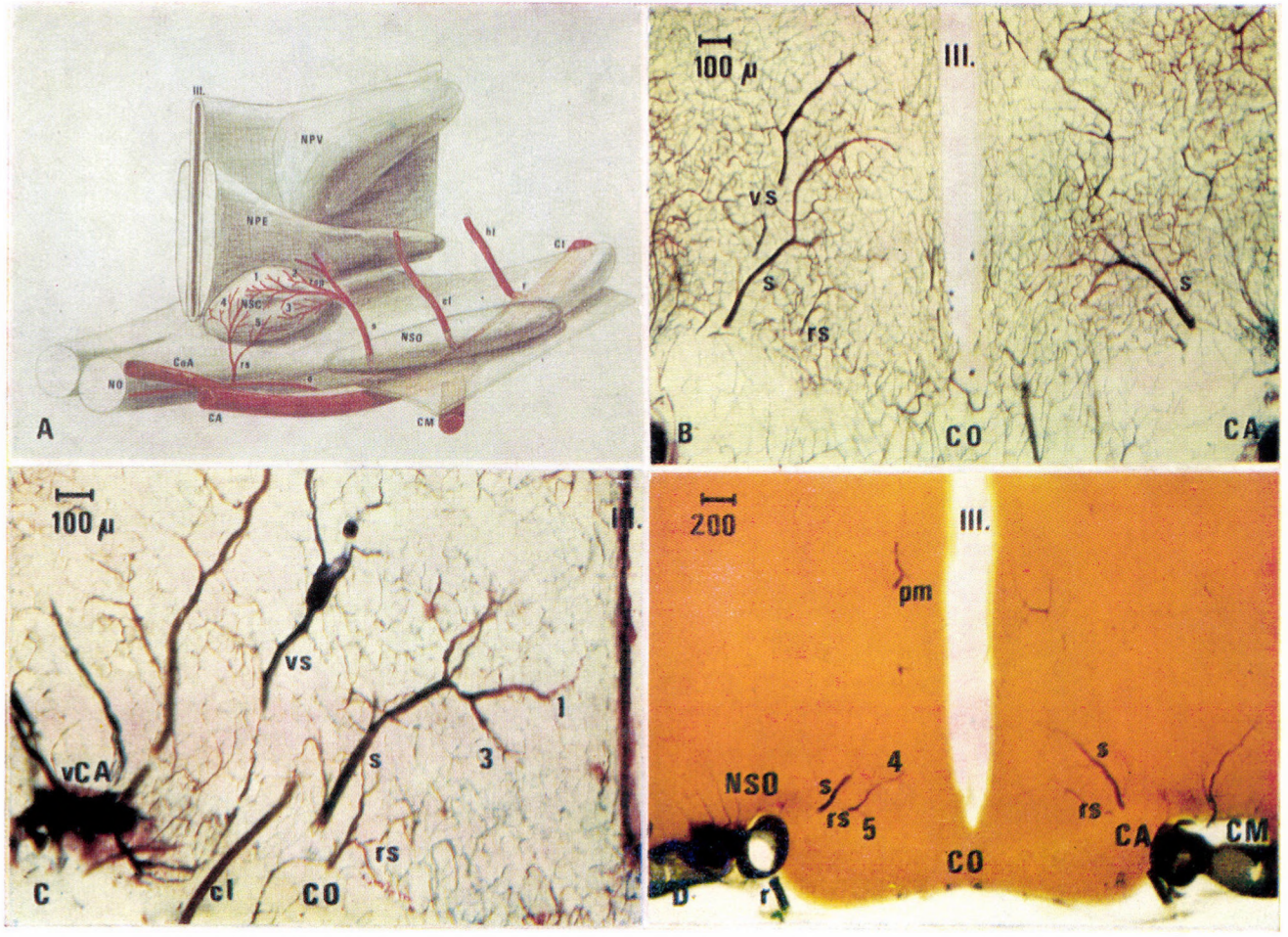


Fig. 2. Arteries of the suprachiasmatic nucleus. Numeration of vessels: 1. r. anterior, 2. r. posterior, 3. r. posterior inferior, 4. r. anterior superior, 5. r. anterior. A. System of suprachiasmatic artery and the area supplied by it. B. Course of suprachiasmatic artery. C. Terminal branches of suprachiasmatic artery. D. Course and branches of ramus suprachiasmaticus. Abbreviations see in text

The nucleus is supplied by the suprachiasmatic artery originating from the retrochiasmatic artery and by the medial branches of the suprachiasmatic branch (Fig. 2A).

The *a. suprachiasmatica*, originating from the *a. retrochiasmatica* 600 μ behind the bregma passes laterally towards the middle through the lateral third of the optic chiasm, then arching upwards and backwards it splits into its main branches in the anterior region of the hypothalamus, 800 to 900 μ behind the bregma (Figs 2A, B). The branch proceeding to the area of the anterior hypothalamic nucleus is the continuation of the main trunk which supplies this group of nuclei and their immediate surroundings. The middle branch runs in the direction of the ventricle and participates in the supply of the periventricular nucleus. The r. paraventricularis inferior running to the anterior part of the paraventricular nucleus originates from this branch [3]. The inferior branches of the suprachiasmatic artery supply the NSC. The *r. posterior superior* reaches the nucleus at its lateral superior part and divides into an anterior and posterior branch. The two branches supply together the upper two-thirds of the nucleus (Figs 2A—C). The *r. posterior inferior* runs from lateral to the nucleus supplying the ventral-posterior two third of the NSC (Figs 2A, B). The branches of the suprachiasmatic artery (mainly the superior ones) have terminals also in the anterior third of the nucleus which beside the r. suprachiasmaticus play a secondary role in this area.

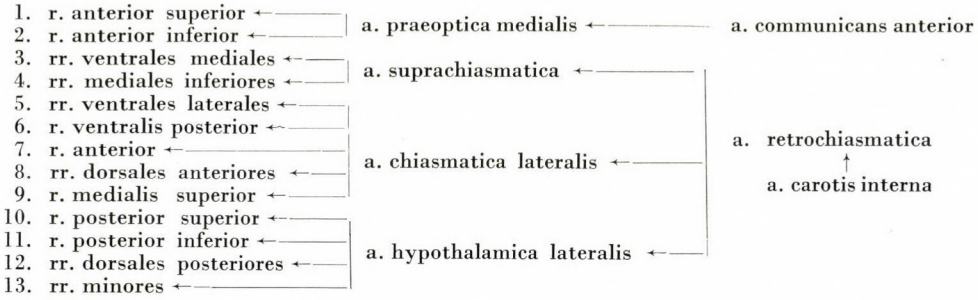
The *ramus suprachiasmaticus* originates directly from the retrochiasmatic artery 300 to 400 μ behind the bregma line, and supplies blood only to the NSC (suprachiasmatic nucleus). This branch is narrower than the suprachiasmatic artery and runs before and medially to the latter above the optic chiasm. Leaving the optic chiasm it proceeds to the anterior part of the NSC where it splits into two branches (Figs 2A, D). The stronger *r. anterior superior* is supplying the anterior superior third of the nucleus in an area of 500 to 800 μ behind the bregma. The *ramus anterior* runs frontally to the lower part of the nucleus. The area supplied by this branch varies, the branch splits as a rule in the anterior inferior third of the NSC. If the lower branch of the suprachiasmatic artery is narrow, its terminal divisions may be observed in the posterior inferior third of the nucleus.

Arteries of the anterior hypothalamic nucleus

The anterior hypothalamic nucleus is the main group of nuclei in the anterior hypothalamus. Rostrally, the nucleus consists of ventral, medial and dorsal subdivisions which begin 500 μ behind the bregma and extend 1100 to 1300 μ backward. The fourth subdivision (caudal part) starts behind the others (from 1100—2000μ) and fills the space below the paraventricular nucleus laterally from the 3rd ventricle to the medial forebrain bundle. The ante-

Table II

Arteries of the anterior hypothalamic nucleus



rior hypothalamic nucleus is supplied by the medial preoptic artery and the branches of the retrochiasmatic artery: the suprachiasmatic, the lateral chiasmatic and lateral hypothalamic arteries (Figs 3A–C, 4A–C, 5A–C; Table II).

The *rami anterior superior* and *anterior inferior* originate from the *a. preoptica medialis* (a branch of the anterior communicating artery, Figs 3A, B). The superior branch runs horizontally backwards and splits in the anterior superior section of the medial part of the anterior hypothalamic nucleus. Its course can be traced dorsally to a distance of 700 to 800 μ. The inferior branch also supplies the medial part running dorsally at the height of the middle and lower third of the 3rd ventricle.

The main trunk of the *a. suprachiasmatica* passes through the lower third, less often through the middle, of the ventral part of the anterior hypothalamic nucleus to the area of the medial part of the NHA (Figs 2B, 3B, C). The three or four branches originating opposite the rr. posterior and superior running to the suprachiasmatic nucleus, the *rr. ventrales mediales*, supply the lower medial section of the ventral part of the NHA to a distance of 1000 μ (Figs 2B, C, 3A, C). Usually one of the branches is stronger and splits in the medial part of the NHA which is (comparatively) poor in cells. Another two to three branches of the same artery provide by means of the *rr. mediales inferiores* the frontal section of the medial part of the anterior hypothalamic nucleus to a distance of 700 to 1100 μ (Figs 2C, 3A, C).

The *a. chiasmatica lateralis* runs through the NHA at 900 to 1100 μ behind the bregma to the paraventricular nucleus (NPV), ramifying in its course and sending branches to the NHA (Figs 3A, 4A; Table II).

The *rami ventrales laterales* proceed ventromedially (Figs 3A, 4B) into the external half of the nucleus and can be traced dorsally to a distance of 1300 μ. They appear to overlap with the branches of the suprachiasmatic artery. The *ramus ventralis posterior* runs dorsally and supplies the posterior end of the ventral part (Figs 3A, 4C). The *ramus anterior* originates from the section of the *a. chiasmatica lateralis* which lies between the ventral and dorsal

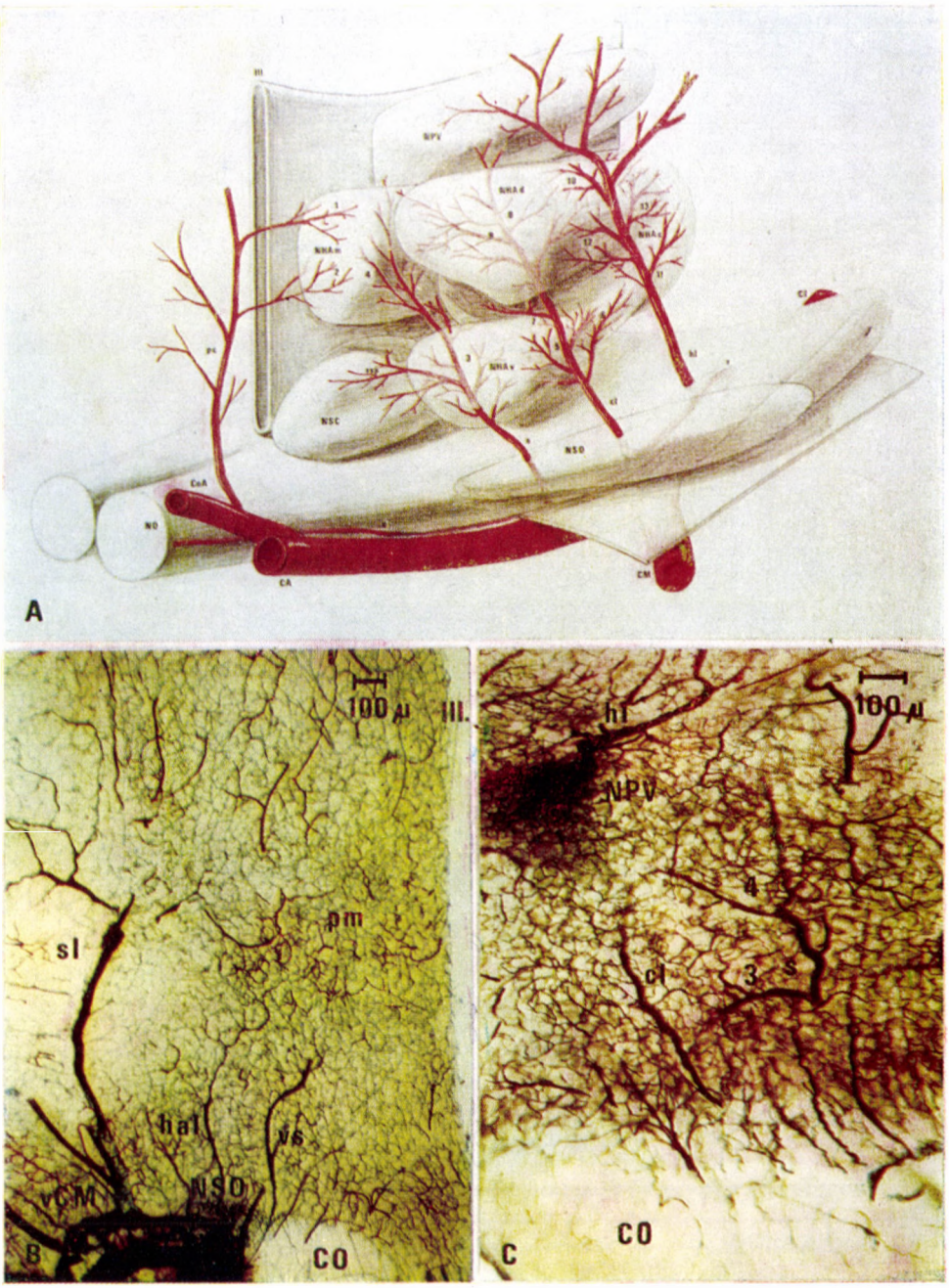


Fig. 3. Arteries of the anterior hypothalamic nucleus. Abbreviations see in text. Indication of vessels see Table II. A. Arterial system of anterior hypothalamic nucleus. B. Terminals of the rr. anteriores of a. preoptica medialis. C. Terminal branches of the a. suprachiasmatica in the anterior hypothalamic nucleus

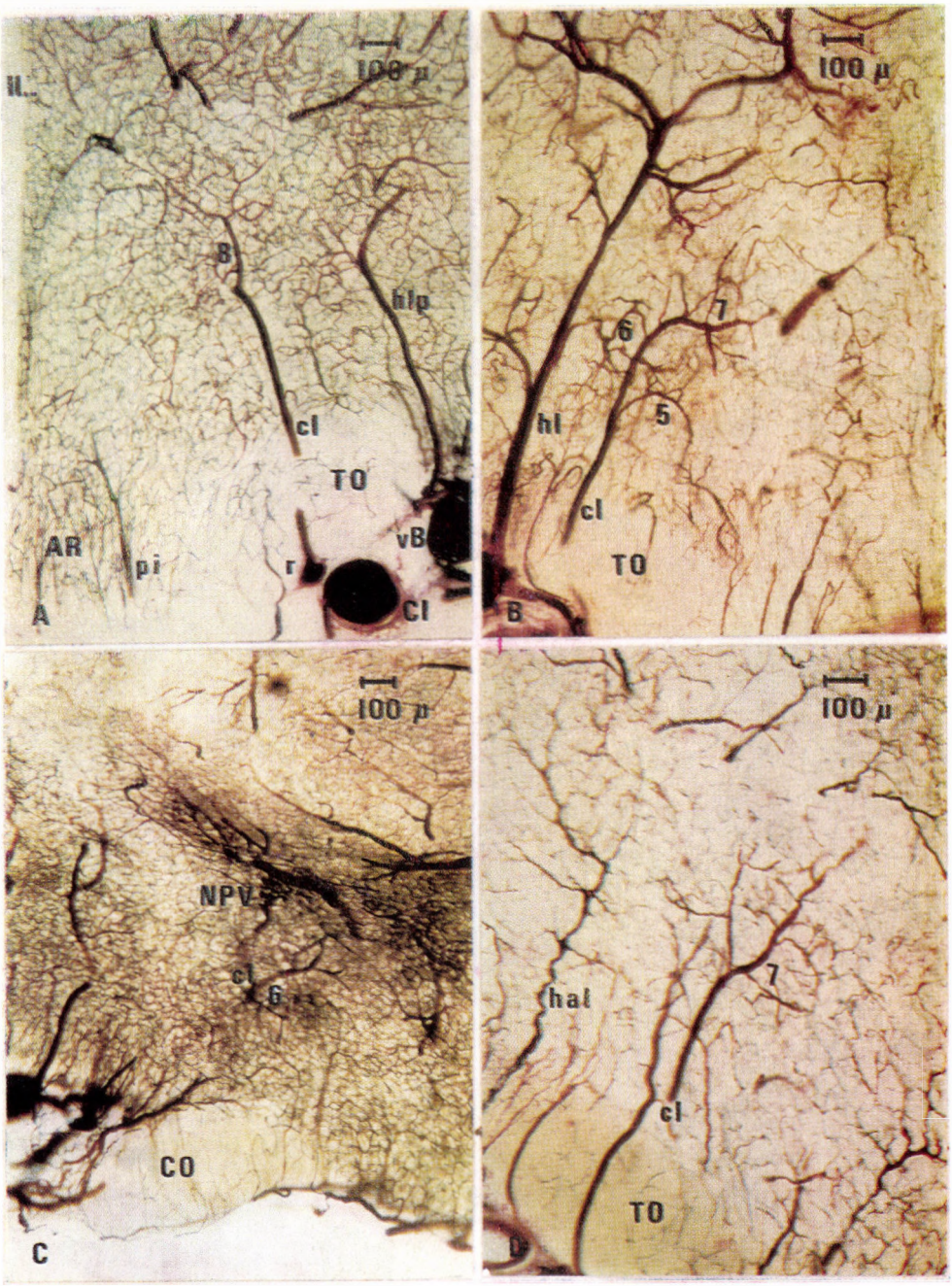


Fig. 4. The a. chiasmatica lateralis and its branches. Indication of vessels see in Table II and Abbreviations. A. Course of lateral chiasmatic artery. B. Rr. ventrales laterales. C. R. ventralis posterior. D. R. anterior

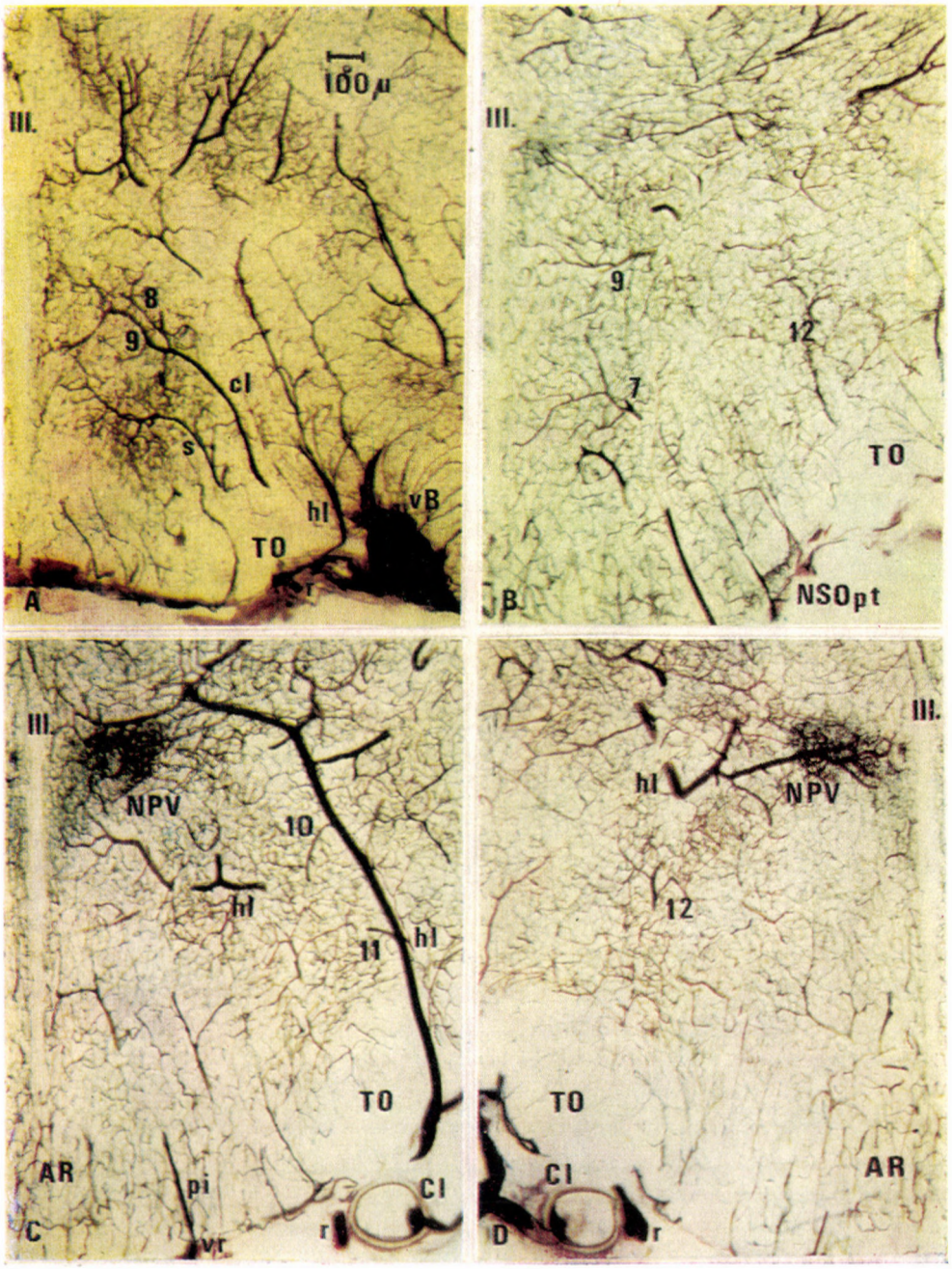


Fig. 5. Course of a. hypothalamica lateralis and its branches. Indication of vessels see in Table II. Abbreviations see in text. A. Rr. dorsales anteriores. B. R. medialis superior. C. R. posterior superior and inferior. D. Rr. dorsales posteriores

parts of the NHA and runs to the frontal section of the caudal part of the NHA where it ramifies into two or three short branches (Figs 3A, 4D). The *rr. dorsales anteriores* can be traced to the posterior section of the dorsal part of the NHA. Several overlappings can be observed with the posterior dorsal branches coming from the lateral hypothalamic artery (Figs 3A, 4A, 5A). The *a. chiasmatica lateralis* usually sends a stronger branch — the *ramus medialis superior* — proceeding laterally from above into the nucleus. This branch ends above and behind the area in which the suprachiasmatic artery ramifies (Figs 3A, C, 5B).

The *a. hypothalamica lateralis* supplies the caudal part of the anterior hypothalamic nucleus. It enters the hypothalamus at 1200 μ behind the bregma and runs then steeply up to the fornix (Figs 3A, 4B, 5C) where it delivers the main arteries of the paraventricular nucleus and finally ramifies in the ventral thalamus. Its branches runnnig to the NHA (Table II) originate from the vascular section between the suprachiasmatic nucleus and the fornix; the upper one, the *ramus posterior superior*, supplies the half of the nucleus under the paraventricular nucleus, while the lower branch, the *ramus posterior inferior*, supplies the inferior half of the latter (Figs 3A, 4B, 5B, C). Short, minor branches — *rr. minores* — are found along the entire length of the vessel. Two branches, the *rr. dorsales posteriores*, run frontally into the posterior end of the dorsal part of the NHA and ramify behind the bregma at a distance of 900 to 1100 μ (Figs 3A, 4A).

Arteries of the periventricular nucleus

The hypothalamic periventricular nucleus (NPE) is situated on the two sides of the frontal part of the 3rd ventricle and is some cell layers thick. It is the (periventricular) caudal continuation of the preoptic periventricular nucleus. There is no sharp transition between the two nuclei which can be separated 400 μ behind the bregma. First, for a length of 400 to 500 μ the nucleus extends dorsally up to the height of the top of the ventricle then gradually narrows down and can be found only below. It can be traced dorsally 2000 μ to the appearance of the medial eminence. The periventricular nucleus is supplied by the terminal branches of the arteries of the medial preoptic, the suprachiasmatic and the anterior hypothalamic nuclei, as well as by the branches of the anterior hypophyseal artery (Figs 3A, 6A). The terminal branches of the *a. preoptica medialis* supply the upper (*rr. anteriores superiores*) and middle third (*rr. anteriores mediae*) to a distance of 700 to 800 μ behind the bregma (Figs 6A, B). The lower third is supplied by the *rr. anteriores inferiores* (Fig. 6A), and the oro-caudal middle part of the nucleus (800 to 1000 μ) by the branches of the suprachiasmatic artery. Below this area are ending the branches of the rami posteriores suprachiasmates (*rr. mediales inferiores*) and

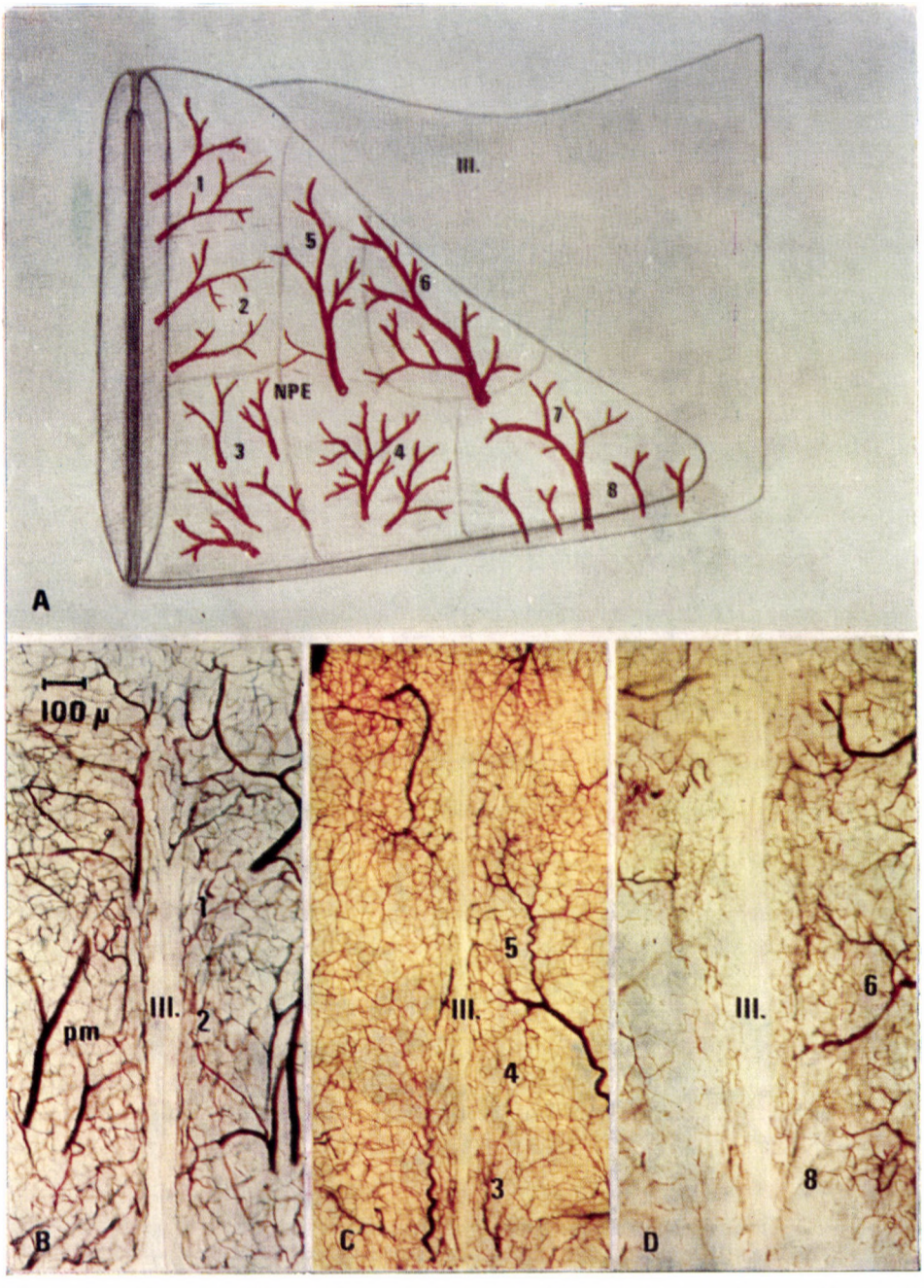


Fig. 6. Arterial supply of the periventricular nucleus. Abbreviations seen in text. Numeration of vessels: 1. rr. anteriores superiores, 2. rr. anteriores mediae, 3. rr. anteriores inferiores, 4. rr. mediales inferiores, 5. rr. mediales superiores, 6. r. periventricularis posterior superior, 7. r. periventricularis posterior major, 8. rr. periventricularis posteriores minores. A. Topography of arteries supplying the periventricular nucleus. B. A. praeoptica medialis rr. anteriores. C. A. suprachiasmatica rr. mediales. D. Rr. periventriculares posteriores

there are above the *rr. mediales superiores* from the suprachiasmatic artery (Figs 6A, C). These latter branches supply, together with the *ramus periventricularis posterior superior* coming laterally in an arch from the lateral chiasmatic artery, the upper part of the periventricular nucleus dorsally to a distance of 1400 μ (Figs 6A, D). The dorsal part of the NPE above the retrochiasmatic area is supplied by branches of the anterior hypophyseal artery which proceed towards the lower dorsal surface of the optic chiasm; the *ramus periventricularis posterior major* runs steeply up the side of the ventricle 1800 μ behind the bregma and ramifies in the narrower dorsal part of the NPE. It supplies only the nucleus and stays all along beside the ventricle. The *rr. periventriculares posteriores minores* are the terminal branches of parallel arteries penetrating into retrochiasmatic area. These branches ramify in the lower dorsal third of the NPE (Figs 6A, D).

VEINS

Veins of the medial hypothalamus

The veins of the hypothalamus meet in the *vena cerebri anterior* and the *vena basalis*, i.e. in the branches of the major venous trunks (Figs 7A, B). The *v. basalis* is the strongest vein of the cerebral base. It originates from the juncture of the *v. cerebri anterior* and *media* 500 to 1000 μ behind the bregma from where it runs backwards on the exterior side of the *circulus arteriosus Willisii*. It by-passes the main branches of the circle on their cortical side (Fig 7B). The course of the *vena cerebri anterior* follows that of the anterior cerebral artery. It runs from the longitudinal fissure of the cerebrum on the external side of the anterior cerebral artery between the optic nerve and the cortex to the frontal side of the division of the *a. carotis interna* (Figs 7A, B), where it merges with the *vena cerebri media* with which it forms the basal vein. Above the optic nerve it anastomoses with several branches with the contralateral vein. Several variations of the middle cerebral vein have been observed. In almost one third of the cases this is a double vein, when a stronger vein appears in front of the middle cerebral artery and another runs 500 to 1000 μ frontally into the anterior cerebral vein. Occasionally, the vein runs behind the middle cerebral artery to the cerebral base. Laterally from the internal carotid artery, 4000 to 5500 μ behind the bregma, the basal vein takes up from the medial side the *vena interpeduncularis anterior* which forms with its contralateral and the *vena interpeduncularis posterior* an X-shaped anastomosis in the fossa interpeduncularis (Fig. 7A). The posterior interpeduncular vein lies closely on top of the cerebral peduncle and joins the *vena cerebri posterior* which runs forward on the external side of the posterior cerebral artery and meets the vena basalis 4500 μ behind the bregma (Figs 7A, B). These trunks are draining the venous blood of the hypothalamus (Table III).

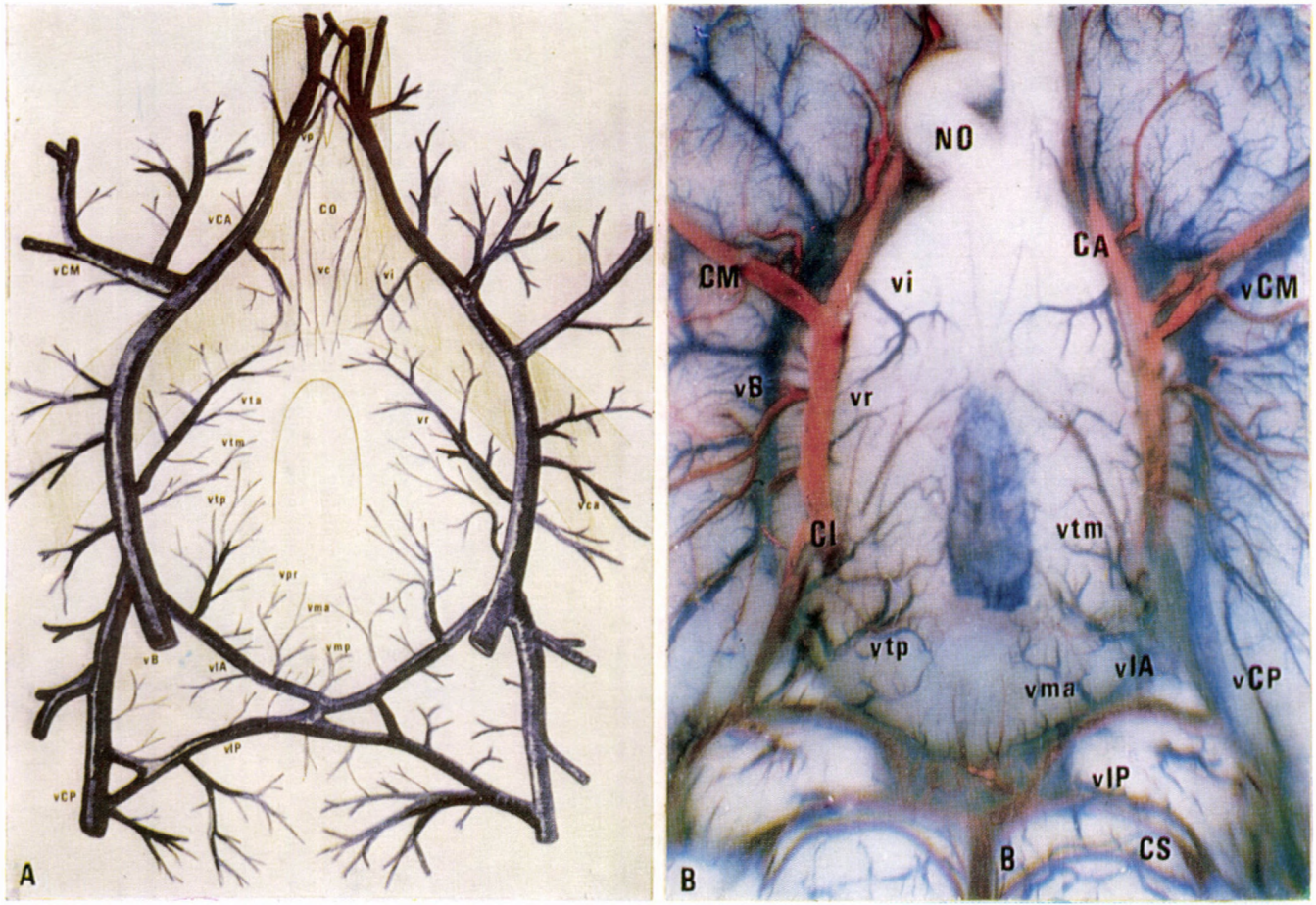
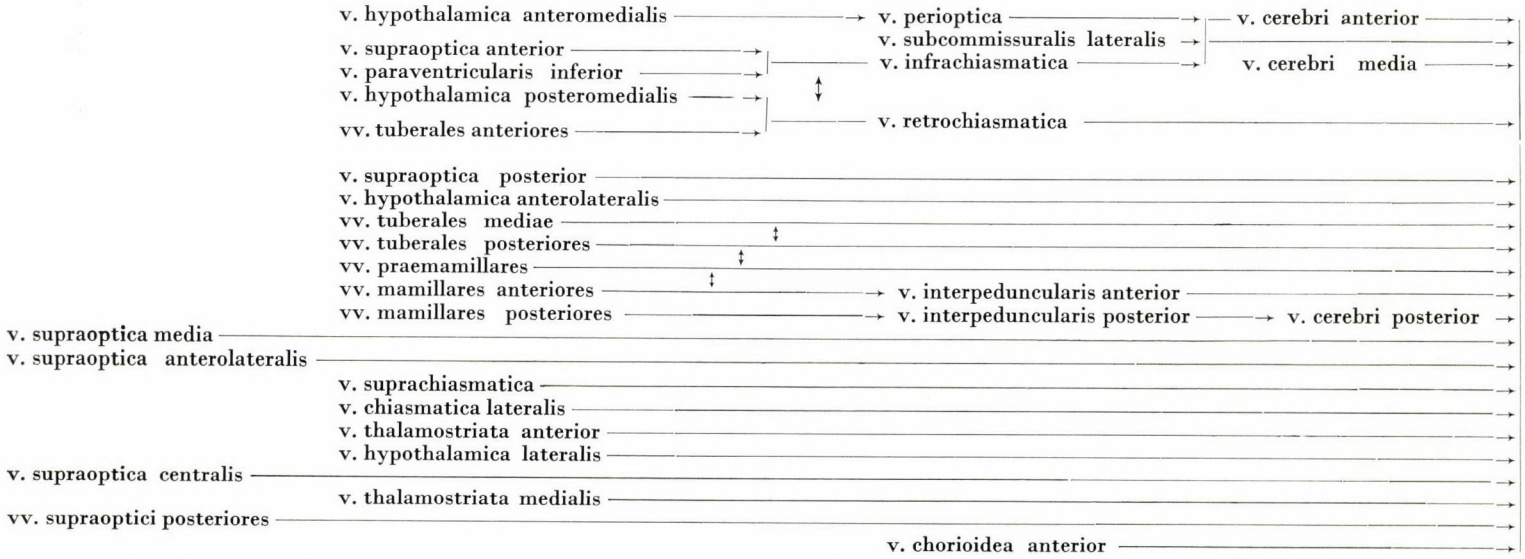


Fig. 7. Veins on the basal surface of the diencephalon. Abbreviations see in text. A. Venous system of the medial hypothalamus. B. Veins on the basal surface of the hypothalamus

Table III

Hypothalamic veins



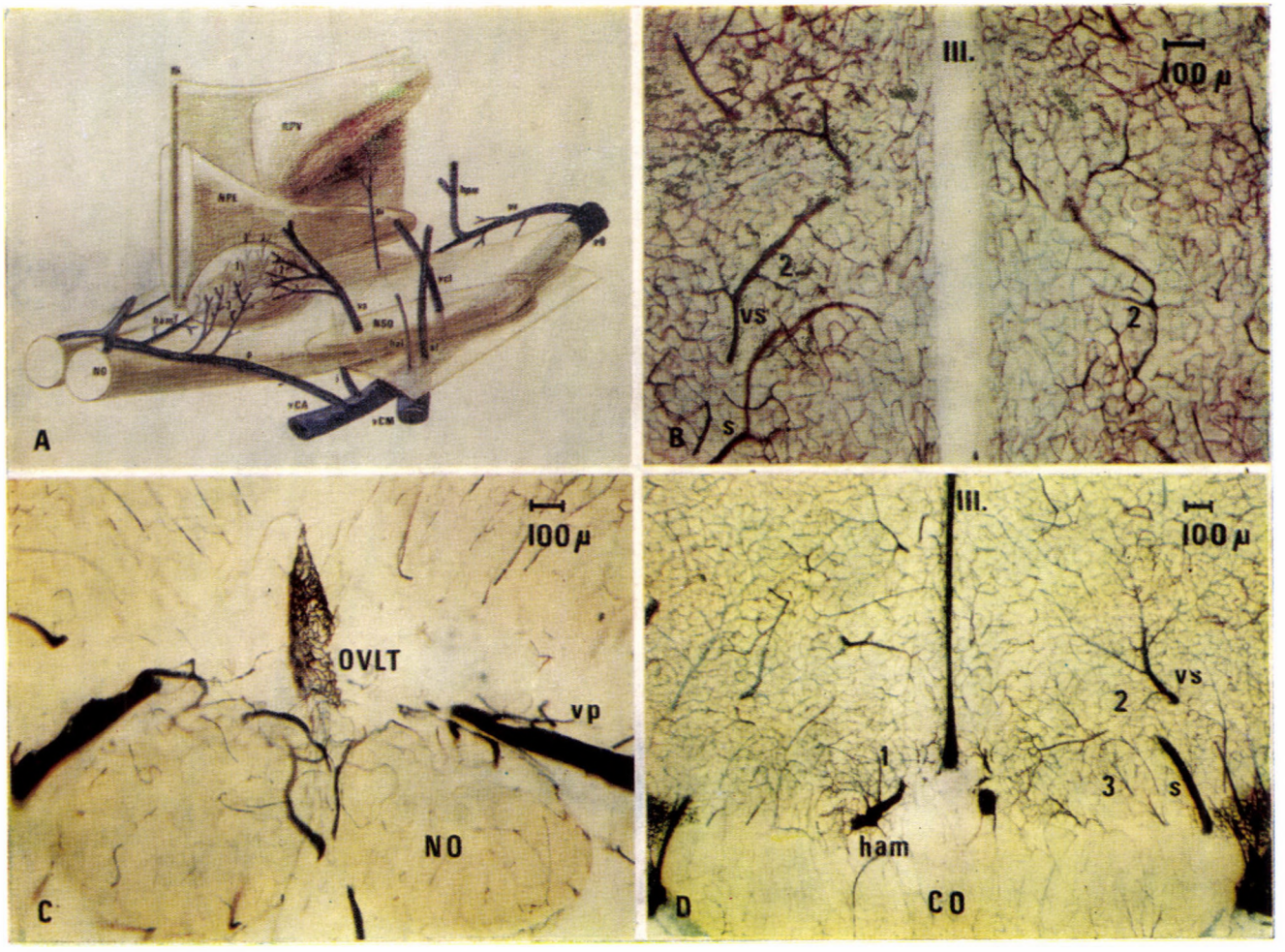


Fig. 8. Veins of the suprachiasmatic nucleus. Numeration of vessels: 1. rr. suprachiasmates, 2. rr. posteriores, 3. rr. anteriores. A. Veins of the NSC. B. The v. suprachiasmatica and its branches. C. V. perioptica. D. Rr. suprachiasmates

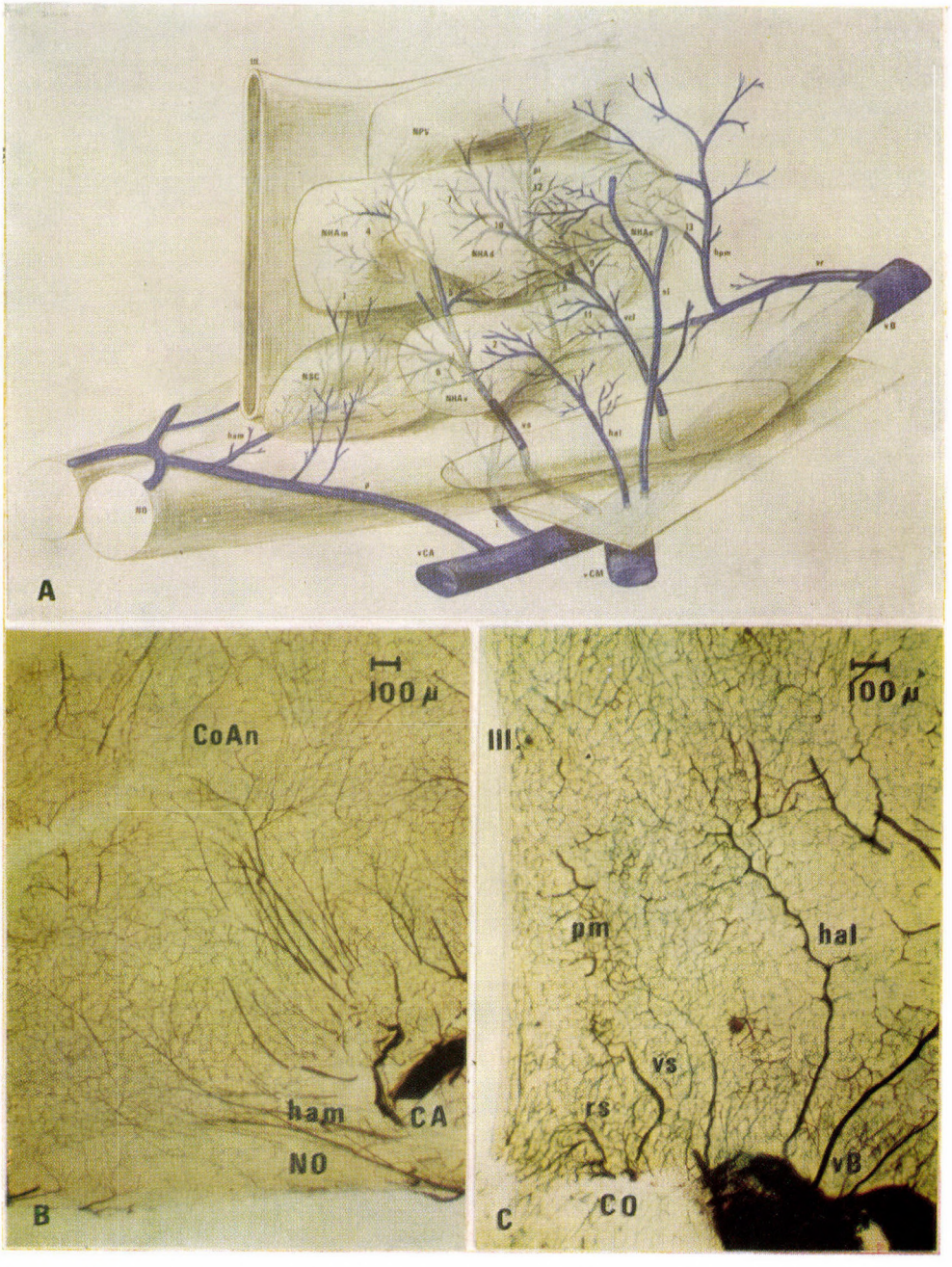


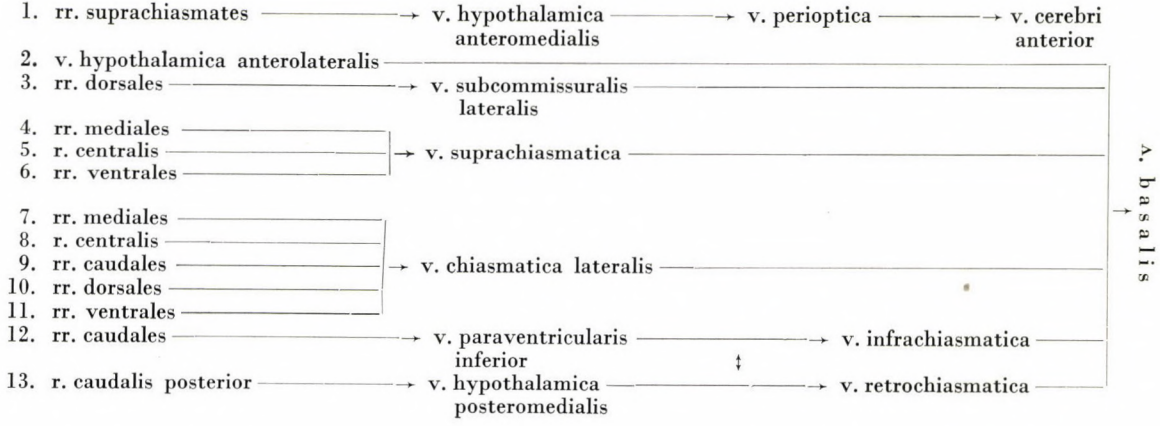
Fig. 9. Veins of the anterior hypothalamic nucleus. Indication of vessels see in Table IV and Abbreviations. A. Veins of the NHA. B. V. hypothalamica anteromedialis. C. V. hypothalamica anterolateralis

Veins of the suprachiasmatic nucleus

The blood of the suprachiasmatic nucleus (NSC) is led off by the branches of the suprachiasmatic and perioptic veins (Figs 8A, 9A). The *v. suprachiasmatica* runs downwards from the anterior paraventricular branch and forwards somewhat laterally from the suprachiasmatic artery to the supraoptic nucleus [3]. At 600 μ the vessel pierces the nucleus and flows into the basal vein. In addition to its branches coming from the NSC it has important branches from the area of the periventricular and anterior hypothalamic nuclei. Its branches originate from the external dorsal half of the nucleus (*rr. posteriores*). In general there are three or four veins which join in the lower part of the anterior hypothalamic nucleus into one or two branches and meet the suprachiasmatic vein (Figs 8A, B, 9A). Considerable venous anastomoses are formed before the optic chiasma, frontally to the optic nerves. The two *vena perioptica* participate in the anastomosis. These veins lie closely on the optic chiasm and run on its cerebral side. (Figs 8A, B). They meet the anterior cerebral vein frontally to the infrachiasmatic vein. The two contralateral perioptic veins meet in the midline by a short anastomosis. The anastomosis is situated near the organon vasculosum laminae terminalis, in the midline 300 to 400 μ before the bregma, in the sulcus in which the two optic nerves meet. From here a vein runs frontally and meets the anterior cerebral vein or a stronger vein joining the latter in the longitudinal fissure of the cerebrum. In addition to the veins of the NSC, the perioptic vein collects the branches originating in the area around and below the anterior commissure, in the preoptic region and in the optic chiasm. Its branches are the overwhelming majority of the branches of the *vena hypothalamica anteromedialis* that originate in the NSC (rr. suprachiasmates), but as it proceeds it takes up minor branches also from the preoptic region. It originates from 5 to 7 minor branches in the dorsal part of the NSC, these branches are then joined by one or two branches from the NPE and NHAm. All these run downwards within the nucleus, then arch forwards below the nucleus forming a common trunk (Figs 8A, D, 9A, B). The small branches originating from the medial third of the anterior half of the nucleus reinforce by their fan-like structure the anteromedial hypothalamic vein. This vein proceeds all along the inferior boundary of the 3rd ventricle and joins the perioptic vein approximately at the height of the bregma (Figs 8A, 9A). The *rr. anteriores* are two or three long veins accompanying the artery (r. suprachiasmaticus) (Fig. 8A). They originate in the anterior external part of the nucleus, run laterally immediately above the optic chiasma and reach the perioptic vein 200 to 300 μ behind the bregma. Quite often two arteries are present, and the veins run always below them.

Table IV

Veins of the anterior hypothalamic nucleus



Veins of the anterior hypothalamic nucleus

The blood of the anterior hypothalamic nucleus is collected by the following veins (Fig 9A; Table IV).

The *v. hypothalamica anteromedialis* originates with one or two longer branches in the antero-inferior section of the medial part the NHA. Below the nucleus they join the rr. suprachiasmates in the area of the NSC (Figs 9A, 8D).

The branches of the *v. hypothalamica anterolateralis* originate in the antero-external section of the ventral part of the NHA, 600 to 1000 μ behind the bregma. The vein runs laterally, by-passes the NSC and meets the vena basalis. It receives outside the nucleus a few thin branches only from the area below the NHA (Fig. 9C).

The upper branches of the *v. subcommissuralis lateralis* originate in the area of the ascending fibres of the stria medullaris. They run laterally to the dorsal part of the NHA, 600 to 900 μ behind the bregma, and meet the basal vein or the anterior cerebral vein near the margin of the NSO. Its lower (dorsal) branches originate in the exterior third of the dorsal part of the NHA and enter the vein laterally (Figs 9A, 10A, 3B).

The *vena suprachiasmatica* collects some of the veins of the NSC, NPV, NPE and NHA. It runs laterally in the area of the NHA, penetrates into the NSO and finally joins the basal vein (Figs 8A, B, 9A, 10B). It takes up branches directly from the medial part of the NHA (*rr. mediales*) and indirectly by the mediation of the ramus paraventricularis which passes through the nucleus (Fig. 9A). The *ramus centralis* collecting the veins from the middle part of the nucleus meets the suprachiasmatic vein dorsally above the NHAv. From the anterior part of the NHAv as a rule 3 or 4 ventral branches reach the suprachiasmatic vein (*rr. ventrales*).

The vena chiasmatica lateralis starts with rr. paraventriculares anterolaterales and runs upwards from the NPV and laterally between the dorsal and ventral parts of the NHA to the NSO (Figs 9A, 10B). It passes through the NSO and meets the basal vein. This vein receives many branches from the NHA: three to four *rr. mediales* originating from the dorsal part of the middle part of the anterior hypothalamic nucleus (NHAm) and running laterally, meet the paraventricular branches (Fig. 9A). The *r. centralis* is usually smaller, originating from the middle area of the nucleus poor in cells and proceeding laterally backwards it meets the lateral chiasmatic vein. The *rr. caudales* originate in the middle section of the anterior caudal part of the NHA. These 2 to 4 branches meet the suprachiasmatic vein dorsally. The 5 or 6 small *rr. dorsales* on the dorsal part of the anterior hypothalamic nucleus (NHAd) run downwards and meet side by side the lateral chiasmatic vein. An anterior and a posterior group of branches can be observed. The 3 or 4

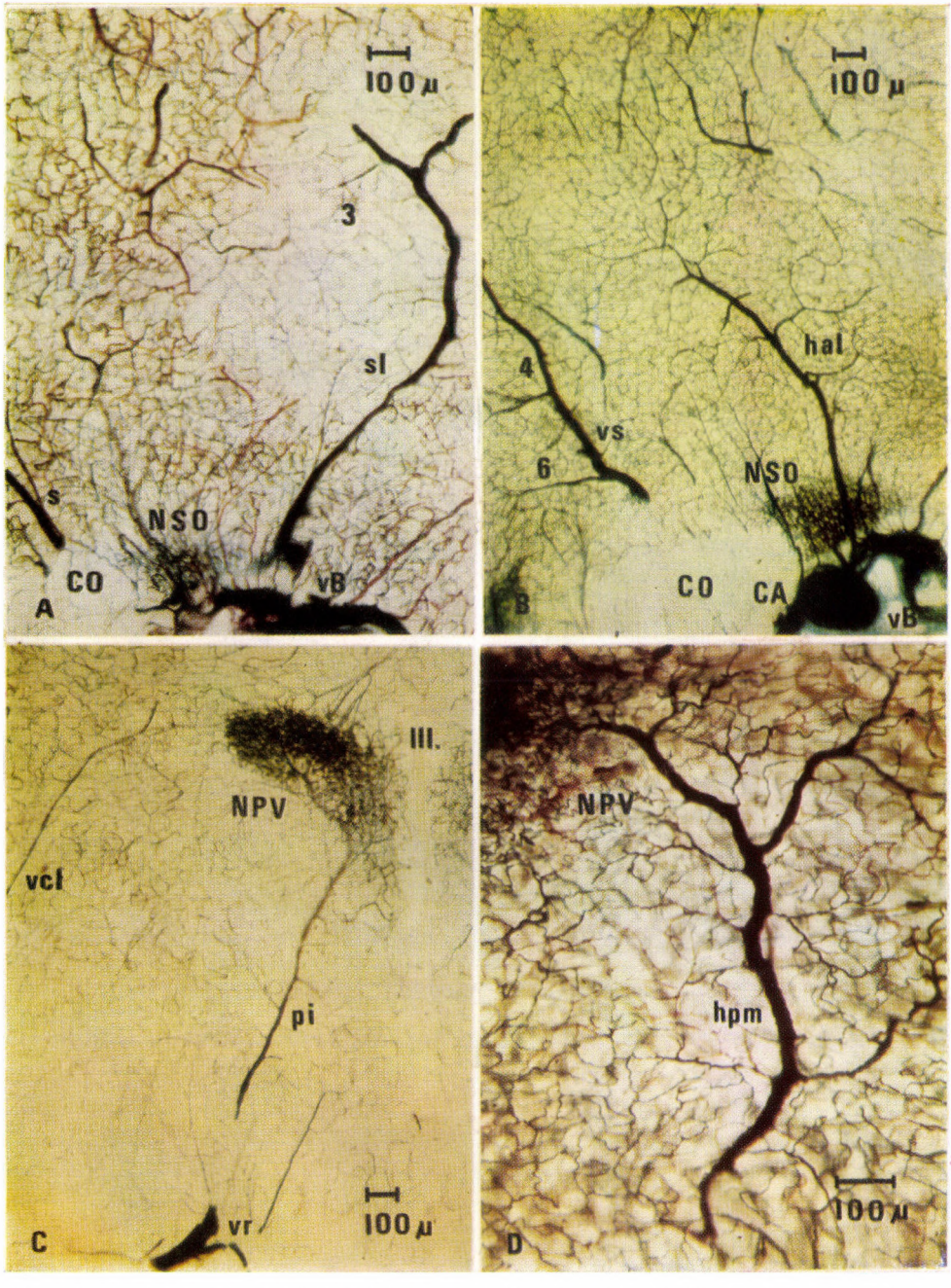


Fig. 10. A. The v. subcommissuralis lateralis and its branches. B. The v. chiasmatica lateralis and its branches. C. The v. paraventricularis inferior and its branches. D. The v. hypothalamica posteromedialis and its branches. Abbreviations see in text. Indication of vessels see Table IV

vv. ventrales originate from the dorsal, lateral and superior parts of the NHAv. After by-passing its ventral part or running through its external part, they are taken up by the lateral chiasmatic vein (Figs 9A, 10B).

The *vena paraventricularis inferior* runs between the medial caudal part of the NHA and the ventricle into the infrachiasmatic vein. Its 4 or 5 small caudal branches issue on the ventricular side of the nucleus and collect in the medial third of the NHAc (Fig. 10C).

The branches of the *vena hypothalamica posteromedialis* collect the blood from the remaining greater part of the NHAc. This vein, proceeding downwards and somewhat laterally, meets about 2000 μ behind the bregma and 700 to 800 μ from the midline the retrochiasmatic vein (Fig 10D). It runs either in the area between the ventromedial, the dorsomedial and the anterior hypothalamic nuclei, or in the posterior section of the dorsal part the NHA and collects 4 to 5 substantial branches from the caudal part of the NHA. In two thirds of the cases a stronger branch (*r. caudalis posterior*) can be observed originating from the external third of this part of the nucleus (Figs 9A, 10D). In other cases this branch runs as an independent vein into the retrochiasmatic vein.

Veins of the periventricular nucleus

The small veins of the periventricular nucleus (NPE) are collected by the veins of the neighbouring nuclei. The nucleus has no independent vein of its own. The blood of the NPE is drained by the anteromedial hypothalamic, the suprachiasmatic, the lateral chiasmatic, the inferior paraventricular veins and the medial retrochiasmatic branches, as well as the thalamic vein (Figs 8A, 9A, 11A). The blood of the inferior anterior part of the nucleus representing the area next to the NSC, up to 1400 μ behind the bregma, is collected by the branches of the anteromedial hypothalamic vein. These are small venous branches leading to the territory of the NSC. Above the suprachiasmatic nucleus, 3 or 4 branches (*rr. periventriculares anteriores*), spread fan-like and leading into the suprachiasmatic vein, are visible. They supply the anterior half of the middle third of the periventricular nucleus, an area 1200 to 1300 μ from the frontal part of the nucleus (Figs 11A, B). In some cases the anterior branches convey also the blood of the fine and long veins running along the 3rd cerebral ventricle backwards and upwards from the organon vasculosum laminae terminalis. The branches of the lateral chiasmatic vein (*rr. periventriculares posteriores*) lie caudally to the former. There are, as a rule, 2 or 3 such branches; they are collected below the anterior part of the NPV one behind the other, in the area of the NPE and then taking a lateral turn meet the lateral chiasmatic vein or its branches coming from the NPV (Figs 11A, C). The blood of the caudal part of the NPE is collected by the small *rr. paraventri-*

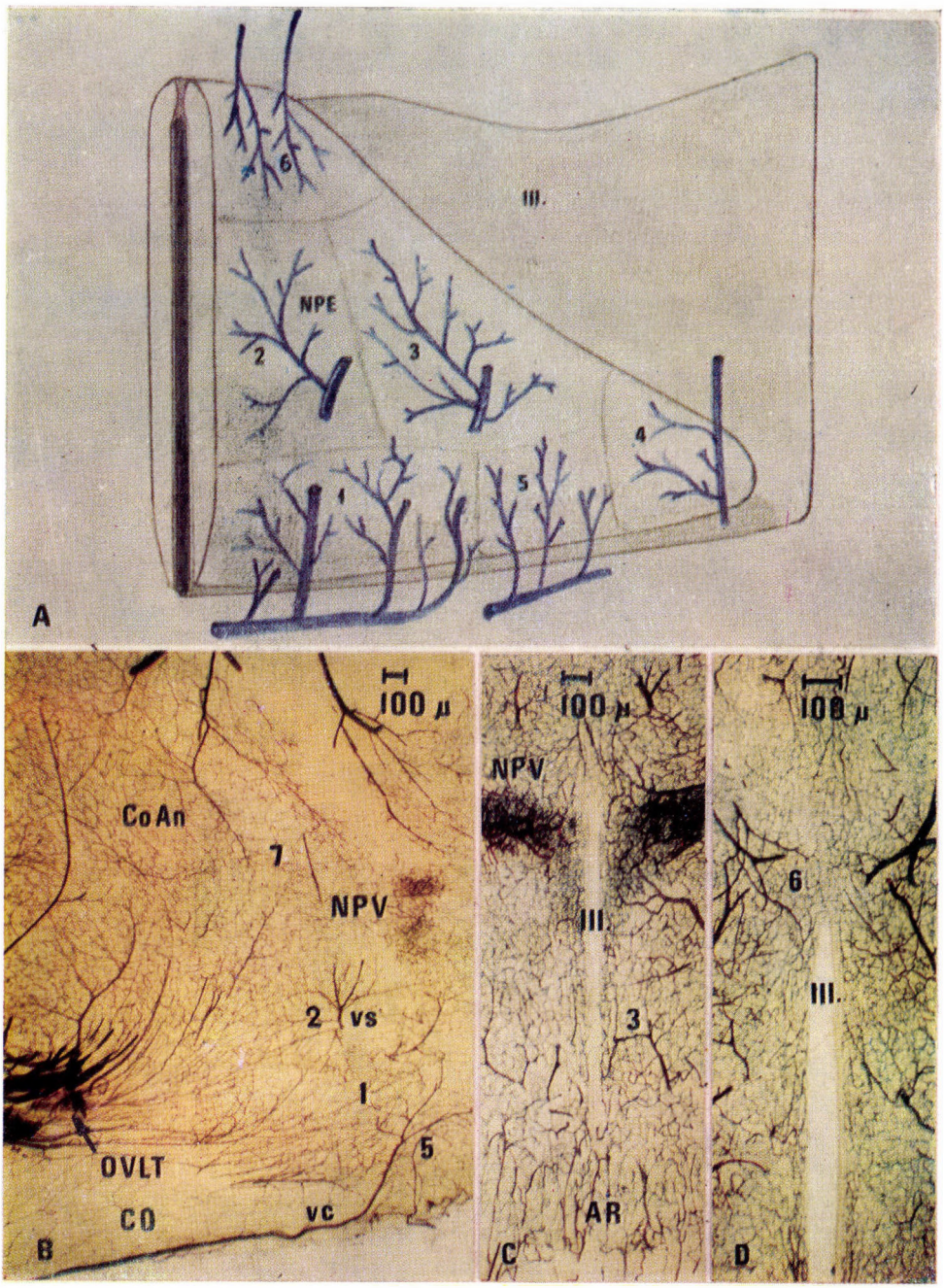


Fig. 11. Veins of the periventricular nucleus. Abbreviations see in text. Numeration of vessels: 1. rr. suprachiasmates, 2. r. periventricularis anterior, 3. rr. periventriculares posteriores, 4. rr. periventriculares inferiores, 5. rr. retrochiasmatici mediales, 6. rr. periventriculares superiores. A. Venous supply of the periventricular nucleus. B. R. periventricularis anterior. C. Rr. periventriculares posteriores. D. Rr. periventriculares superiores

culares inferiores of the inferior paraventricular vein which run next to the nucleus parallel to the 3rd ventricle (Fig. 11A). (In one third of the cases, one or two longer branches of the rr. retrochiasmatici mediales also participate in the drainage of this dorsal part.) In the superior anterior third of the NPE the *rr. periventriculares superiores* collect the blood. These 2 or 3 branches run upwards opposite to the former and meet the thalamic vein 500 to 700 μ behind the bregma (Figs 11A, D). The thalamic vein follows the upper curvature of the thalamus from behind the fornix and runs backwards into the great vena Galeni.

Discussion

Surprisingly few data are available concerning the vascular network of the rat hypothalamus, and the majority refers to the major arterial trunks [8, 9] without discussing the vessels of the individual nuclei of the hypothalamus. AKMAYEV's data [1] are the only one known to describe the capillary structure of the rat hypothalamus. The angiotopography of the hypothalamus of species other than the rat has been worked out in far greater detail; this is particularly true for the rabbit [5 —7] and the dog [4]. In several cases, especially in the case of the branches of major arterial and venous trunks, we had to adopt the nomenclature used for these species.

The arterial blood supply of all the nuclei of the anterior hypothalamus originates from the basal arteries and in all cases many smaller or larger vessels supply the nuclei. In none of the investigated nuclei could an artery supplying alone the given area be detected. A number of small, topographically rather overlapping branches form the network of the nuclei, thus injury to a single artery has practically no influence on their blood supply. This repeatedly ensured vascularity might explain the low incidence of ischaemic necrosis of individual nuclei after experimental surgical interventions (lesions, dissections).

All the nuclei of the anterior hypothalamus obtain blood from arteries of inferior origin (Table I). Only some of these branches participate exclusively in the vascular network of the hypothalamus, the terminal branch of the majority is situated in the basal ganglia. This is not generally true for the veins which, though their majority is collected basally, include, as in the case of the paraventricular nucleus, dorsally running veins.

The *nucleus suprachiasmatis* obtains arterial blood from the retrochiasmatic artery. Four branches penetrate into the nucleus. Two of them are branches of the suprachiasmatic artery which supplies also other areas of the hypothalamus, and two are branches of the midlde suprachiasmatic artery which supplies only the NSC. The bicoloured perfusion method has made it

possible to recognize the basal branches described by SCREMIN [8] as an artery, and the vessel running to the inferior-posterior part of the nucleus, as veins. The venous system of the nucleus is only partly in topographic agreement with the arteries. In addition to the suprachiasmatic veins there is also the perioptic vein which, running on the dorsal part of the optic chiasma, meets the anterior cerebral vein in front of the infrachiasmatic vein.

The *nucleus hypothalamicus anterior* is one of the largest nuclei of the hypothalamus and is made up of four subdivisions. It is supplied by the branches of four arteries (Fig 3A; Table II) of which the branches of the midde preoptic artery reach the nucleus frontally and the branches of the retrochiasmatic artery from below. Many small branches form the repeatedly ensured capillary network of the NHA. The blood of the nucleus is collected by 7 venous trunks, all of which proceed towards the basal surface of the cerebrum.

The *nucleus periventricularis* has no independent artery; it is supplied by the terminal branches of the three arteries which supply the neighbouring areas (Fig 6A), and by the anterior hypophyseal artery. This is the most oral area of the hypothalamus supplied by the anterior pituitary artery. The periventricular nucleus has no independent vein; the small branches coming from it are collected by the veins of the neighbouring nuclei. In contrast to arteries, some of the veins are collected dorsally by the thalamic vein.

The angio-architecture of the anterior hypothalamus is no closed system caudally, but is in connection with the middle part of the hypothalamus. The special vascular system of the retrochiasmatic area separating the two parts is closely linked to the anterior hypothalamus. Their separate treatment is, however, justified didactically hence the forthcoming parts of this series (AMBACH and PALKOVITS, Blood supply of the rat hypothalamus, IV. and V. in preparation) will be the continuation of the present work.

ABBREVIATIONS

ai	=	a. infrachiasmatica
AR	=	area retrochiasmatica
B	=	a. basilaris
ca	=	a. chorioidea anterior
CA	=	a. cerebri anterior
CI	=	a. carotis interna
cl	=	a. chiasmatica lateralis
CM	=	a. cerebri media
CO	=	chiasma opticum
CoA	=	a. communicans anterior
CoAn	=	commissura anterior
CoP	=	a. communicans posterior
CP	=	a. cerebri posterior
CS	=	a. cerebelli superior

ha	=	a. hypophysea anterior
hal	=	v. hypothalamica anterolateralis
ham	=	v. hypothalamica anteromedialis
hl	=	a. hypothalamica lateralis
hlp	=	a. hypothalamica lateralis posterior minor
hm	=	a. hypophysea media
hp	=	a. hypophysea posterior
hpm	=	a. hypothalamica posteromedialis
i	=	v. infrachiasmatica
ma	=	a. mamillaris anterior
mp	=	a. mamillaris posterior
NHAc	=	nucleus hypothalamicus anterior pars caudalis
NHAd	=	nucleus hypothalamicus anterior pars dorsalis
NHAm	=	nucleus hypothalamicus anterior pars medialis
NHAv	=	nucleus hypothalamicus anterior pars ventralis
NO	=	nervus opticus
NPE	=	nucleus periventricularis
NPV	=	nucleus paraventricularis
NSC	=	nucleus suprachiasmatis
NSO	=	nucleus supraopticus
NSOpt	=	nucleus supraopticus pars tuberalis
o	=	a. ophthalmica
OVLT	=	organon vasculosum laminae terminalis
p	=	v. perioptica
pi	=	v. paraventricularis inferior
pm	=	a. praeoptica medialis
PT	=	pars tuberalis
r	=	a. retrochiasmatica
rc	=	rr. chiasmates
rm	=	r. meningeus
rr	=	rr. retrochiasmates
rs	=	r. suprachiasmatis anterior
rsp	=	r. suprachiasmatis posterior superior
s	=	a. suprachiasmatica
sl	=	v. subcommissuralis lateralis
tl	=	a. thalamostriata lateralis
tm	=	a. thalamostriata medialis
TO	=	tractus opticus
tua	=	a. tuberalis anterior
tum	=	a. tuberalis media
tup	=	a. tuberalis posterior
vB	=	v. basalis
vCA	=	v. cerebri anterior
vca	=	v. chorioidea anterior
vcl	=	v. chiasmatica lateralis
vCM	=	v. cerebri media
vCP	=	v. cerebri posterior
vIA	=	v. interpeduncularis anterior
vIP	=	v. interpeduncularis posterior
vma	=	v. mamillaris anterior

vmp = v. mamillaris posterior
vp = v. perioptica
vpr = vv. praemamillares
vr = v. retrochiasmatica
vs = v. suprachiasmatica
vta = v. tuberalis anterior
vtm = v. tuberalis media
vtp = v. tuberalis posterior
III. = ventriculus tertius

REFERENCES

1. AKMAYEV, I. G.: (1971) Morphological aspects of the hypothalamic-hypophyseal system. III. Vascularity of the hypothalamus, with special reference to its quantitative aspects. Z. Zellforsch. **116,** 195—204. — 2. AMBACH, G., PALKOVITS, M.: (1974) Blood supply of the rat hypothalamus. I. Nucleus supraopticus. Acta morph. Acad. Sci. Hung., **22,** 291—310. — 3. AMBACH, G., PALKOVITS, M.: (1974) Blood supply of the rat hypothalamus. II. Nucleus paraventricularis. Acta morph. Acad. Sci. Hung., **22,** 311—320. — 4. BÖLÖNYI, F., BARTA, I.: (1954) Contribution of the angio-architecture of the hypothalamus. Acta morph. Acad. Sci. Hung., **4,** 293—299. — 5. HASAGEWA, K.: (1954) On the vascular supply of the hypophysis and of the hypothalamus of rabbits. I. The arterial supply. Fukuoka Acta med., **45,** 430—437. — 6. HASEGAWA, K.: (1954) On the vascular supply of the hypophysis and of the hypothalamus of rabbits. II. On the systematic and the portal veins. Fukuoka Acta med., **45,** 504—511. — 7. PROLO, D. J., STILWELL, D.: (1962) Arterial supply to the diencephalon and some associated areas of the rabbit brain. J. comp. Neurol., **119,** 224—254. — 8. SCREMIN, O. U.: (1970) The vascular anatomy of the rat's hypothalamus in stereotaxic coordinates. J. comp. Neurol., **139,** 31—52. — 9. SPENGLER, I., HUBER, P.: (1959) Topographische Beziehungen zwischen neurosecretorischen Ganglienzellen und arteriellen Gefässen im Rattenhypothalamus. Pflüger's Arch. path. Anat., **269,** 31—37.

DIE BLUTVERSORGUNG DES HYPOTHALAMUS BEI RATTEN, III.

REGIO ANTERIOR HYPOTHALAMI (NUCLEUS SUPRACHIASMATIS, NUCLEUS HYPOTHALAMICUS ANTERIOR, NUCLEUS PERIVENTRICULARIS)

G. AMBACH und M. PALKOVITS

Mit der Methode der doppelten Gefäßauffüllung wurde die arterielle und venöse Blutversorgung des vorderen Hypothalamus untersucht. An Serienschnitten wurde unter Berücksichtigung der räumlichen Koordinaten die Angiotopographie des Hypothalamus mit dessen Zytoarchitektur verglichen und die an der Gefäßversorgung des Nucleus suprachiasmatis, Nucleus hypothalamicus anterior und Nucleus periventricularis beteiligten Arterien und Venen ausführlich besprochen. Die Topographie der auf der basalen Oberfläche des Ratten-Dienzephalons verlaufenden Arterien- und Venenstämme, ferner die in den vorderen Hypothalamus von unten eintretenden und durch diesen verlaufenden größeren Zweige werden beschrieben.

КРОВОСНАБЖЕНИЕ ГИПОТАЛАМУСА У КРЫС. III. ПЕРЕДНЯЯ ОБЛАСТЬ ГИПОТАЛАМУСА

(nucleus suprachiasmatis, nucleus hypothalamicus anterior, nucleus periventricularis)

Г. АМБАХ и М. ПАЛЬКОВИТШ

Методом двойного наполнения сосудов авторами было изучено артериальное и венное кровоснабжение передней части гипоталамуса. На серийных срезах — с учетом пространственных координат — ангиотопография гипоталамуса была сопоставлена с его цитоархитектурой. Дается подробное описание артерий и вен, участвующих в кровоснабжении nucleus suprachiasmatis, nucleus hypothalamicus anterior, nucleus periventricularis. Обсуждается топография артериальных и венных стволов, наблюдаемых на базальной поверхности диэнцефалона крысы, а также входящие в гипоталамус снизу и проходящие через гипоталамус более крупные ветви.

Dr. György Ambach
Dr. Miklós Palkovits

I. Anatómiai Intézet,
1450 Budapest, Tűzoltó u. 58., Hungary

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Makerere Medical School, Kampala

EFFECT OF PROSTAGLANDIN F2 ALPHA ON THE NEUROHYPOPHYSIS

A HISTOCHEMICAL STUDY

U. Singh and M. Katzarski*

(Received October 28, 1974)

Ten micrograms of PG F2 alpha in distilled water were injected intraperitoneally into thirty non-pregnant female rats and their hypophysis was studied after aldehyde-fuchsin and performic acid–alcian blue staining. A definite depletion of posterior pituitary principle was observed in the hypophysis. The role of oxytocin in relation to the oxytocic effect of prostaglandin F2 alpha is discussed.

Introduction

The prostaglandins have successfully been used to induce therapeutic abortion and labour (Embrey, 1971; Karim and Sharama, 1971; Arnold et al., 1972), but their mode of action is not fully understood. This study has been carried out as a continuation of our preliminary investigations (Singh and Sebuwufu, 1973) in order to ascertain any possible relationship which might exist between the oxytocic effect of these compounds and the release of posterior pituitary principle from the neurohypophysis.

Materials and methods

The experiments were carried out on 30 non-pregnant female rats of approximately the same weight. They were arranged in groups of six using two as control in each group. Ten μg of PG F2 alpha in 1 ml of distilled water was administered intraperitoneally to all rats except the controls which were injected with a similar amount of distilled water.

After an interval of half and one hour from the time of injection, the rats in each group were anaesthetized with ether and decapitated. The brain was immediately removed together with the hypophysis, and fixed in formol-saline. The hypophysis was separated from the brain and kept for further fixation in formol-saline, then embedded in paraffin. Five μ thick sections were cut and stained with aldehyde fuchsin (Gomori, 1950) and alcian blue–performic acid (Adams and Sloper, 1956). Sections from control and experimental animals were stained together to avoid any difference during staining.

* Present address: Dept. of Anatomy, Medical School Plovdiv, Bulgaria.

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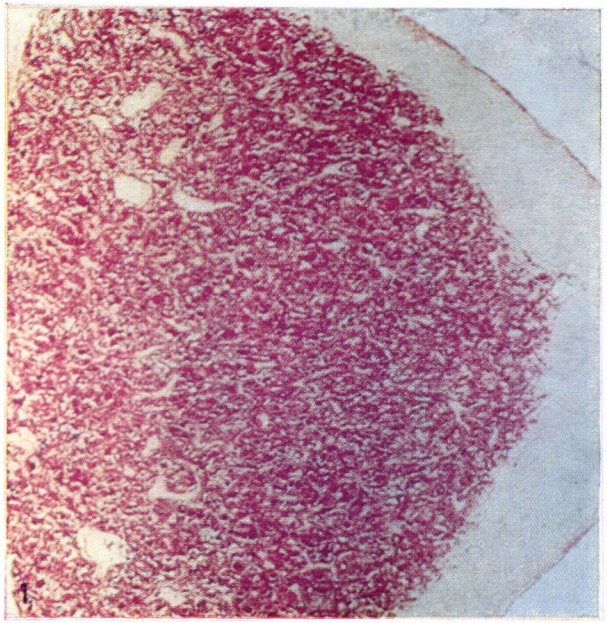


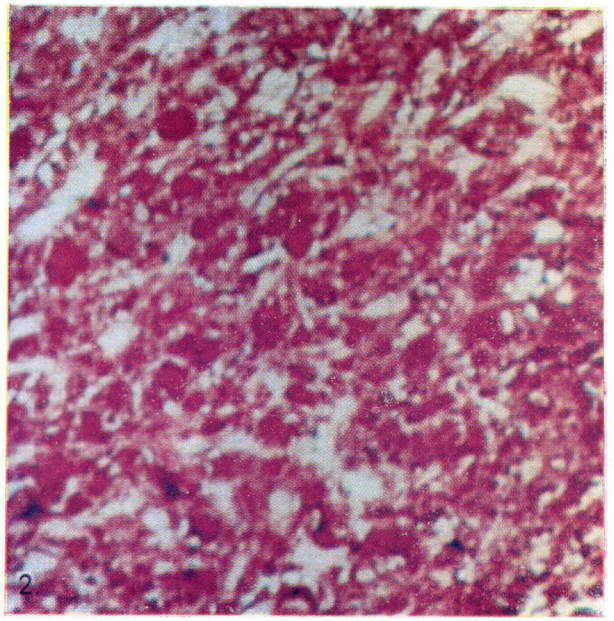
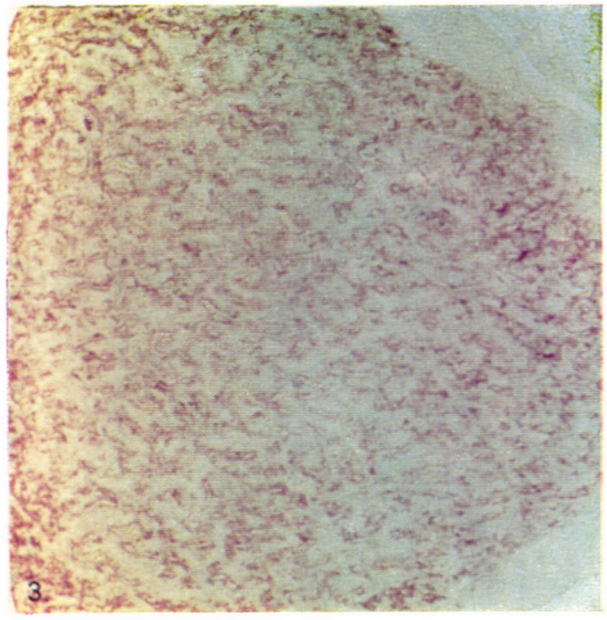


Fig. 1. Horizontal section of pars nervosa of hypophysis of control rat, 30 minutes after injection with distilled water, showing N.S.M. densely filled. Aldehyde-fuchsin × 100

Fig. 2. Horizontal section of part of pars nervosa of control rat, 30 minutes after injection with distilled water, showing N.S.M. densely filled. Aldehyde-fuchsin, × 200



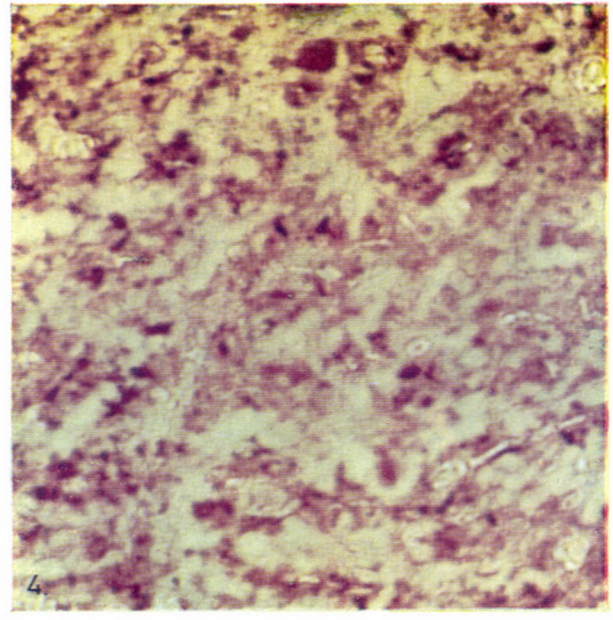
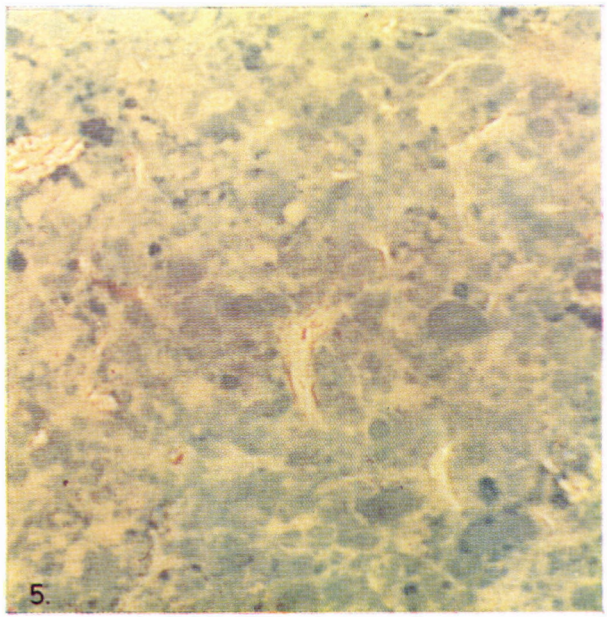


Fig. 3. Horizontal section of pars nervosa of hypophysis of rat, 30 minutes after injection with PG F2 alpha, showing depletion of N.S.M. Aldehyde-fuchsin × 100

Fig. 4. Horizontal section of part of pars nervosa of hypophysis of rat, after injection with PG F2 alpha, showing depletion of N.S.M. Aldehyde-fuchsin × 200



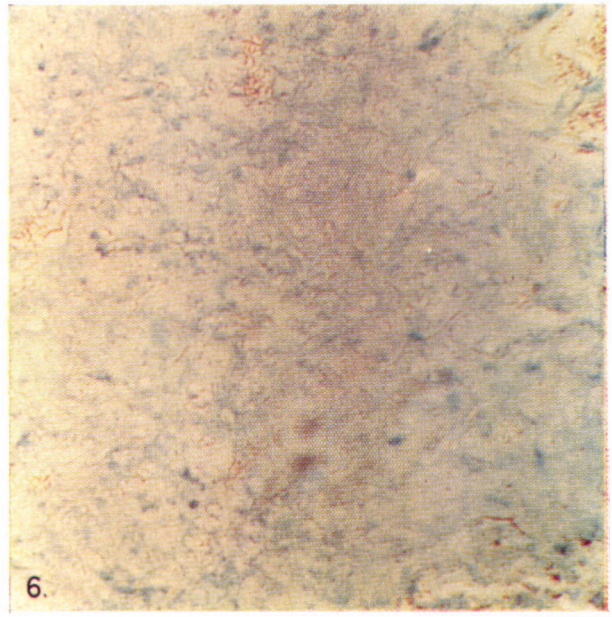


Fig. 5. Horizontal section of part of pars nervosa of hypophysis of control rat, one hour after injection with distilled water, showing N.S.M. Performic acid–alcian blue, × 400

Fig. 6. Horizontal section of part of pars nervosa of hypophysis of rat, one hour after injection with PG F2 alpha showing depletion of N.S.M. and empty spaces. Performic acid–alcian blue, × 400

Observations

a) In the sections obtained from control animals, the neural lobe was densely filled with neurosecretory material (N.S.M.) which appears purple-blue in the case of aldehyde-fuchsin and bluish-green with performic acid–alcian blue stains (Figs 2 and 5).

b) Sections obtained 30 minutes after the administration of PG F2 alpha showed less N.S.M. than in the controls (Figs 3 and 4).

c) Sections obtained one hour after the administration of PG F2 were almost devoid of N.S.M. and showed empty spaces (Fig. 6).

Discussion

Despite the limitations and doubts concerning the reliability of histochemical reactions put forward by ENESTROM (1969) they have been used for the demonstration of N.S.M. and are considered specific (BANDARANAYAKE, 1971; CHOUDHURY, 1971; PEARSE, 1968). As the experimental conditions for control and experimental animals were similar, the depletion of N.S.M. seen in the latter group of rats was probably not due to either physical or osmotic stress which may also cause a depletion of N.S.M. (RODECK and BRAUKMANN, 1966; CHOUDHURY, 1971; ROTHBALLER, 1953). PG F2 alpha causes pain on intramuscular injection (HORTON and MAIN, 1967) but is tolerated well by intravenous drip (KARIM, 1971) and it is not known whether its intraperitoneal administration is painful. Considering, however, the doses, time interval and absence of physical signs indicating pain (hair raising, trembling) in the rats, it is believed that the drug is tolerated well.

It is difficult to conclude, from histochemical reactions alone, whether the changes seen in the neuro-hypophysis were due to depletion of oxytocin or vasopressin, both being polypeptides rich in cystine (PEARSE, 1968). The results are therefore discussed in the light of the interrelationship between the physiological and biochemical activity of these polypeptides and prostaglandins. Whereas prostaglandin E antagonizes the vasopressin-induced activation of adenyl cyclase–cylic A.M.P. system to cause diuresis (HORTON and MAIN, 1967), PG F2 alpha does not affect this system presumably due to the lack of a keto group at position 9 of the cyclopentane ring (FRANKS, et al., 1971). Unlike PG and A, PG F2 alpha is a vasopressor compound; it causes constriction of blood vessels and thereby increases venous return and cardiac output (DAYER, 1970; DUCHARME and WEEKS, 1967; HORTON and MAIN, 1967). It has been suggested that this effect on blood pressure is not mediated by the renin-angiotensin—A. D. H. system (LEE et al., 1971). Thus there exists an antagonism between the PG F2 alpha and vasopressin, as far as their effects on the renal tubules and blood vessels are concerned.

The effects of PG F2 alpha on the uterus are more closely related to oxytocin. PG F2 alpha has synergistic action on uterine contractions when administered with oxytocin (EMBREY, 1971). These compounds have successfully been used for the induction of abortion and labour (ARNOLD, et al., 1972; BYGDEMAN, 1971; EMBREY, 1971; KARIM, 1971; KARIM and SHARMA, 1971). None of these workers have observed an abnormal rise of blood pressure or any other cardiovascular abnormality even after prolonged perfusion with the compound. Recent reports indicate increased levels of oxytocin in blood following perfusion with PG F2 alpha for induction of labour in pregnant women (ARNOLD et al., 1972).

It is quite likely, particularly in the light of the observation of ARNOLD et al., that in addition to their direct action on the myometrium (KARIM and SHARMA, 1971) these compounds probably affect also secondarily the hypothalamo-hypophysial system and cause an endogenous release of oxytocin from the neural lobe. Such a hypothesis has already been suggested by SPERROF (1971). To substantiate it, radio-immuno-assay and electron microscopic studies have to be carried out on other species and preliminary work is in progress for such investigations.

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REFERENCES

1. ADAMS, C. W. M., SLOPER, J.: (1956) The hypothalamic elaborations of posterior pituitary principles in man, the rat and the dog. Histochemical evidence derived from a performic acid alcian blue reaction for cystine. J. Endocr. **13,** 221—228. — 2. ARNOLD, G., HILARY, C. B., CARD, T.: (1972) Oxytocin release by infused prostaglandin. Brit. med. J., **1,** 543—544. — 3. BANDARANAYAKE, R. C.: (1971) Morphology of the accessory neurosecretory nuclei and of the retrochiasmatic part of the supraoptic nucleus of the rat. Acta anat. (Basel) **80,** 14—22. — 4. BYGDEMAN, M., TOPPOZADA, M., WIQVIST, N.: (1971) Introduction of the mid trimester abortion by intra-amniotic administration of prostaglandin F2 alpha. Acta physiol. scand. **82,** 415—416. — 5. CHOUDHURY, S. R.: (1971) Response of hypothalamic secretory neurones to trauma. Acta anat. (Basel) **79,** 84—92. — 6. DAYER, D. C.: (1970) Comparison of constricting actions produced by serotonin and prostaglandin on isolated sheep umbilical arteries and veins. Gynec. Invest. **1,** 204—209. — 7. DUCHARME, D. W., WEEKS, J. R.: (1967) Cardiovascular pharmacology of prostaglandin F2 alpha, a unique pressor agent. In: Proc. 2nd Nobel Symp. Almqvist and Wiksell, pp. 173—181. — 8. EMBREY, M.: (1971) PGE compounds for induction of labor and abortion. Ann. N.Y. Acad. Sci. **180,** 518—523. — 9. ENESTROM, S.: (1969) Selective stain of the neurosecretory substance. Acta anat. (Basel) **73,** 4—9. — 10. FRANKS, D. J., MACMANUS, J. P., WHITFIELD, I. F.: (1971) Effect of prostaglandins on cyclic AMP production and cell proliferation in thymic lymphocytes. Biochem. biophys. Res. Commun. **44,** 1177—1183. — 11. GOMORI, G. A. M.: (1950) Aldehyde fuchsin: a new stain for elastic tissue. Amer. J. clin. Path. **20,** 665. — 12. HORTON, E. W., MAIN, I. H. M.: (1967) Identification of prostaglandins in central nervous tissues of the cat and chicken. Brit. J. Pharmacol. **30,** 582—602. — 13.

KARIM, S.: (1971) Action of prostaglandin in pregnant women. Ann.N.Y. Acad. Sci. **180,** 483—498. — 14. KARIM, S. M. M., FILSHIE, G. M.: (1970) Therapeutic abortion using prostaglandin F2 alpha. Lancet **1,** 157. — 15. KARIM, S. M. M., SHARMA, S. D.: (1971) Oral administration of prostaglandin for induction of labour. Brit. med. J. **1,** 260—262. — 16. LEE, J. B., COVINO, B. G., TAKMAN, B. H., SMITH, E. R.: (1965) Renomedullary vasodepressor substance, medullin: Isolation, chemical characterization and physiological properties. Circulat. Res. **17,** 57. — 17. LEE, J., KANNEGIESSER, M., O'TOOLE, J., WESTURA, E.: (1971) Hypertension and renomedullary prostaglandins: A human study of the antihypertensive effects of PGal. Ann. N.Y. Acad. Sci. **180,** 218—240. — 18. PEARSE, A. G. E.: (1968) Histochemistry; theoretical and applied. 3rd ed. J. Churchill Co., London Vol. **1,** 239—240. — 19. RODECK, H., BRAUKMANN: (1966) Neurosekretion — Stress — Adaptation. I. Wirkung von Schmerz auf das neurosekretorische System. Z. ges. exp. Med. **141,** 33—44. — 20. ROTHBALLER, A. B.: (1953) Changes in the rat neurohypophysis induced by painful stimuli, with particular reference to neurosecretory material. Anat. Rec. **115,** 21—36. — 21. SINGH, U., SEBUWUFU, P. H. S.: (1973) Prostaglandin and neurosecretion. A histochemical study. East Afr. med. J. **50,** 199—206. — 22. SPEROFF, L.: (1971) Discussion of session on prostaglandin in female reproductive physiology. Ann. N.Y. Acad. Sci. **100,** 513—517.

DIE WIRKUNG VON PROSTAGLANDIN F2 ALPHA AUF DIE NEUROHYPOPHYSE EINE HISTOCHEMISCHE STUDIE

U. SINGH und M. KATZARSKI

30 nichtschwangeren Ratten wurde die wäßrige Lösung von 10 μg Prostglandin F2 Alpha intraperitoneal eingeführt und anschließend die Hypophyse der Tiere mittels Aldehydfuchsin und Performsäure-Alzyanblau-Färbung untersucht. In der Hypophyse ließ sich dem hinteren Teil entsprechend eine charakteristische Verminderung wahrnehmen. Die Rolle des Oxytozins wird im Zusammenhang mit der oxytoxischen Wirkung des Prostaglandins F2 Alpha besprochen.

ДЕЙСТВИЕ ПРОСТАГЛАНДИНА F2 ALPHA НА НЕЙРОГИПОФИЗ ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ

У. СИНГ и М. КАТЦАРСКИ

30 небеременным крысам авторы впрыскивали водный раствор из 10 мк/г F2 alpha простагландина внутрибрюшинно и изучали гипофиз животных методом окрашивания альдегидфуксином и перформокислым—альциановым голубым. Соответственно задней части в гипофизе наблюдалось характерное уменьшение. Роль окситоцина обсуждается в связи с окситоксическим эффектом F2 alpha простагландина.

SINGH, U., M.B., B.S., M.S.
KATZARSKI, M., M.D.
Makerere Medical School, Kampala, Uganda

Acta morphologica Acad. Sci. hung. 23 (1), pp. 59–69, 1975

Institute of Anatomy, Histology and Embryology, University Medical School, and Institute of Mineralogy, Kossuth Lajos University, Debrecen

CONNECTIVE TISSUE ALTERATIONS FOLLOWING NEONATAL THYMECTOMY

VI. CALCIUM HISTOCHEMICAL, GROWTH-DYNAMICAL AND THERMOANALYTICAL INVESTIGATIONS ON BONE TISSUE OF THYMECTOMIZED RATS

B. Mándi, M. Petkó, Gy. Szöőr and T. Glant

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Thymectomy was performed in newborn rats and the changes occurring in the epiphyseal cartilage and bone were investigated by Ca histochemical and thermoanalytical methods, one, two and six weeks following operation. Formation of Ca complexes was slowed down in the epiphyseal cartilage and the rate of growth decreased. At the same time the inorganic substance content decreased considerably in the bone tissue of operated rats as compared to the controls.

Introduction

For a long time there have been few data available on the effect of the thymus on the development and structure of connective tissue, though attention had turned long ago to the growth regulating function of the organ [10, 13]. Then in the past 15 years, the question of the relation between thymic activity and supporting tissues has been the subject of several studies.

According to the results, neonatal thymectomy brings about a decrease of the proliferative activity of the epiphyseal cartilage [1, 2, 18] and retards the process of ossification [2, 18]. Submicroscopic changes occur in the cells of the epiphyseal cartilage [16] resulting in an altered composition of the cartilage matrix [19]. The bones of thymectomized animals are fragile and occasionally skeletal deformities are encountered [12]. In the bone cells and matrix ultrastructural changes can be observed [17]. Radiological examinations suggested a decrease of the Ca content of bones [3] and the development of a rickets like condition [14, 21] but these were checked by conventional histological methods which failed to give sufficient insight in the finer changes of the chemical composition of bone tissue following thymectomy. It seemed therefore interesting to perform Ca histochemical and thermoanalytical investigations. As far as we know, the latter procedure has not yet been applied in bone research.

Material and method

Thymectomy according to MILLER's [20] method was performed on a total of 200 albino rats within the first 24 hours after birth. The operation resulted in a characteristic wasting syndrome, with retardation of growth, loss of weight and atrophy of the lymphatic system in about 30% of the animals. Since a great number of operated animals died from advanced wasting, the experiments were carried out on 36 rats. In addition to the wasting syndrome an impairment of the cellular immune response was also noted, as judged from the decrease in the proportion of rosette forming cells (sRFC) of the spleen. As controls 36 rats from the same litters as the operated ones were used. They were subjected to a sham operation consisting of the exposure of the thymus without removing it. No wasting syndrome developed in these rats.

The animals were killed and the material processed, one, two, and six weeks following surgery. For the examinations the tibia and the femur were used.

I. *Histochemical demonstration of Ca*

1. *Demonstration of ionic Ca with N,N-naphthalylhydroxylamine according to* VOIGT [28]

The proximal epiphysis of the tibia was embedded in paraffin and sectioned at 10 μ.

The sections were stained with 0.1% N,N-naphthalylhydroxylamine (Fluka A.G., Buchs, Switzerland) and examined under the polarisatiom microscope.

2. *Demonstration of bound Ca according to* KOSSA [23]

The proximal epiphysis was fixed in alcohol, embedded in paraffin and sectioned. Then Kossa's Ca reaction was performed followed by haematoxylin staining.

II. *Studies on growth dynamics*

From all age groups 5 thymectomized and 5 control rats were injected intraperitoneally with 25 mg/kg oxytetracycline hydrochloride (Chinoin, Budapest) 48 hours, and with 50 mg/kg Fluorexon® (2,4 bis N,N-dicarbomethyl-aminomethyl-fluorescein Chemapol, Prague) 24 hours before sacrifice. The proximal epiphysis of the tibia was removed, fixed in absolute alcohol, embedded in paraffin and sectioned. After dehydration, the sections were mounted in Fluoromont. Micrometric measurements were performed under a fluorescence microscope with BG 3/4 and OG 1/1 filters, by means of an ocular micrometer. Oxytetracycline produced a yellow, and Fluorexon a green fluorescence. The two fluorochromes, well separated from each other, were localized as stripes in the lower, calcifying part of the epiphyseal cartilage disc. The distance measured between the lower margins of the two fluorescent stripes by means of an ocular micrometer, corresponds — depending on the conditions of the experiment — to the growth rate of bone in 24 hours. From each material 10 sections were prepared and 20 measurements were taken on each section. From the data so obtained we have calculated the mean values.

III. *Thermoanalytic determination of the proportion of organic and inorganic substances in the bone*

The femurs of thymectomized and control rats were removed, cleaned of their soft parts and dried over P_3O_5. After weighing and length measurement of the bones, the two epiphyses were removed and the diaphyses measured again. For the examinations only the diaphyseal parts of the femurs were used. The bones were then reduced to granules 0.06 mm in diameter. The examinations were carried out in 0.015—0.100 g material by means of a derivatograph (MOM) designed by PAULIK et al. [22]. Heating was done at a rate of 10°C/min in air at a temperature ranging from 20 to 1000°C.

Results

Demonstration of ionic Ca with N,N-naphthalylhydroxylamine

One-week control rats. In the zone of calcification of the epiphyseal cartilage (Fig. 1) and in the metaphysis, large amounts of coarsely granulated N,N-naphthalylhydroxylamine crystals showing marked birefringence are present. The metaphyseal trabeculae are long and well-developed. In other parts of the disc, especially in the zone of proliferation, the crystals are considerably less birefringent than in the zone of calcification.

One-week thymectomized rats (Fig. 2). Similarly as in the controls, birefringent crystals — though much smaller in number — can be noted in the calcifying cartilage zone of the epiphyseal disc and in the metaphysis. The bone trabeculae are strikingly short. Some cells of the zone of proliferation and marrow cavity display a certain activity.

Two-week control rats. In the epiphyseal cartilage (Fig. 3) a positive reaction is displayed not only by the trabeculae of the zone of calcification and in the metaphysis, but also in the cartilaginous trabeculae of the zone of hypertrophy.

Two-week thymectomized rats. The bone trabeculae of the epiphysis (Fig. 4) show less birefringent N,N-naphthalylhydroxylamine crystals than in the controls. At the same time the crystals seem to have increased in amount in the zone of proliferating cartilage.

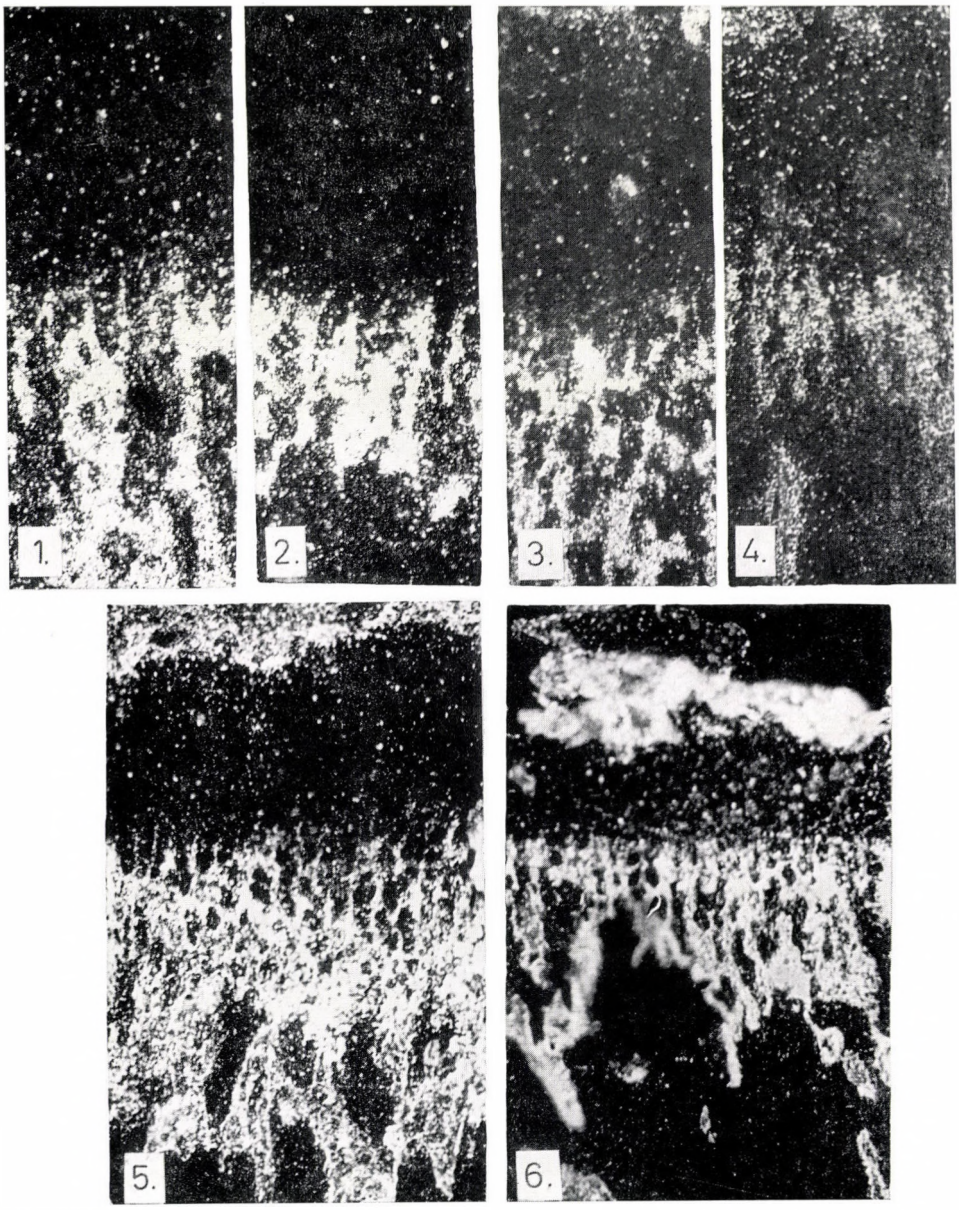
Six-week control rats (Fig. 5). In the cartilaginous trabeculae of the lower part of the proliferation zone birefringent crystals are visible. For this reason, the line of calcium incorporation has an irregular course.

Six-week thymectomized rats (Fig. 6). Appearance of birefringent crystals begins uniformly in the ground substance of the calcification zone and the line of calcium incorporation is at the same level in the entire territory of the cartilage disc.

Demonstration of bound Ca

One-week controls (Fig. 7). In the epiphyseal cartilage, only the zones of maturation and calcification are separated morphologically. In the ground substance of the calcification zone insoluble, bound Ca is present. The metaphyseal trabeculae are thin, showing a parallel course. At some sites they are connected with each other.

One-week thymectomized rats (Fig. 8). The cells of the calcification zone are close to each other with little ground substance between them. There are few trabeculae showing a positive reaction; these trabeculae are thinner and shorter than in the controls.



Figs 1—6. Polarization photomicrographs of epiphyseal cartilages stained with N,N-naphthalylhydroxylamine. × 63

Fig. 1. One-week control rat. Large amounts of birefringent crystals in calcification zone and in metaphyseal trabeculae

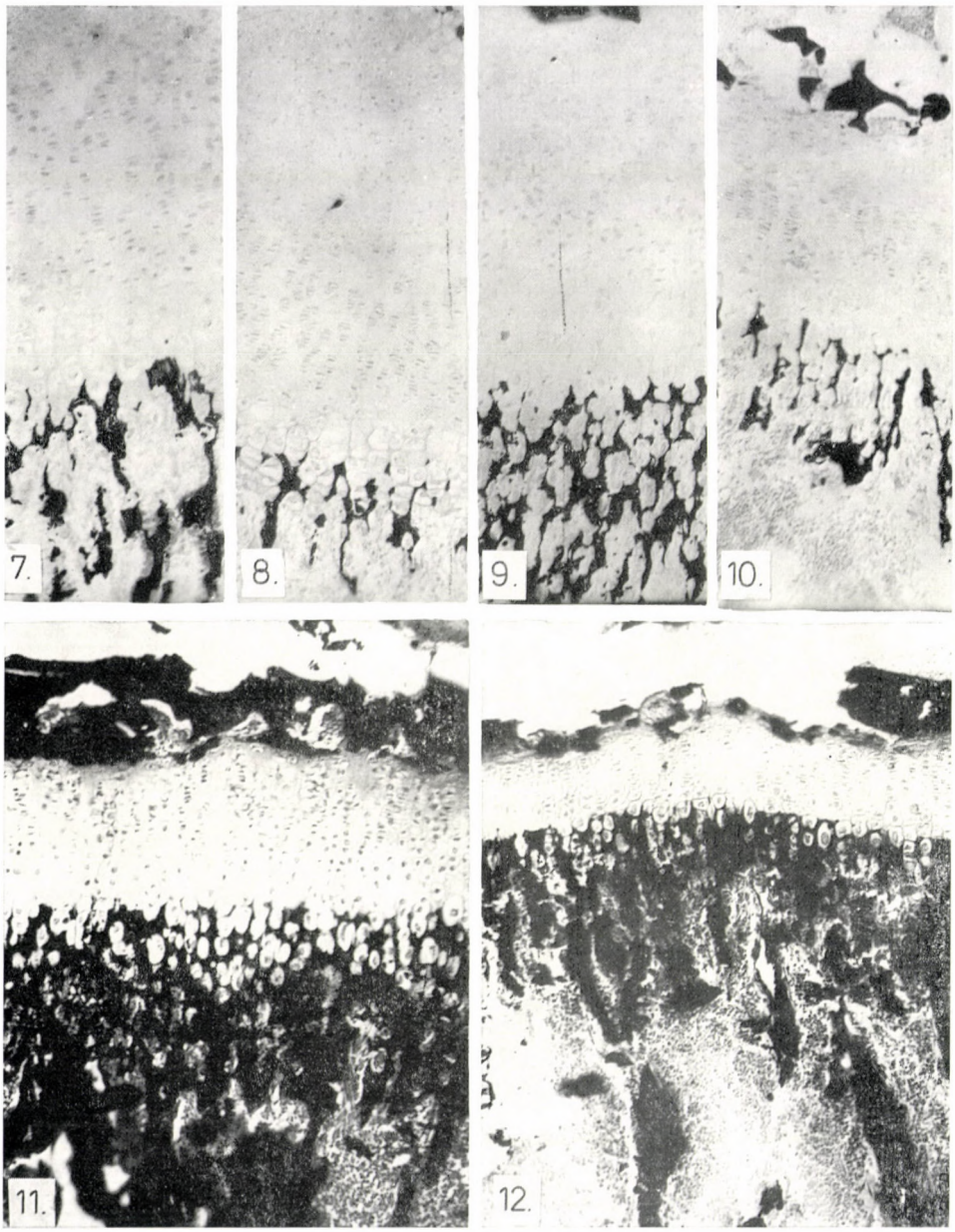
Fig. 2. One-week thymectomized rat. Smaller amount of birefringent crystals in calcification zone and metaphysis

Fig. 3. Two-week control rat. In addition to the cartilaginous trabecules of the calcification zone and metaphysis, those in hypertrophic cartilage also contain small amounts of birefringent crystals

Fig. 4. Two-week thymectomized rat. Few birefringent crystals, in the bone trabecules more in the proliferation zone of the epiphyseal cartilage

Fig. 5. Six-week control rat. Birefringent crystals are visible also in the lower part of the proliferation zone

Fig. 6. Six-week thymectomized rat. Birefringent crystals are localized in the zone of calcification and in the area distal to this zone. The epiphyseal cartilage disc is narrow



Figs 7—12. Kossa's reaction in epiphyseal cartilage. × 80

Fig. 7. One-week control. Bound Ca in ground substance of calcification zone and in the metaphyseal trabecule showing a parallel course

Fig. 8. One-week thymectomized rat. Thin metaphyseal trabeculae with low bound Ca content

Fig. 9. Two-week control rat. Bound Ca also in the lower part of the zone of maturing cartilage. Numerous bone trabeculae rich in bound Ca

Fig. 10. Two-week thymectomized rat. A few bone trabeculae containing small amount of bound Ca

Fig. 11. Six-week control rat. Bound Ca in the area extending to the upper margin of maturing cartilage. Dense network of thick bone trabeculae rich in calcium

Fig. 12. Six-week thymectomized rat. Bound Ca only in the calcification zone and distally to it. The bone trabeculae are short and less numerous than in the controls

Two-week control rats (Fig. 9). The zonal structure of the epiphyseal cartilage disc is well discernible. Bound Ca is present even in the lower part of the zone of maturation. The trabeculae forming a network are more numerous than at the one-week stage.

Two-week thymectomized rats (Fig. 10). Some areas of the ground substance of the calcification zone are devoid of positivity. In the metaphysis, the bone trabeculae containing bound Ca are short, thin and few in number.

Six week control rats (Fig. 11). Positivity extends to the upper part of the zone of maturation. Cartilage cells displaying nuclear staining are visible among the cartilaginous trabeculae containing calcium. The thick bone trabeculae form a dense network.

Six-week thymectomized rats (Fig. 12). Positive reaction mainly in the zone of calcification, sparsely in the ground substance of maturing cartilage. There are fewer bone trabeculae than in the controls.

Growth dynamics

The method of Ca demonstration in vivo allowed to study the growth dynamics. Results are summarized in Table I.

Table I

Growth in μm per 24 hr in control and thymectomized rats

Age	Control	Thymectomized
1 week	135.2 ± 5.2	130.8 ± 8.3
2 weeks	113.4 ± 9.4	95.4 ± 10.1
6 weeks	102.6 ± 7.8	88.3 ± 9.9

As can be seen from Table I, there was no appreciable difference in growth between the control and the thymectomized rats. At 2 weeks, however, the daily growth rate was significantly lower in the thymectomized than in the control rats. The difference was still significant at 6 weeks of age.

Thermoanalytical assay

For the examinations only materials from the two- and six-week groups used as the materials obtainable at one week was not sufficient. As can be seen in Table II, the femurs of thymectomized rats were lighter in both age groups. In the 2-week group, the quotient of weight and length in the controls was 4.51 mg/mm, and in the thymectomized rats 2.1 mg/mm. In the 6-week group the values were 8.91 mg/mm and 6.69 mg/mm, respectively.

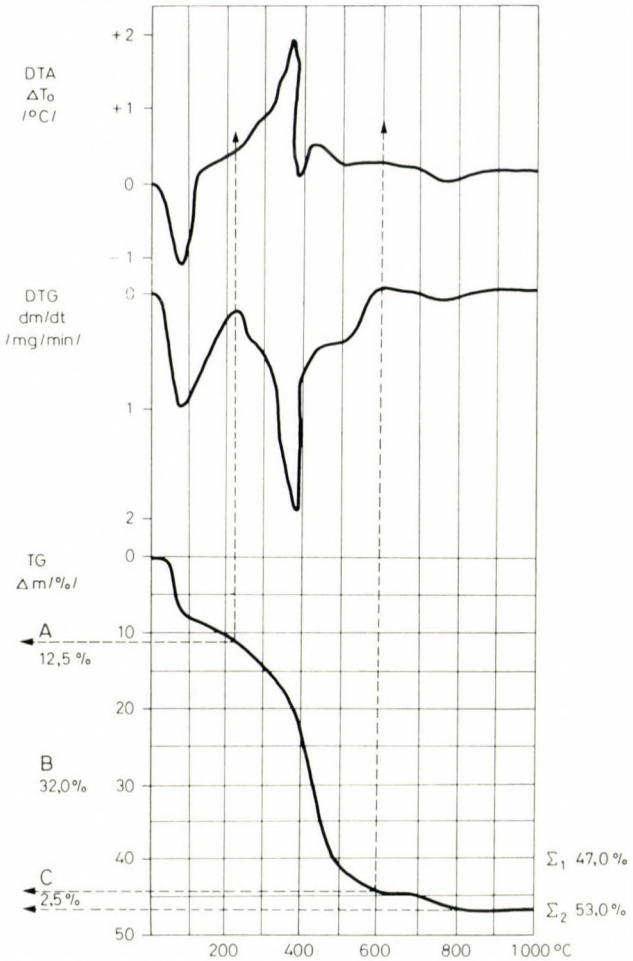


Fig. 13. Derivatogram of control animal. DTA ΔT_0 (°C) = differential thermoanalytical curve; DTG dm/dt/mg/min = derivative thermoanalytical curve; TG Δm (%) = thermogravimetric curve, loss of substance at TG curve, in per cent; A = quantity of water evaporated at 20—220°C; B = quantity of organic substance lost at 220—600°C; C = initial breakdown of carbonate hydroxyapatite at 600—1000°C, loss of $OHCO_2$; Σ_1 = total loss of substances (A + B + C) up to 1000°C; Σ_2 = $Ca_3(PO_4)_3$ + CaO + MgO residue

The derivatographic curves showed that the reactions taking place during heating are qualitatively identical. The water content of the bone tissue is represented by the value obtained at 20—220°C (A), the organic matter by the value obtained at 220—600°C (B), whereas the inorganic substance content is represented by the sum of the heating residue (Σ_2) and the amount of inorganic substances removed at 600—1000°C (C), as shown in Fig. 13.

On the basis of the results calculated (Table III) a significant difference can be noted in the distribution of organic and inorganic substances in the

Table II

Mean length and weight of femur in control and thymectomized rats at 2 and 6 weeks after surgery

Specimen	Femur length (mm)	Femur weight (g)
2 weeks, control	18.17 ± 0.5	0.082 ± 0.005
2 weeks, thymectomized	11.93 ± 0.4	0.026 ± 0.002
6 weeks, control	26.43 ± 0.5	0.235 ± 0.003
6 weeks, thymectomized	23.06 ± 0.7	0.154 ± 0.002

Table III

Composition of bone tissue in the femur of control and thymectomized rats, in per cent of weight

Specimen	A	B	C	Σ_1	Σ_2
2 weeks, control	13.14 ± 0.2	39.20 ± 0.6	4.07 ± 0.02	56.41 ± 0.9	43.59 ± 0.8
2 weeks, thymectomized	11.42 ± 0.3	51.77 ± 0.2	5.87 ± 0.08	69.06 ± 0.7	30.94 ± 0.9
6 weeks, control	13.23 ± 0.5	31.76 ± 0.4	2.13 ± 0.05	47.12 ± 0.5	52.88 ± 1.0
6 weeks, thymectomized	12.91 ± 0.4	34.07 ± 0.4	2.24 ± 0.07	49.22 ± 0.6	50.78 ± 0.6

A = water content (20—220°C)
B = organic substances (220—600°C)
C = inorganic substances (600—1000°C)
Σ'_1 = A + B + C (20—1000°C);
Σ'_2 = inorganic residue $(Ca_3/PO_4)_3$ + CaO + MgO

bone tissue between the controls and thymectomized rats. The bones of the latter are characterized by a relative abundance of organic, and a decrease of inorganic substances.

Discussion

According to the results, the localization of ionic, unbound Ca is nearly identical in the epiphyseal cartilage of control and thymectomized rats. On the other hand, the metaphyseal bone trabeculae of thymectomized animals are thin and short and display a weaker positivity. In the control group Kossa's reaction was positive in the zones of calcification and maturation, while in the thymectomized group marked positivity appeared only in the zone of calcification. Thus, thymectomy seems to exert an injurious effect on the formation of Ca-complexes.

In connective tissue research the interesting results offered by thermoanalytical assay [4, 5, 9] have justified the application of this method. In our experimental material the procedure revealed quantitative differences. The total inorganic substance content of bone decreased in the thymectomized animals as compared to the controls. Similar observations were made with ^{32}P [26]. It should be noted that part of the water evaporating in the domain between 20 and 220°C (Fig. 13A) derives from water bound to organic macromolecules, but its loss coincides with that of the adsorbed moisture. From the derivatographic curve it can be seen that the usual drying temperature of 100—110°C is not sufficient in the case of bone as they will retain considerable amounts of water at these temperatures.

On the 7th postoperative day the growth rate did not show appreciable differences between the thymectomized and control groups. This means that the growth retardation occurring later was probably due to the thymus deficiency and not to the surgical injury because this would have manifested itself earlier.

Thermoanalytical and growth dynamical investigations revealed marked differences between thymectomized and control animals in the two-week stage. The difference, though in a slighter degree, was still remarkable at 6 weeks. This is probably due to the fact that the animals with severe wasting disease die before the age of 6 weeks and only those with a milder form of the syndrome reach this age.

The cause of growth retardation and decrease of the amount of inorganic substances in the bone after thymectomy seems to be attributable first of all to endocrine changes. The alpha cells of the pituitary, after a temporary hyperactivity [8] decrease in number and undergo degranulation [7, 24, 25]. A similar decrease was noted in the activity of the thyroid [7, 8] and the adrenal glands [6—8]. On the basis of recent results it cannot, however, be excluded that the lack of biologically active substances produced by the thymus may be the cause of the changes described above. Of these substances, thymosin [11] should be mentioned first. In fact, this hormone-like substance produced by the epithelial cells of the thymus has been found by us to be able to prevent the wasting syndrome, and by administering to rats antithymosin rabbit serum we could induce symptoms characteristic of the wasting disease [15]. Further investigation of this question is in progress.

REFERENCES

1. Asher, L., Landolt, E.: (1934) Die Wirkung der Thymusexstirpation auf das Wachstum bei vitaminarmer Nahrung. Pflügers Arch. ges. Physiol. **235,** 605—613. — 2. Berek, L.: (1967) Osseal changes in mice following neonatal thymectomy. Experientia (Basel) **24,** 721. — 3. Berek, L.: (1969) A method for osseal radiomicrometric measurements in mice. Experientia (Basel) **25,** 633—634. — 4. Bihari-Varga, M.: (1971) Thermoanalytical character-

ization of the stability of cross-linked proteins. Acta biochim. biophys. Acad. Sci. hung. **6,** 265—270. — 5. BIHARI-VARGA, M.: (1971) Thermoanalytical assay of glycosaminoglycans. Acta biochim. biophys. Acad. Sci. hung. **6,** 271—276. — 6. COMSA, J.: (1957) Effect of thymectomy upon the functional condition of the adrenal cortex in guinea pigs. Nature (Lond.) **179,** 872—873. — 7. COMSA, J.: (1971) The thymic hormones. Hormones **2,** 226—255. — 8. COMSA, J., HOOK, R. R. jr.: Thymectomy. In: Thymic hormones. Ed. Luckey, T. D. Univ. Park Press, pp 1—18, 1973. — 9. FARKAS, T., BIHARI-VARGA, M., BIRÓ, T.: (1974) Thermoanalytical and histological study of intraarticular papain-induced degradation and repair of rabbit cartilage. I. Immature animals. Ann. rheumat. Dis. **33,** 385—392. — 10. FRIEDLEBEN, A.: (1858) Die Physiologie der Thymusdrüse in Gesundheit und Krankheit, vom Standpunkte experimenteller Forschung zur Lebensgeschichte der Kindheit. Literarische Anstalt, Frankfurt am Main. — 11. GOLDSTEIN, A. L., GUHA, A., ZATZ, M. M., HARDY, M. A., WHITE, A.: (1972) Purification and biological activity of thymosin, a hormone of the thymus gland. Proc. nat. Acad. Sci. (Wash.) **69,** 1800—1810. — 12. HARMS, J. W.: (1952) Morphologische Untersuchungen über den Thymus von Xenopus laevis. Morph. Jb. **92,** 256—338. — 13. KLOSE, H., VOGHT, H.: (1910) Klinik und Biologie der Thymusdrüse mit besonderer Berücksichtigung ihrer Beziehungen zum Knochen und Nervensystem. Bruns. Beitr. klin. Chir. **69,** 1—200. — 14. KLOSE, H.: (1914) Thymusdrüse und Rachitis. Path. Anat. bl. allg. **25,** 1. — 15. MÁNDI, B., GLANT, T.: (1973) On the thymosin producing cells of the thymus gland. Nature New Biol. **246,** 25. — 16. MÁNDI, B., GYARMATI, J., HADHÁZY, CS., LACZKÓ, J.: (1973) Effect of postnatal thymectomy on the submicroscopic structure of the epiphyseal cartilage in the rat. Acta morph. Acad. Sci. hung. **21,** 39—48. — 17. MÁNDI, B., GYARMATI, J., VARGA, S.: (1974) Changes of the submicroscopic structure of the bone in neonatally thymectomized rats. Acta Morph. Acad. Sci. hung. **22,** 265—274 — 18. MÁNDI, B., HADHÁZY, CS., MÁNDI, A., GLANT, T.: (1971) Effect of postnatal thymectomy on enchondral ossification. Acta morph. Acad. Sci. hung. **19,** 259—268. — 19. MÁNDI, B., VERECZKEI, L., BALOGH, K.: (1973) Mucopolysaccharide histochemistry of the epiphyseal cartilage of neonatally thymectomized rats. Acta morph. Acad. Sci. hung. **21,** 359—369. — 20. MILLER, J. F. A. P.: (1962) Role of thymus in transplantation immunity. Ann. N.Y. Acad. Sci. **99,** 340—354. — 21. PAPPENHEIMER, A. M.: (1914) Further experiments upon the effects of extirpation of the thymus in rats, with special reference to the alleged production of rachitic lesion. J. exp. Med. **20,** 477—498. — 22. PAULIK, F., PAULIK, J., ERDEY, L.: (1958) Der Derivatograph. Z. analyt. Chem. **160,** 241—261. — 23. PEARSE, A. G. E.: Histochemistry Theoretical and Applied. 2nd ed. Little Brown and Co., Boston 1962, pp. 934. — 24. PIERPAOLI, W., SORKIN, E. (1967a) Cellular modification in the hypophysis of neonatally thymectomized mice. Brit. J. exp. Pathol. **48,** 627—631. — 25. PIERPAOLI, W., SORKIN, E.: (1967b) Relationship between thymus and hypophysis. Nature (Lond.) **215,** 834—837. — 26. TOMA, E., TOMA, V. V., OROS, J.: (1962) Incorporation du P^{32} dans certains os du rat ethymisé. J. Physiol. (Paris) **54,** 400—404. — 27. SORKIN, E., PIERPAOLI, W., FABRIS, N., BIANCHI, E.: (1972) Relation of growth hormone the thymus and the immune response. In: Growth and growth hormone. Ed.: Pecile, A., Müller, E. Excerpta Medica, Amsterdam, pp. 132—142. — 28. VOIGT, G. E.: (1957) Ein neuer histotopochemischer Nachweis des Calciums mit Naphthalhydroxamsäure. Acta histochem. (Jena) **4,** 122—131.

VERÄNDERUNGEN DES STÜTZGEWEBES BEI THYMEKTOMIERTEN TIEREN, VII CA-HISTOCHEMISCHE UND THERMOANALYTISCHE UNTERSUCHUNGEN DER WACHSTUMDYNAMIK AM KNOCHENGEWEBE VON NEONATAL THYMEKTOMIERTEN RATTEN

B. MÁNDI, M. PETKÓ, GY. SZÖŐR und T. GLANT

An neugeborenen Ratten wurde eine Thymektomie ausgeführt und 1, 2 und 6 Wochen nach dem Eingriff mittels Ca-histochemischer sowie thermoanalytischer Verfahren die in der Knorpelscheibe und im Knochengewebe der Epiphysen vor sich gehenden Veränderungen untersucht. Den Ergebnissen zufolge erfährt in der Epiphysenknorpel der thymektomierten Tiere die Entstehung des Ca-Komplexes eine Verlangsamung. Das Ausmaß des täglichen Wachstums nimmt ab. Im Knochengewebe der operierten Tiere ist der Gehalt an anorganischen Stoffen wesentlich geringer als im Knochengewebe der Kontrolltiere.

ИЗМЕНЕНИЯ ОПОРНОЙ ТКАНИ У ЖИВОТНЫХ ПОСЛЕ ТИМЭКТОМИИ, VII Ca-ГИСТОХИМИЧЕСКИЕ И ТЕРМОАНАЛИТИЧЕСКИЕ ИССЛЕДОВАНИЯ ДИНАМИКИ РОСТА В КОСТНОЙ ТКАНИ КРЫС ПОСЛЕ НЕОНАТАЛЬНОЙ ТИМЭКТОМИИ

Б. МАНДИ, М. ПЕТКО, Д. СЁР и Т. ГЛАНТ

Авторы произвели у новорожденных крыс тимэктомию и спустя 1, 2 и 6 недель после вмешательства изучали Ca-гистохимическими и термоаналитическими методами изменения, возникшие в эпифизарном хрящевом диске и в костной ткани. Согласно результатам после тимэктомии в эпифизарном хряще животных наблюдается замедление образовании Ca-комплекса. Уменьшается динамика суточного роста. В костной ткани оперированных животных содержание неорганического вещества значительно меньше, чем у контрольных животных.

Dr. Barnabás MÁNDI
Dr. Mihály PETKÓ
DOTE Anatómiai Intézet,
4012 Debrecen, Nagyerdei krt. 98., Hungary

Dr. Tibor GLANT
Dr. Gyula SZŐÖR
KLTE Ásványtani Intézet,
4010 Debrecen, Hungary

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Institute of Pathology, University Medical School, Debrecen

MORPHOLOGICAL EXAMINATION OF THE JUXTAGLOMERULAR APPARATUS FOLLOWING THE FLUORESCENCE MICROSCOPIC DEMONSTRATION OF SYMPATHETIC NERVES

Z. NEMES, EDIT BODOLAY, K. PECZE and P. ENDES

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Several catecholamine demonstrating fluorescence techniques based on the formaldehyde-condensation principle have been examined for the possibility of application of juxtaglomerular granulum demonstrating methods. Freezing and thawing cause a distruption of juxtaglomerular granules. Short formalin fixation does not prevent this damage. Juxtaglomerular granules were successfully stained after freeze-drying.

The adaptation of ENDES's trichrome method on the basis of the classical catecholamine technique of FALCK and OWMAN resulted in a reliable practical method. Procedures introduced into the trichrome methods to increase the colour and intensity contrast of the staining of juxtaglomerular granules and to stain the elastic membranes are described. Some aspects of the staining mechanism of juxtaglomerular specific granules are also discussed.

Catecholamines cause renin release by stimulating the beta-adrenergic receptors of renin-containing juxtaglomerular cells [1, 2, 22, 25, 28]. A series of morphological examinations prove the adrenergic innervation of juxtaglomerular cells [3, 11, 13, 17, 21, 27,]. Elimination or the influence of renal symphatetic nerves results in an inhibition or reduction of the renin release produced by a number of experimental stimuli [5, 6, 16, 19, 20, 26, 30]. These effects depend on the liberation of the sympathetic neural transmitter, i.e. noradrenaline, produced either by extrarenal stimuli via sympathetic reflexes or by intrarenal regulating mechanisms.

The above observations suggest that the morphological evaluation of the relation between the granular storage of renin and the noradrenaline content of juxtaglomerular nerves may be a useful tool in the study of renin secretion.

In order to be able to demonstrate renin containing granules in the juxtaglomerular cells following the fluorescence microscopic demonstration of sympathetic nerves, we have studied the application of juxtaglomerular (JG) granule-staining methods in sections used for catecholamine demonstrating techniques. The most promising combination has been developed into a reliable practical method.

Material and methods

Albino mice of both sexes weighing 40—50 g were used. The frequency of arteriolar granulated cells in mice, a favourable condition for studying the staining of JG granules, accounted for the choice of the experimental animal.

The mice were decapitated under ether anaesthesia and $2 \times 3 \times 5$ mm transversal kidney sections were obtained. Corresponding to the techniques of formaldehyde-condensation reaction some kidney slices were immediately quenched in a dry ice—isopentane mixture and 1. cyrostat sections were prepared for the method of EL-BADAWI and SCHENK (1967), and 2. for the freeze-drying method of CSILLIK and KÁLMÁN (1967), or 3. they were freeze-dried in block for the classical method of FALCK and OWMAN (1965). Other slices were fixed in cold aqueous solutions of 1—4% formalin for the method of SAKHAROVA and SAKHAROV (1968).

Kidney blocks were freeze-dried for 5 days at —40°C below 10^{-3} torr, in the presence of P_2O_5 by means of an Edwards—Pearse EPD 2 tissue drier. Following freeze-drying the blocks were treated with formaldehyde gas of 60% relative humidity [15] at 80°C for one hour and vacuum-embedded in previously degassed paraffin. 3—6 μ paraffin sections were prepared, fixed to slides with egg white–glycerol and mounted in paraffin oil.

Fluorescence microscopic examination of the catecholamine-specific reaction was carried out by means of a Leitz Orthoplan microscope equipped with a Ploem's incident light fluorescence device [23]. Violet exciting light was produced by a HBO 200 high pressure mercury lamp, with 3 mm BG 3 exciting filter combined with 4 mm GB 38 red absorption filter. The TK 455 interference dividing plate built into Ploem's illuminator reflects 90% at 405 nm and transmits 80% at 480 nm. The K 460 barrier filter in the slot above the illuminator absorbs remaining weak violet light reflected from the specimen (Fig. 1A). The catechol-

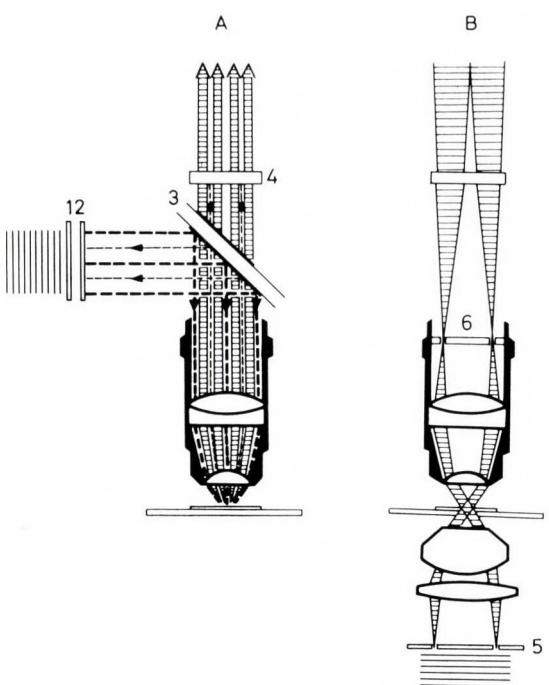


Fig. 1. Beam path for incident light fluorescence (A) and for phase contrast (B). ||||| visible spectrum, ≡≡≡ violet light. 1. 3 mm BG 3 filter, 2. 4 mm GB 38 filter. 3. TK 455 interference dividing plate, 4. K 460 barrier filter, 5. annular stop, 6. phase ring

amine-specific intense yellowish green fluorescence can easily be distinguished from the damped yellow-white backround autofluorescence.

The use of phase contrast objectives for fluorescence microscopy allowed the phase contrast microscopic examination of the kidney structure in unstained sections simply by switching on the transmitted light (Fig. 1B). When appropriate fields for microphotography were selected under phase contrast microscopic control, the transmitted light was switched out and exposures were made with incident illumination. Coordinates of the photographed fields were read off the scales of the object stage for a further examination after staining.

Subsequent to fluorescence microphotography the coverslip was gently removed, the paraffin oil dissolved in xylene and the sections rehydrated through ethanol. These sections with and without post-fixation were stained with different methods.

Post-fixation was carried out in Bouin's fixative for 4—6 hours, or in Zenker-formol for 24 hrs as recommended by WILSON (1952) for his modified Bowie-staining, or in 2.5% potassium dichromate overnight as used by WAGERMARK et al. (1968) in their Bowie-modification.

The combined trichrome staining of ENDES et al. (1969) and WILSON's modified Bowie-stain was used for the demonstration of JG granules. Modifications of ENDES' original method were made 1. to improve the contrast in colour and/or intensity between the staining of JG granules and that of nuclear chromatin in juxtaglomerular cells, and 2. to achieve adequate elastic staining for distinguishing between different parts of the arterial and arteriolar vasculature. For this purpose, Weigert's resorcin-fuchsin stain, Romeis' orcein stain, Verhoeff's elastica method and Gömöri's aldehyde-fuchsin stain [18] were applied interposed between different steps of Endes' trichrome technique.

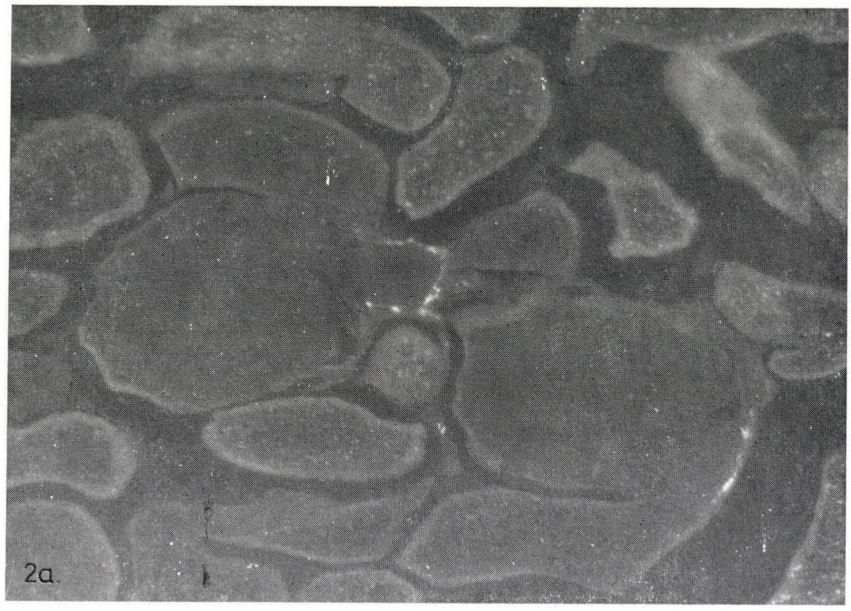
To improve the staining contrast of JG granules the following procedures were carried out: 1. Mallory's phosphotungstic acid—haematoxylin (PTAH) treatment with the ionic strength adjusted by 0.1—0.8 M NaCl; 2. methylation for 1—8 hrs by 1% HCl in absolute methanol at 60°C prior to staining.

If adequately stained the fields corresponding to the fluorescence microphotograms were photographed again.

Results

The method of EL-BADAWI and SCHENK (1967) gave intense specific fluorescence but its reproducibility was inferior to that of the classic method of FLACK and OWMAN (1965). The reproducibility of the technique of SAKHAROVA and SAKHAROV (1968) proved to be satisfactory but the intensity of fluorescence was generally weak to moderate. Although the method of CSILIK and KÁLMÁN (1967) produced intense specific fluorescence with good reproducibility, the sections came off the slides during further reatment. The classical freeze-drying technique of FALCK and OWMAN (1965) was reliable as regards fluorescence intensity and reproducibility.

There were nerve fibres and varicosities that exhibited a yellowish-green fluorescence specific for monoamines around arteries and arterioles in the immediate vicinity of most glomeruli (Fig. 2). It appeared that in the preglomerular granulated part of arterioles the varicosities were more numerous than in the proximal non-granulated part containing internal elastic membrane. In longitudinally sectioned afferent arterioles, fluorescent fibres appeared as double chains due to varicosities whereas the varicose fibres formed beaded closed circles when cross-sectioned and open circles when cut obliquely. Efferent arterioles could be recognized only when sectioned longitudinally, by a single or occasionally double chain running tangentially to the glomerulus (Fig. 3).



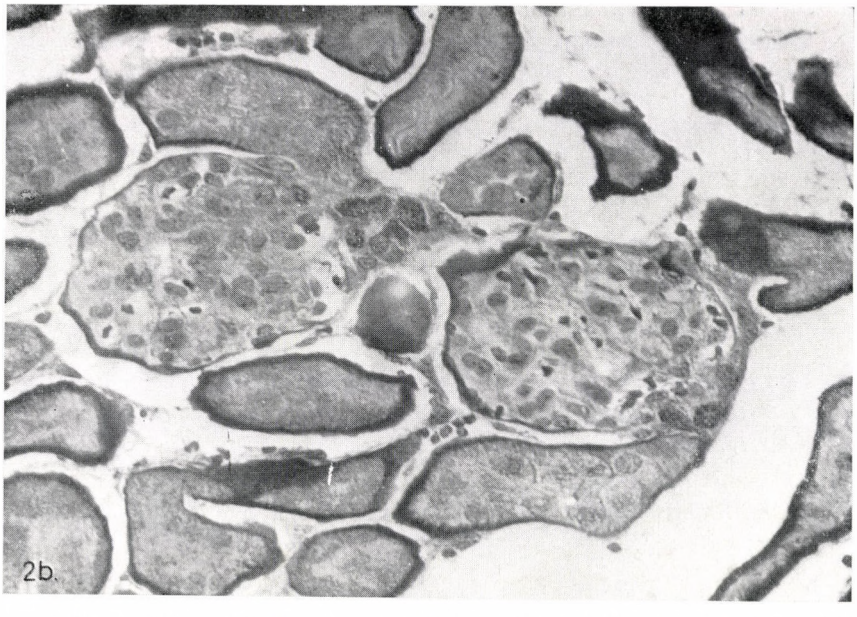
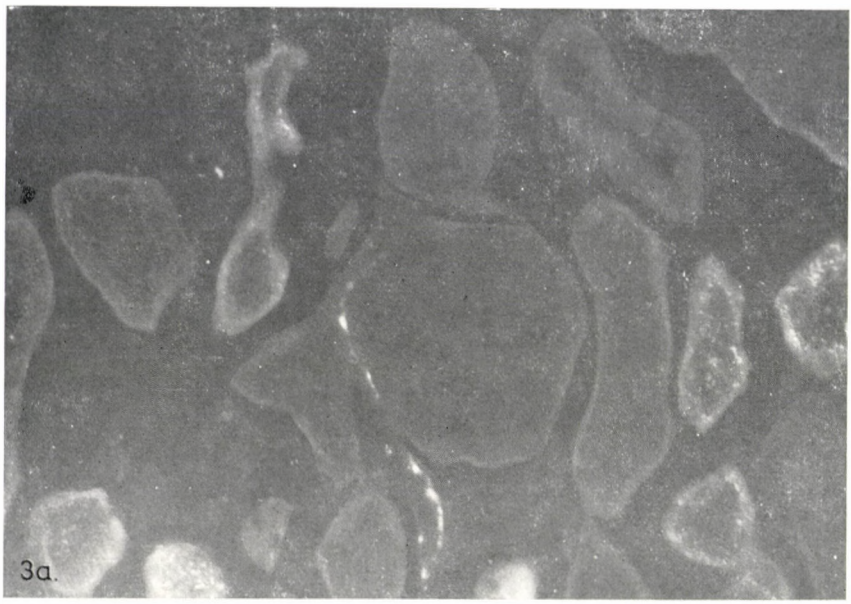


Fig. 2. (a) Mouse kidney. Falck—Owman technique. Formaldehyde-induced catecholamine fluorescence around two glomeruli. Weak autofluorescence in the surrounding tubules. (b) Identical section stained with Endes' trichrome method. The fields encircled by fluorescent fibres correspond to oblique-sectioned afferent arterioles with richly granulated juxtaglomerular cells. ×500



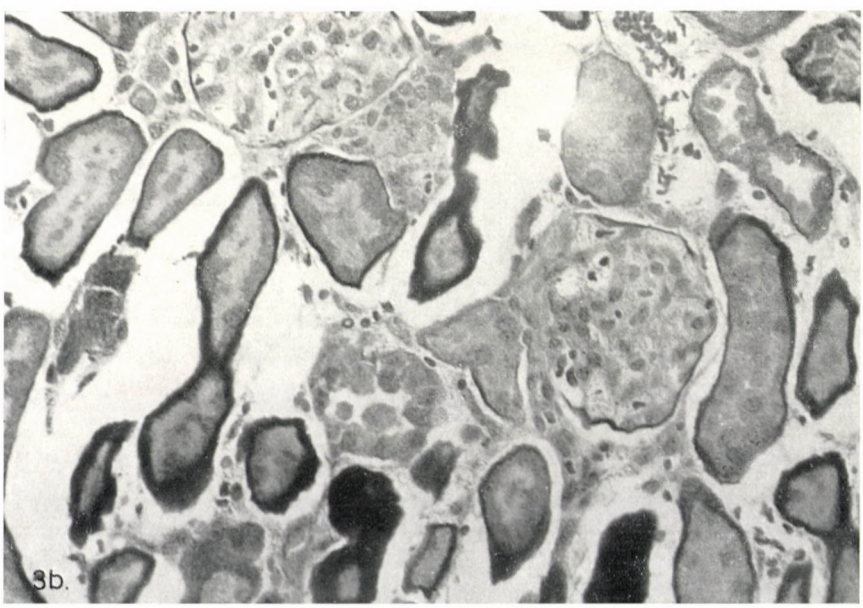


Fig. 3. (a) Mouse kidney. Falck—Owman technique. Varicose fluorescent fibres running longitudinally to the glomerulus. ×600. (b) Identical section stained with Endes' trichrome method. The fluorescent fibres in (a) indicate a longitudinally sectioned efferent arteriole. ×600

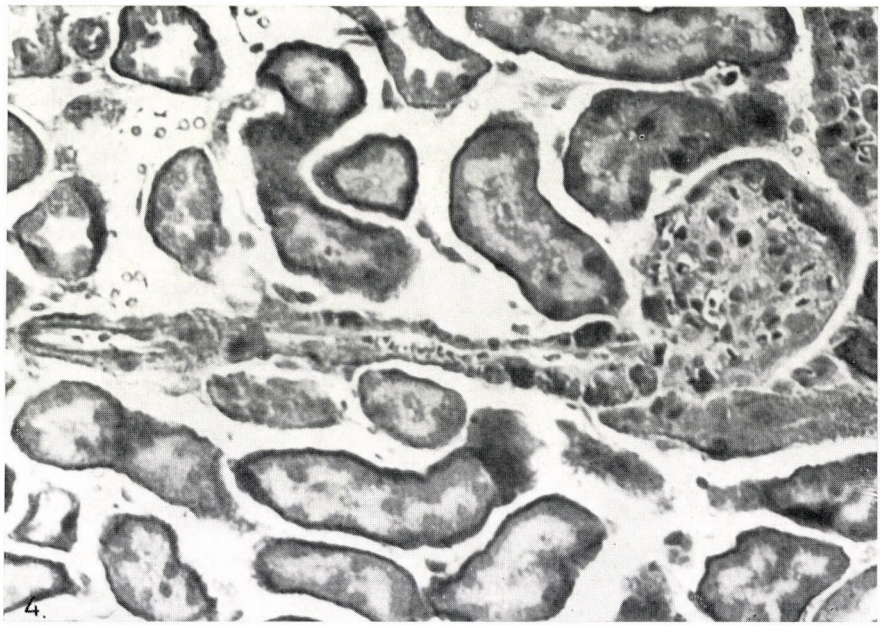


Fig. 4. Mouse kidney. Longitudinally sectioned afferent arteriole. The preglomerular part is long and richly granulated. The proximal part contains well-defined elastic membrane. Endes' trichrome; aldehyde-fuchsin-orcein-PTAH modification. ×120

Under the fluorescence microscope the structural relations of arterioles and glomeruli cannot be determined, neither can the specific granules of juxtaglomerular cells be recognized. For these purposes phase contrast microscopic examination is necessary. Poorly granulated juxtaglomerular cells cannot be recognized with certainty even by phase contrast because of the similar appearance of the nuclear chromatin. It is also difficult to judge the internal elastic membrane in interlobular arteries and afferent arterioles by their autofluorescence or by phase contrast. These problems can be solved only by differential staining.

JG granules were stainable only in the sections prepared according to Falck and Owman (1965). Irrespective of post-fixation, these granules could neither be recognized by phase contrast, nor stained in the sections obtained by the fluorescence techniques of El-Badawi and Schenk (1967) and Sakharova and Sakharov (1968).

The staining intensity and contrast of JG granules appeared to be inadequate with the crystal violet technique of Wilson (1952) even after post-fixation in dichromate as recommended by Wagermark et al. (1968). The combined trichrome method of Endes et al. (1969) stained JG granules intensely and selectively but the colour of chromatin granules was an equally strong blue which made it difficult to recognize poorly granulated cells. The

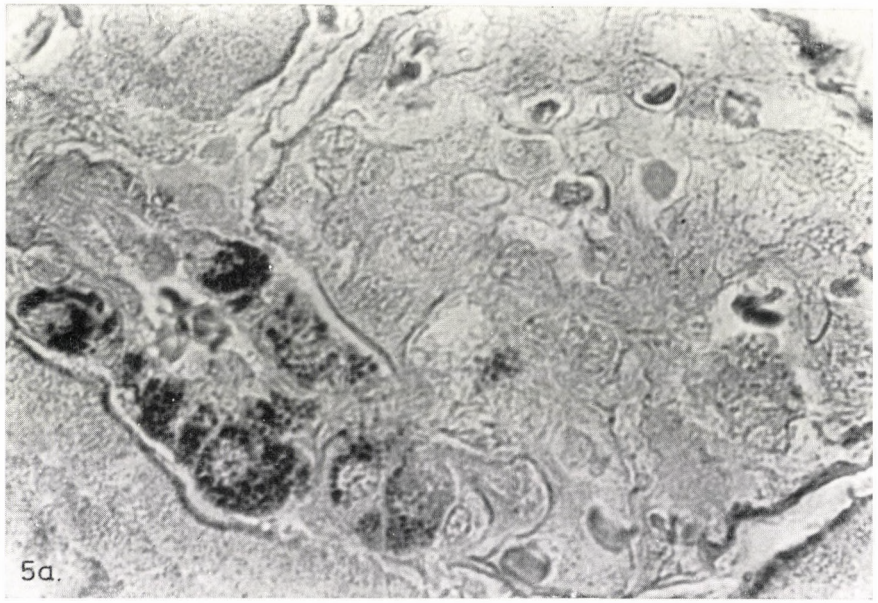
Table I

Effects of elastic stains combined with Endes' trichrome method

Sequence of stains	Staining of JG granules	Staining of nuclear chromatin	Elastic staining	
			arteriolar	arterial
PTAH	blue +++	blue +++	greyish-brown −, ±	+
Resorcin–fuchsin–PTAH	 −	greyish-brown +	brown ++	++
PTAH–resorcin–fuchsin	 −	 −	brown ++	++
Orcein–PTAH	blue +++	brown +	brownish-red −, ±	++
PTAH–orcein	 −	brownish-red +	brownish-red ++	++
Verhoeff's stain–PTAH	grey +	greyish-blue ++	greyish-brown ±, +	+
PTAH–Verhoeff's stain	grey ++	grey ++	grey +	++
Aldehyde-fuchsin–PTAH	blue +++	blue ++	blue-lilac ++	++
PTAH–aldehyde-fuchsin	 −	 −	blue-lilac ++	++
Aldehyde-fuchsin–orcein–PTAH	blue +++	brown +	blue-lilac ++	++
Orcein–aldehyde-fuchsin–PTAH	blue +++	blue ++	blue-lilac ++	++

thicker elastic membranes could be distinguished by their faint greyish-brown staining.

The effects of elastic stains introduced into ENDES method to improve the staining of elastic membranes are seen in Table I. For convenience these methods are related to the most important step, the phosphotungstic acid—haematoxylin (PTAH) treatment. In some cases the staining was not limited to elastic membranes but effected also the staining contrast of JG granules by their affinity for nuclei. Orcein prior to PTAH stained the nuclei brownish-red and the blue colour of JG granules was left unchanged. The elastic staining, however, is not satisfactory. Aldehyde-fuchsin (ripened for 10 days) applied prior to PTAH produced excellent elastic staining. The best combination was achieved by orcein interposed between aldehyde-fuchsin and PTAH when the elastic staining and the colour contrast of JG granules were equally satisfactory (Fig. 4).



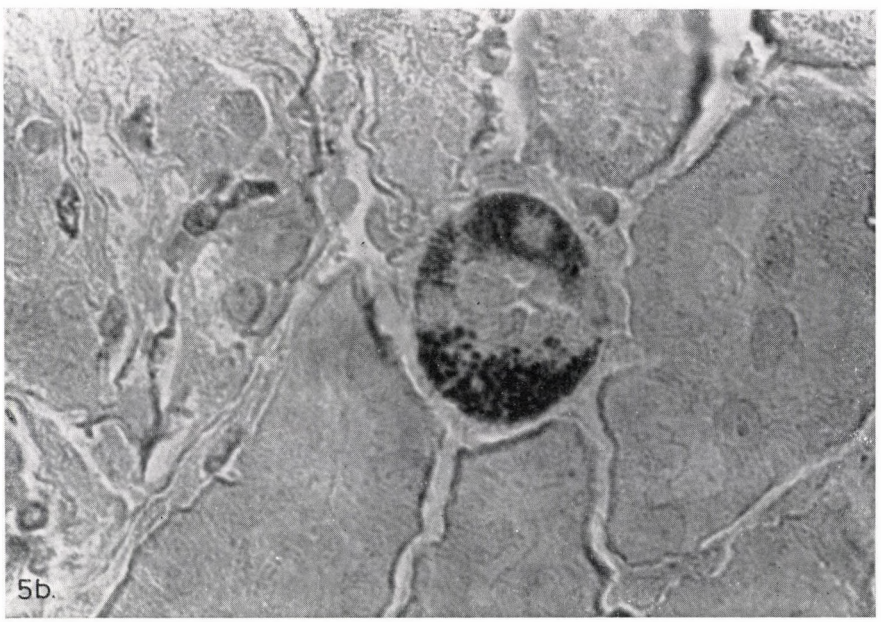


Fig. 5. Mouse kidney. Endes' trichrome method after methylation. Longitudinally (a) and cross sectioned (b) afferent arterioles. Strongly stained juxtaglomerular granules againts a pale background. ×800

Raised ionic strength was not found to increase the contrast of staining intensity between JG granules and nuclear chromatin with PTAH. The staining of both juxtaglomerular granules and nuclear chromatin decreased above 0.4 NaCl concentration.

Methylation for 4 hrs eliminated the staining of nuclei and tubular mitochondria without any appreciable weakening of the colour of JG granules. Strong blue JG granules were seen against a pale reddish-brown background (Fig. 5).

Discussion

All kinds of catecholamine demonstrating techniques based on the formaldehyde-condensation principle have been examined. For their relative simplicity and the easiness of serial sectioning, the methods of EL-BADAWI and SCHENK (1967), CSILLIK and KÁLMÁN (1967), and SAKHAROVA and SAKHAROV (1968) are frequently used. In our experience these techniques cannot, however, be compared to the classical method of FALCK and OWMAN (1965) as regards the intensity and sharpness of fluorescence and reproducibility.

The technique of EL-BADAWI and SCHENK (1967) involves thawing of unfixed cryostat sections onto slides. The thawing causes disruption of JG granules [12]. In keeping with this observation we could not demonstrate JG granules by phase contrast or staining, even after postfixation.

The method of SAKHAROVA and SAKHAROV (1968) uses cryostat sections from short formalin-fixed blocks. Thawing cannot be avoided. We found that the catecholamine-specific fluorescence was prevented when the fixation with 4% formalin had exceeded 60 min or with 2% formalin exceeding 120 min. This limited fixation at 0°C prior to freezing and thawing failed to preserve the granules.

The method of CSILLIK and KÁLMÁN (1967) applies freeze-drying of cryostat sections. The avoidance of thawing, however, eliminates the possibility of spreading of the sections on and fixing them to, slides. Thus, for practical reasons, these sections were not used for staining.

The classical method of FALCK and OWMAN (1965) meets the requirement of preservation of JG granules and provides all practical conditions for staining.

The crystal violet staining of Bowie needs special fixation and a long staining time with extremely dilute solutions of the dye, and a critical differentiation period. In our experience, the fixation of blocks with formaldehyde vapor is not a suitable method for Bowie-staining even if the recommended post-fixations are used [27, 29]. The granules stained only moderately and without the required contrast.

The method of ENDES, however, which has the advantage of formalin-requirement, gave a strong staining of JG granules, which then has focussed our attention on this technique.

In order to recognize the poorly granulated juxtaglomerular cells as well, we sought to increase the colour and/or intensity contrast between JG granules and nuclear chromatin. To recognize and locate afferent arterioles in the arterial tree we set out to develop a good elastic staining effect too.

PTAH treatment mostly removes the stain from the elastic membrane, only the ripened aldehyde-fuchsin persists. Although the elastic staining of orcein is liable to extraction by PTAH, the affinity to the nuclei of orcein is stronger than that of PTAH. Orcein can be used for increasing the colour contrast with ENDES' trichrome stain because it does not affect the staining of JG granules. An aldehyde-fuchsin – orcein – PTAH sequence results both in an adequate elastic staining and in a good staining contrast of JG granules.

In order to compare the catecholamine content of arteriolar nerves with the granularity of juxtaglomerular arterioles on microphotograms, especially when poorly granulated cells are seen, JG granule intensity-contrast is necessary. Intensity-contrast could not be achieved by increasing the ionic strength because the PTAH staining of nuclear chromatin is reduced parallel with that of JG granules.

The required intensity contrast was achieved by methylation which completely eliminated the background staining with PTAH without reducing the staining of JG granules. The resistance to methylation of the staining of JG granules with PTAH points to a minor importance of protein carboxyls in dye binding. The staining mechanism of crystal violet, haematoxylin–acridine orange, haematoxylin–thioflavin-T and haematoxylin–Rivanol is different in this respect [14].

Our results confirm in general the observations of GOMBA et al. (1969) on the sympathetic innervation of juxtaglomerular afferent and efferent arterioles of mice. The increased frequency of varicosities in the preglomerular granulated part of afferent arterioles as compared to that of the non-granulated part that contains internal elastic membrane may be an expression of the increased functional activity of sympathetic nerves around renin producing cells. This would agree with the ultrastructural data of BARAJAS and MÜLLER (1973) in that the granulated cells of afferent arterioles of the rat are in contact with more numerous nerve terminals than the arteriolar non-granulated cells.

REFERENCES

1. ALEXANDRE, J. M., MENARD, J., CHEVILLARD, C., SCHMITT, H.: (1970) Increased plasma renin activity induced in rats by physostigmine and effects of alpha- and beta-receptor blocking drugs thereon. Europ. J. Pharmacol. **12,** 127—131. — 2. ASSAYKEEN, T. A., CLAYTON,

P. L., GOLDFIEN, A., GANONG, W. F.: (1970) Effect of alpha- and beta-adrenergic blocking agents on the renin response to hypoglycemia and epinephrine in dogs. Endocrinology **87,** 1318—1322. — 3. BARAJAS, L.: (1964) The innervation of the juxtaglomerular apparatus. An electron microscopic study of the innervation of the glomerular arterioles. Lab. Invest. **13,** 916—929. — 4. BARAJAS, L., MÜLLER, J.: (1973) The innervation of the juxtaglomerular apparatus and surrounding tubules. A quantitative analysis by serial section electron microscopy. J. Ultrastruct. Res. **43,** 107—132. — 5. BIRBARI, A.: (1971) Effect of sympathetic nervous system on renin release. Amer. J. Physiol. **220,** 16—18. — 6. BUNAG, R. D., PAGE, I. H., MCCUBBIN, J. E.: (1966) Neural stimulation of release of renin. Circulat. Res. **19,** 851—858. — 7. CSILLIK, B., KÁLMÁN, GY.: (1967) Fagyasztva-szárítás vacuum nélkül. Morph. Ig. Orv. Szle **7,** 278—284. — 8. EL-BADAWI, A., SCHENK, E. A.: (1967) Histochemical methods for separate, consecutive and simultaneous demonstration of acetylcholinesterase and norepinephrine in cryostat sections. J. Histochem. Cytochem. **15,** 580—588. — 9. ENDES, P., GOMBA, SZ., DÉVÉNYI, I.: (1969) Specific staining and exact quantitative evaluation of the granulation in the juxtaglomerular cells. Acta morph. Acad. Sci. hung. **17,** 47—53. — 10. FALCK, B., OWMAN, C.: (1965) A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic amines. Acta Univ. Lund, Sect. II. No. 7. 1—23. — 11. FOURMAN, J.: (1970) The adrenergic innervation of the efferent arterioles and the vasa recta in the mammalian kidney. Experientia (Basel) **26,** 293—294. — 12. GOMBA, SZ., SOLTÉSZ, M. B., ENDES, P.: (1964) Studies of the granulated cells of the juxtaglomerular apparatus. III. Effect of fixatives and freezing on the granules. Acta morph. Acad. Sci. hung. **13,** 19—24. — 13. GOMBA, SZ., BOSTELMANN, W., SZOKOLY, V., SOLTÉSZ, M. B.: (1969) Histochemische Untersuchungen der adrenergen Innervation des juxtaglomerulären Apparates. Acta biol. med. germ. **22,** 387—392. — 14. HARADA, K.: (1973) The staining and histochemical reaction for the acid nature of protein in juxtaglomerular granules. Mikroskopie **29,** 77—101. — 15. HODGMAN, C. D.: (1959) Handbook of Chemistry and Physics. Chemical Rubber Publishing Co., Cleveland 41sted., p. 2500. — 16. KANEKO, Y., TAKEDA, T., IKEDA, T., TAGAWA, H., ISHII, M., TAKABATAKE, Y., UEDA, H.: (1970) Effect of ganglion-blocking agents on renin release in hypertensive patients. Circulat. Res. **27,** 97—103. — 17. LÓRÁNTH, CS., UNGVÁRY, GY., DONÁTH, T.: (1969) The innervation of the juxtaglomerular apparatus. Acta morph. Acad. Sci. hung. **17,** 131—141. — 18. LILLIE, R. D.: (1954) Histopathologic Technic and Practical Histochemistry. The Blakiston Co., New York. — 19. MOGIL, R. A., ITSKOVITZ, H. D., RUSSELL, J. H., MURPHY, J. J.: (1969) Renal innervation and renin activity in salt metabolism and hypertension. Amer. J. Physiol. **216,** 693—697. — 20. MOHAMMED, S., FASOLA, A. F., PRIVITERA, P. J., LIPICKY, R. J., MARTZ, B. L., GAFFNEY, T. E.: (1969) Effect of methyldopa on plasma renin activity in man. Circulat. Res. **25,** 543—548. — 21. NILSSON, O.: (1965): The adrenergic innervation of the kidney. Lab. Invest. **14,** 1392—1395. — 22. PASSO, S. S., ASSAYKEEN, T. A., GOLDFIEN, A., GANONG, W. F.: (1971) Effect of alpha- and beta-adrenergic blocking agents on the increase in renin secretion produced by stimulation of the medulla oblongata in dogs. Neuroendocrinology **7,** 97—103. — 23. PLOEM, J. S.: (1969) A new microscopic method for the visualisation of blue formaldehyde induced catecholamine fluorescence. Arch. int. Pharmacodyn. **182,** 421—424. — 24. SAKHAROVA, A. V., SAKHAROV, D. A.: (1968) The fluorescence of biogenic monoamines in sections of the nervous tissue fixed with aqueous formaldehyde. Cytologia **10,** 389—391. — 25. VANDER, A. J.: (1965) Effect of catecholamines and the renal nerves on renin secretion in anesthetized dogs. Amer. J. Physiol. **209,** 659—662. — 26. VANDER, A. J., LUCIANO, J. R.: (1967) Neural and humoral control of renin release in salt depletion. Circulat. Res. **20—21,** Suppl. II-69-77. — 27. WAGERMARK, J., UNGERSTEDT, U., LJUNGQVIST, A.: (1968) Sympathetic innervation of the juxtaglomerular cells of the kidney. Circulat. Res. **22,** 149—153. — 28. WATHEN, R. L., KINGSBURY, W. S., STOUDER, D. A., SCHNEIDER, E. G., ROSTOFER, H. H.: (1965) Effect of infusion of catecholamines and angiotensin II on renin release in anesthetized dogs. Amer. J. Physiol. **209,** 1012—1024. — 29. WILSON, W.: (1952) A new staining method for demonstrating the granules of the juxtaglomerular complex. Anat. Rec. **112,** 497—507. — 30. WINER, N., CHOKSHI, D. S., WALKENHORST, W. G.: (1971) Effects of cyclic AMP, sympathetic amines, and adrenergic receptor antagonists on renin secretion. Circulat. Res. **29,** 239—248.

VERFAHREN ZUR MORPHOLOGISCHEN UNTERSUCHUNG DES JUXTAGLOMERULÄREN APPARATS NACH FLUORESZENZMIKROSKOPISCHER DARSTELLUNG DER SYMPATHISCHEN NERVEN

Z. NEMES, E. BODOLAY, K. PECZE und P. ENDES

Zahlreiche, auf dem Prinzip der Formaldehyd-Konzentration beruhende fluoreszenzmikroskopische Verfahren zum Nachweis der Katecholamine wurden im Hinblick darauf untersucht, auf welche Weise sie an das Verfahren zum Nachweis der juxtaglomerulären Granula angeschlossen werden können. Das Einfrieren- und Schmelzenlassen führen zur Disruption der juxtaglomerulären Granula. Diese Schädigung läßt sich auch durch kurzzeitige Fixierung nicht vermeiden. Nach Gefriertrocknung konnten die juxtaglomerulären Körnchen erfolgreich gefärbt werden.

Das Endessche Trichrom-Verfahren ergab nach Adaptation an das klassische Falck—Owmansche Verfahren zum Katecholamin-Nachweis eine zuverlässige Methode für die Praxis. Die der Erhöhung des Farben- und Intensitäts-Kontrastes der Granula sowie der Färbung der elastischen Membranen dienenden Modifikationen werden mitgeteilt, ferner auch einige Besonderheiten des Färbungsmechanismus der spezifischen Körnchen des juxtaglomerulären Apparats besprochen.

ТЕХНИКА МОРФОЛОГИЧЕСКОГО ИЗУЧЕНИЯ ЮКСТАГЛОМЕРУЛЯРНОГО АППАРАТА ПОСЛЕ ФЛЮОРЕСЦЕНТНОМИКРОСКОПИЧЕСКОГО ВЫЯВЛЕНИЯ СИМПАТИЧЕСКИХ НЕРВОВ

Л. НЕМЕШ, Е. БОДОЛАИ, К. ПЕЦЕ, П. ЭНДЕШ

Авторы изучали ряд методов для выявления флюоресцентных катехоламинов, основанных на принципе конденсации формальдегида, в целях их сочетания с методом для выявления юкстагломерулярных зернышек. Замораживание и оттаивание приводит к разрыву юкстагломерулярных зернышек. Кратковременная фиксация не предотвращает этого повреждения. После лиофилизации окрашивание юкстагломерулярных зернышек было успешным.

В результате адаптации трихромного метода Эндеша к классическому методу Фалька и Оумена для выявления катехоламинов был получен надежный метод для применения на практике. Сообщаются модификации, служащие повышению контрастности красок и интенсивности окрашивания зернышек, а также окрашиванию эластических мембран. Обсуждаются некоторые особенности механизма окрашивания специфических зернышек юкстагломерулярного аппарата.

Dr. Zoltán Nemes
Dr. Edit Bodolay
Dr. Károly Pecze
Prof. Dr. Pongrác Endes

DOTE Kórbonctani Intézet,
4012 Debrecen, Pf. 14, Hungary

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2nd Department of Pathology, Semmelweis Medical University, Budapest

PERMEABILITY OF THE POST-ISCHEMIC RAT AORTA

T. Kerényi, G. Horváth, Z. Detre, S. Kurunczi and H. Jellinek

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The time course of the vascular permeability disorder following upon acute hypoxia and the fate of plasma substances entering the vessel wall in consequence of increased permeability were studied on the rat abdominal aorta rendered hypoxic for one hour by double ligature and recirculated for periods ranging from one hour to 30 days. The distribution and quantity of mural plasma inhibition were determined histochemically by means of a colloidal iron tracer and Prussian blue reaction and by photometric analysis, respectively. Plasma imbibition reached its maximum after recirculation for 24 to 48 hours and fell to an almost normal level after 10 days.

Administration of the colloidal iron tracer on the second day of recirculation, when the permeability disorder was at its peak, showed plasma imbibition in every layer of the vessel wall. At seven days it was restricted to the outer third of the media and the adventitia.

The endothelium is acting as the main barrier to mural plasma imbibition and in the case of a permeability disorder only the elastic lamellae constitute a temporary mechanical obstacle to the ingression of plasma.

During the recent decade interest has increasingly been centred on the relationship between hypoxia and arteriosclerosis and it has been generally accepted that temporary or lasting hypoxia in due course leads to arteriosclerotic changes. The role of hypoxia in the pathogenesis of arteriosclerosis was first assumed by Hueper (1944). Later, investigations into human (Ormos, 1957; Defaria, 1967; Lapperouza, 1962) and experimental arteriosclerosis (Burck and Hartmann, 1963; Kjeldsen et al., 1969; Helin and Lorenzen, 1969; Garbarsch et al., 1969) have unequivocally shown that certain forms of the condition are initiated or accentuated by hypoxia. It has also been shown that hyperlipidaemia, one of the accepted causal factors of arteriosclerosis, also acts towards the decrease of oxygen saturation and carbon dioxide diffusion (Talbott and Frayser, 1963) and, vice versa, incorporation of ^{14}C into triglycerides is enhanced by oxygen deficiency (Kesse, 1969). The first lesion developing in early sclerosis, oedema of the aortic intima, has been partly attributed to a hypoxia-induced disorder of endothelial permeability (Meesen, 1939; Rotter, 1949; Müller and Loewenich, 1961) and imbibition of the hypoxic arterial wall by various plasma proteins (Dixon, 1961; Kerényi and Jellinek, 1971) has also been reported. There is reason to believe that

any local trauma eliciting increased plasma flow across the vessel wall acts towards the reduction of mural oxygen supply, thereby potentiating the influence of other noxious factors, e.g. hyperlipidaemia (WHEREAT, 1967). In order to obtain more information on the problem, examinations were carried out on rat abdominal aortic segments rendered hypoxic for given periods. This model proved especially suitable for the study of the endothelial permeability disturbance, which plays a decisive role in determining the morphological appearance of acute vascular lesions.

Materials and methods

Acute mural hypoxia was produced by double ligature of a 1 cm long segment of the infrarenal aorta in rats of both sexes, weighing 200—300 g. The ligatures were removed after one hour and the ligated segment was recirculated for periods ranging from one hour to 30

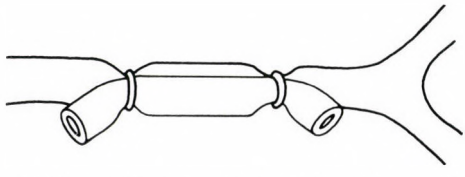

Fig. 1. Double ligation of infrarenal aorta. A piece of rubber catheter prevents the mechanical injury of the vessel wall

days. A thick silk thread was used for the ligation and a piece of a rubber catheter was placed under it to prevent injury of the vessel wall (Fig. 1). The colloidal iron preparation Ferrlecit® (Nattermann, Köln) was used as a tracer of plasma imbibition (JELLINEK et al., 1969). The penetration, deposition and elimination of plasma substances entering the vessel wall were examined in two groups of rats (Fig. 2). Following recirculation for periods ranging from 1 hour to 30 days (1, 2, 4, 16, 24 hours, 2, 3, 4, 8, 10, 12, 16, 20, 30 days), rats of the first group were treated intravenously with 1 ml/100 g Ferrlecit® one hour before sacrifice, to determine the actual permeability of the vessel wall. Aortic specimens of three rats at each recirculation period were dried to constant weight, reduced to ashes and examined photometrically by means of the TPZT reagent (BOTWELL and MALLET 1955, as modified by BENEDEK and SOÓS, 1967) for the quantities of iron demonstrable in the vessel wall. The extinctions were read at 595 mμ in a Spektromom MOM 360 apparatus and expressed in terms of μg Fe/mg dry matter.

The rats of the second group were treated intravenously with 1 ml/100 g Ferrlecit® on the second day of recirculation and were killed at periods ranging from 30 minutes to 20 days after the administration of the colloidal iron (for details see Fig. 2). This group served for the study of the further fate of plasma substances entering the vessel wall.

The aortic specimens were fixed in formalin, embedded in paraffin and stained with haematoxylin-eosin, Prussian blue, alcian blue—PAS or Azan. Corresponding aortic segments from untreated and sham-operated rats served as controls.

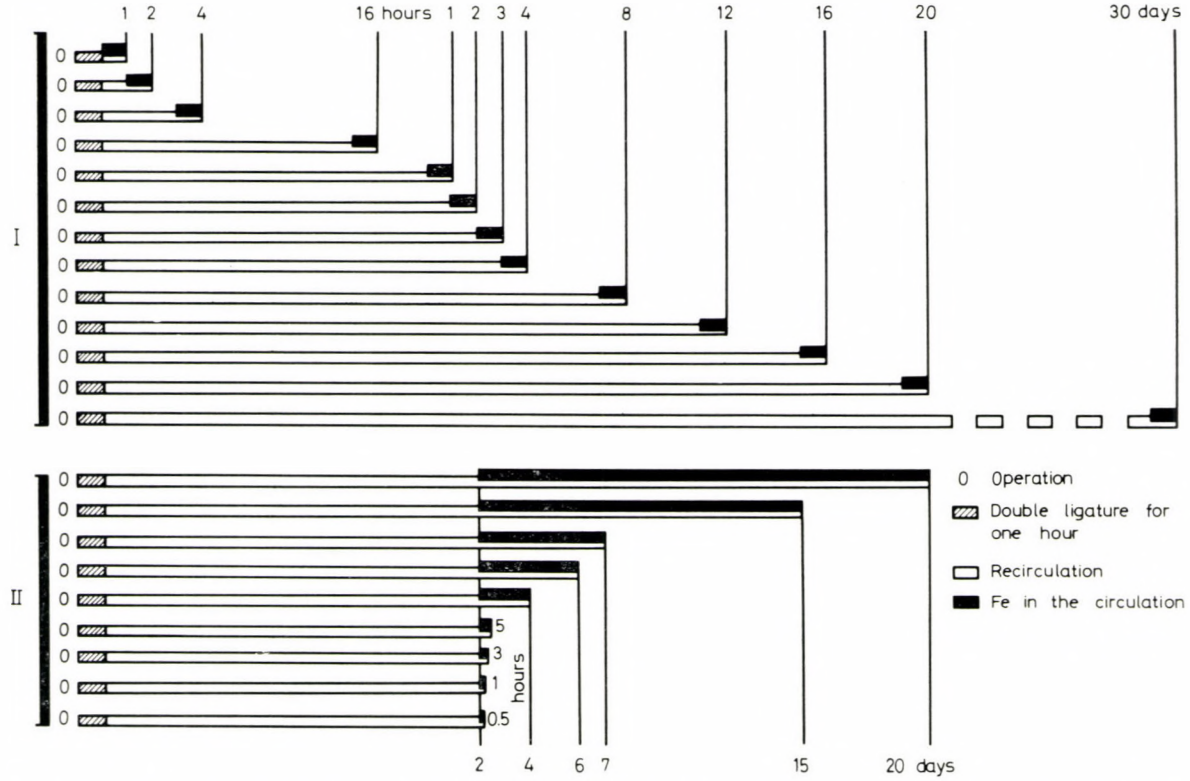

Fig. 2. The project of the two experimental groups. Group I, to determine the actual permeability of the vessel wall; group II, to follow the further fate of the iron traced plasma substances entering the vessel wall on the second day of recirculation

Results

Group 1. In sections from rats killed after one hour of recirculation, a positive Prussian blue reaction was found in endothelial cells and subendothelial spaces as well as in the media directly beneath the first elastic lamella (Fig. 3). After 2-hour recirculation, deposition of iron was found in several cases also in the deeper layers of the media. The iron-traced plasma was localized in the intercellular spaces, especially along the elastic membranes, and showed a homogeneous Prussian-blue positive reaction.

After recirculation for two days, two forms of iron deposition could be distinguished in the media, viz. delicate granules within the cells and a homo-

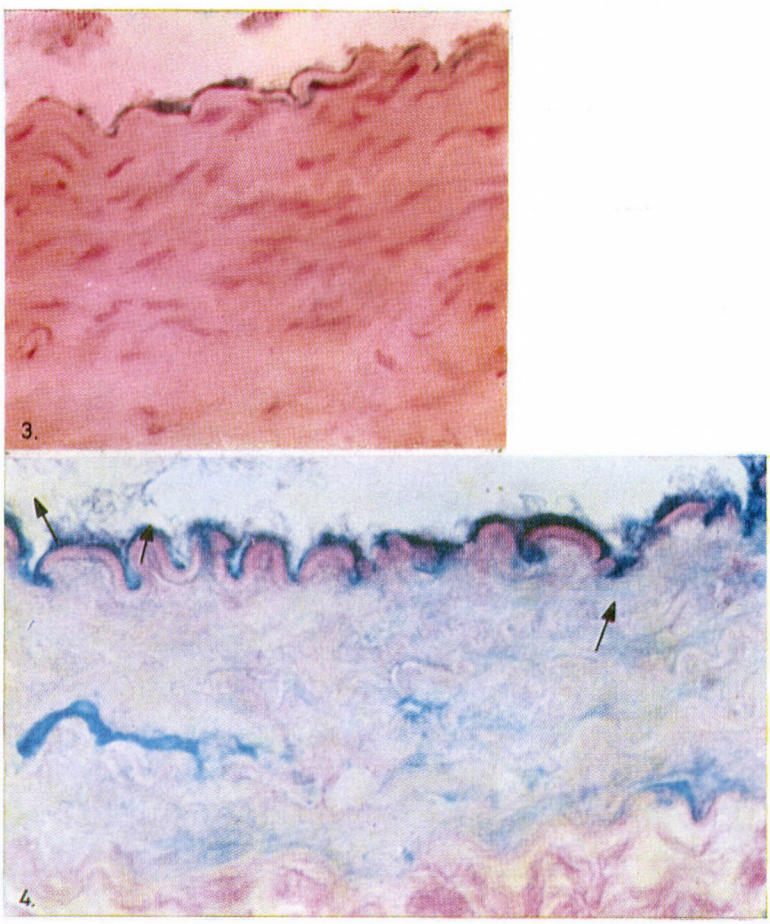

Fig. 3. Prussian blue reaction in the endothelial cells and beneath the first elastic lamella after one hour recirculation

Fig. 4. Intensive homogeneous and granular Prussian blue positivity in the whole vessel wall on the second day of recirculation. Elastic lamellae act as barriers to plasma imbibition (arrows)

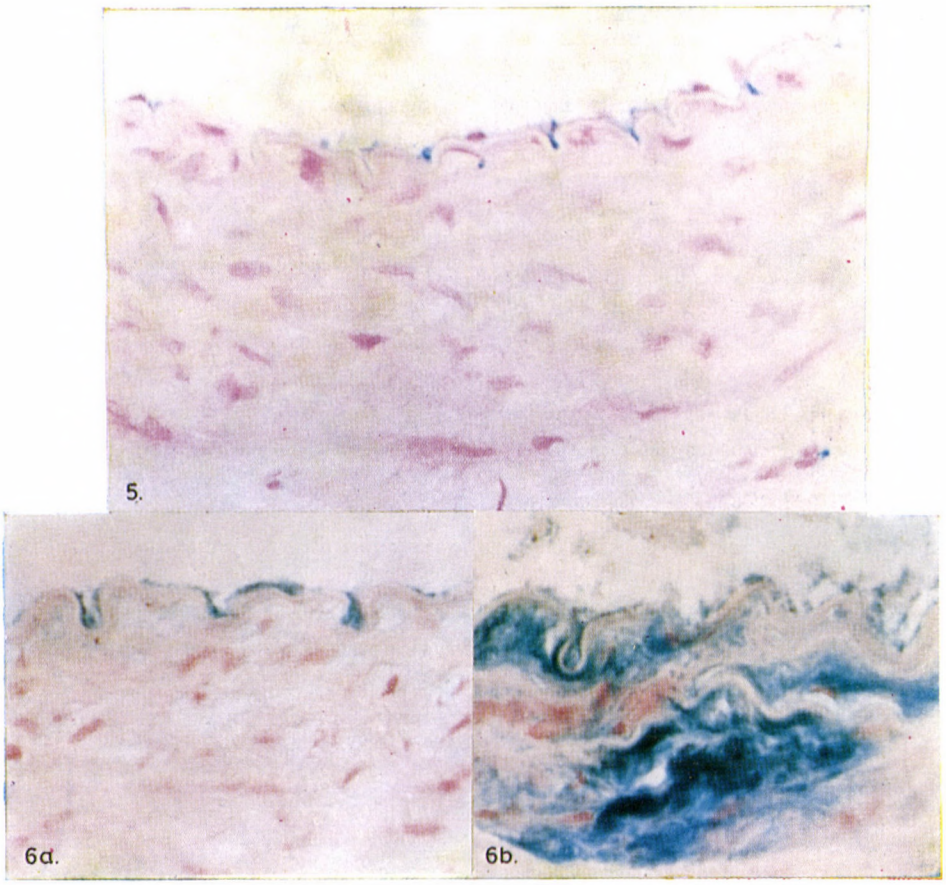

Fig. 5. Slight permeability disturbance after recirculation for ten days

Fig. 6. Minimum (a) and maximum (b) permeability after two days recirculation

geneous mass extracellularly (Fig. 4). The elastic fibres seem to act as a barrier to plasma imbibition, temporarily preventing the entrance of plasma substances into certain layers of the media (Fig. 4). The Prussian blue positivity of the intima was of a much lesser degree at two hours than after shorter periods of recirculation.

After recirculation for 8 to 10 days, the presence of the tracer was limited to endothelial cells, subendothelial space and the stomata of the first elastic lamella (Fig. 5).

The intima showed a sparse, diffuse Prussian blue reaction in both recirculated and control specimens of aorta.

The histologically demonstrable iron content of the intima and media varied widely in each experimental group, even in the uniformly injured portion between the two ligatures, as judged from the great variety of Prussian

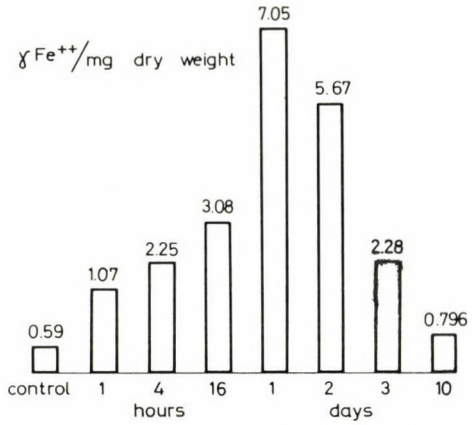

Fig. 7. Photometrically estimated iron content of the recirculated aortas shows the intensity of actual permeability disturbance

blue positive reactions in the areas showing minimum and maximum imbibition (Fig. 6a, b). Serial sections were therefore prepared to assess the average degree of mural injury.

To exclude the subjective error involved in histological evaluation, the iron contents of the injured sections were determined quantitatively by photometric evaluation of ashed specimens, using TPTZ reagent. The values determined after the different recirculation periods were in μg iron/mg dry matter: 1.07 at 1 hour; 2.25 at 4 hours; 3.08 at 16 hours; 7.05 at 24 hours; 5.67 at 48 hours; 2.28 at 3 days; 0.796 at 10 days (Figs 7 and 8). The value for the sham-operated control was 0.59 μg iron/mg dry matter).

Group 2. Aortic specimens from rats killed three hours after the administration of Ferrlecit® showed the presence of iron-traced plasma substances over the entire width of the media. In this series too, the elastic lamellae were found to act as temporary barriers (Fig. 9a). Part of the colloidal

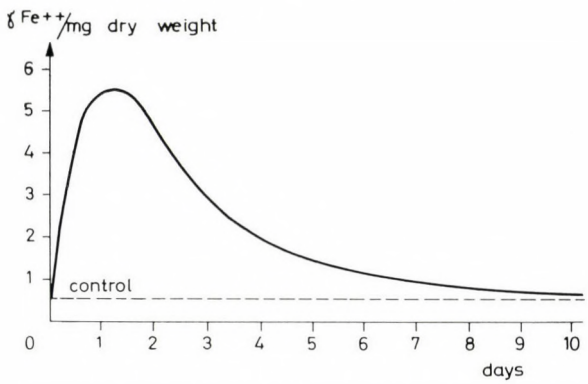

Fig. 8. Time course of the permeability disturbance after 1 to 10 days recirculation

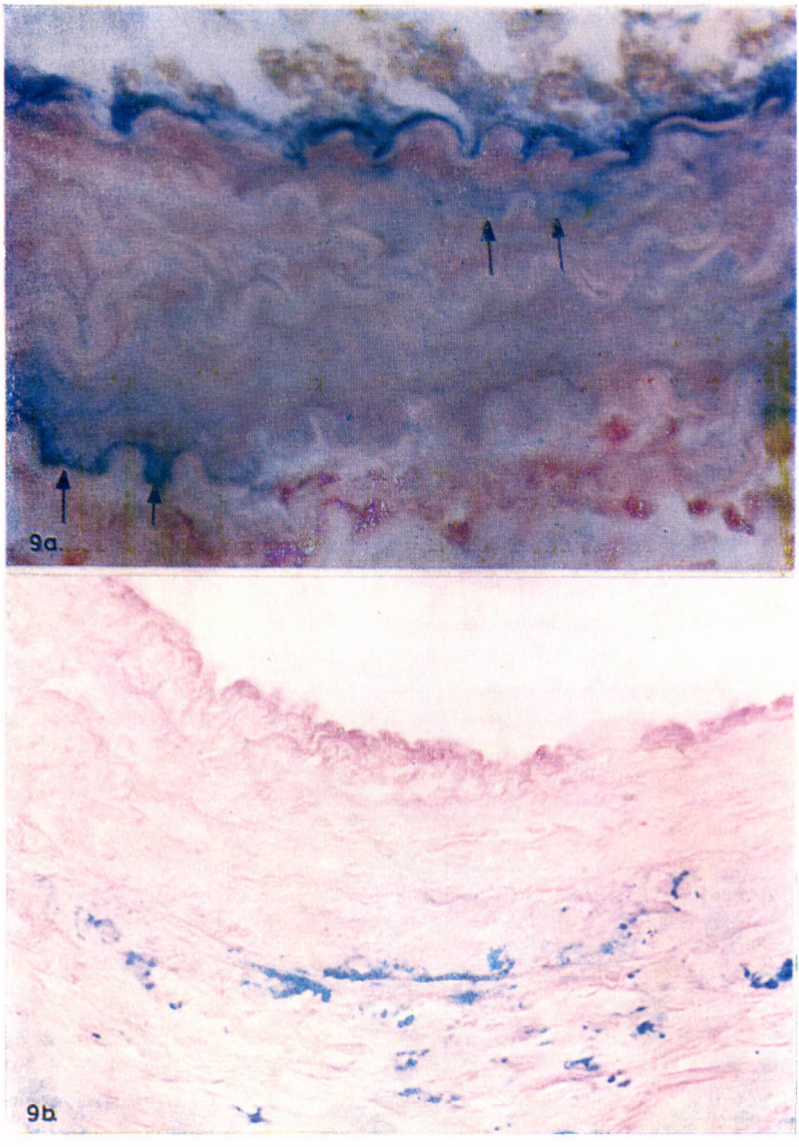

Fig. 9. Aortas from the second experimental group. a) Great amount of colloidal iron in the vessel wall 3 hours after administration of plasma tracer. Elastic lamellae act as barriers (arrows). b) Iron traced plasma gives Prussian blue positivity only in the outher third of the media and in the adventitia on the 7th day following the administration of colloidal iron preparation

iron was deposited intracellularly in the form of granules, part was distributed homogeneously in the extracellular space. The flow of iron traced plasma across the stomata of the elastic lamellae could easily be followed up.

Rats killed seven days after Ferrlecit® treatment, viz. on the ninth day of recirculation, had colloidal iron only in the outer third of the media and in the adventitia, in which it was usually present in the form of granules and coarse clumps (Fig. 9b).

Sections treated with staining techniques other than the Prussian blue reaction showed in both experimental groups an initial vacuolization of the medial smooth muscle cells, progressing to a disseminated muscle cell necrosis by four to eight days. The localization of dystrophic changes in the media only infrequently coincided with the areas heavily imbibed by plasma.

Discussion

It is clear from the present results that in rat abdominal aortic segments the maximal plasma imbibition was found after 24 to 48 hours, both histologically and by quantitative determination of the iron tracer. Although according to CONSTANTINIDES and ROBINSON (1969), the inter-endothelial junctions open up as soon as after 16 minutes of hypoxia, in the present experiments only a minimum increase of permeability was found in the acute stage, viz. immediately after the release of the ligatures. There are two possible explanations of this phenomenon, viz. (1) the endothelial cells and the closing structures between them (maculae occludentes, overlaps, interdigitation) constitute an incomplete permeability barrier (SCHWARTZ and BENDITT, 1972), in which case the increase in permeability might be due to basement membrane disbalance resulting from hypoxic cell injury. (2) Regenerative processes begin to predominate over the degenerative processes of the irreversibly damaged endothelium only after two days. Clarification of the problem will require further studies.

It was also found that plasma substances, once they have reached the subendothelial space, continue to penetrate into the adventitia across the interstitital gaps of the media and stomata of the elastic lamellae. Unlike colloidal carbon, the saccharated iron oxide particles of Ferrlecit ® are not retained by the basement membranes (COTRAN et al., 1967), so that the Prussian blue positive reaction indicates the actual amount of plasma deposition. A small amount of the tracer is, however, rapidly incorporated by the media cells, which store it probably in the lysosomal organelles; this particularity of the media cells has been observed in various types of vascular wall injury (OHTA et al., 1959; WISSLER, 1967; WEISS, 1968). Adventitital fibroblasts and histiocytes also show a similar storage phenomenon.

In the second experimental group the fate of the tracer administered at the time of maximum permeability disorder was followed up in the vessel wall. Elimination of the bulk of the tracer towards the adventitia took place in a relatively short time, although some Prussian blue positive substance was retained in necrotic smooth muscle cells and at sites also within granules localized in the adventitia.

The pale, diffuse Prussian blue reaction, found in the adventitia of control specimens and at certain periods of recirculation as well, probably corresponded to the intra-vital Hale-reaction of adventitial acid mucopolysaccharides (YARDLEY and BROWN, 1965).

The permeability disturbance showed a focal distribution, although the injury involved equally the entire area of the ligated segments. The degree of plasma imbibition could nevertheless reliably be assessed by the quantitative determination of iron by a chemical method which, along with the histological studies, allowed to analyse the time course of the permeability disturbance.

REFERENCES

BENEDEK, E., SOÓS, G. (1967): Serum vas egyszerű meghatározási eljárása. Orv. Hetil. **108,** 2137—2139. — BENISCH, B. M., WISNIEWSKI, M. (1971): Muscular cushions of intramyocardial arteries: their relationship to coronary arteriosclerosis. Angiology **22,** 305—310. — BOTWELL, T. H., MALLET, B. (1955): The determination of iron in plasma or serum. Biochem. J. **59,** 599—602. — BURCK, H. C., HARTMANN, F. (1960): Atmungsgröße der Aortenwand und experimentelle Arteriosklerose. Zbl. allg. Path. path. Anat. **104,** 564—565. — CONSTANTINIDES, P., ROBINSON, J. (1969): Ultrastructural injury of arterial endothelium. I. Effects of pH, osmolarity, anoxia and temperature. Arch. Path. **88,** 99—105. — COTRAN, R. S., SUTER, E. R., MAJNO, G. (1967): The use of colloidal carbon as a tracer for vascular injury. Vasc. Dis. **4,** 107—127. — DEFARIA, J. L. (1961): Aortenmedia-Nekrose mit sekundärer Arteriosklerose bei den kongenitalen zyanotischen Herzkrankheiten. Beitr. path. Anat. **125,** 129—147. — DIXON, K. C. (1961): Deposition of globular lipid in arterial cells in relation to anoxia. Amer. J. Path. **39,** 65—74. — GARBARSCH, C., MATTHIESSEN, M. E., HELIN, P., LORENZEN, I. (1969): Arteriosclerosis and hypoxia. I. Gross and microscopic changes in rabbit aorta induced by systemic hypoxia. Histochemical studies. J. Atheroscler. Res. **9,** 283—294. — GUTSTEIN, W. H., FARREL, G. A., ARMELLINI, C. (1973): Blood flow disturbance and endothelial cell injury in preatherosclerotic swine. Lab. Invest. **29,** 134—149. — HELIN, P., LORENZEN, I. (1969): Arteriosclerosis in rabbit aorta induced by systemic hypoxia. Angiology **20,** 1—12. — HUEPER, W. C. (1944): Arteriosclerosis. The anoxemia theory. Arch. Path. **38,** 173—181. — JELLINEK, H., NAGY, Z., HÜTTNER, I., BÁLINT, A., KÓCZÉ, A., KERÉNYI, T. (1969): Investigations of the permeability changes of the vascular wall in experimental malignant hypertension by means of a colloidal iron preparation. Brit. J. exp. Path. **50,** 13—18. — KERÉNYI, T., JELLINEK, H. (1972): Fibrin deposition in smooth muscle cells of muscular type small arteries under temporary conditions of hypoxia. Exp. molec. Path. **17,** 1—5. — KJELDSEN, H. (1969): Smoking and atherosclerosis. Munksgaard, Copenhagen, p. 145. — KJELDSEN, K., WANSTRUP, J., ASTRUP, P. (1968): Enhancing influence of arterial hypoxia on the development of atheromatosis in cholesterol-fed rabbits. J. atheroscler. Res. **8,** 835—845. — KRESSE, H., FILIPOVIC, I., BUDDECKE, E. (1969): Gesteigerte ^{14}C-Inkorporation in die Triacylglycerine (Triglyceride) des Arteriengewebes bei Sauerstoffmangel. Hoppe Seylers Z. physiol. Chem. **350,** 1611—1618. — LAPEROUZA, (1962): Zwei Fälle von Ceroidpigmentbildung des Mesenterialfettes und der glatten Muskulatur mit schwerer pseudoperniziöser Anämie bei Parkinsonismus. Virchows Arch. path. Anat. **335,** 544—555. — MEESEN, H. (1939): Experimentelle Untersuchungen zum Kollaps-Problem.

Beitr. path. Anat. **102,** 191—267. — MÜLLER, E., LOEWENICH, V. (1961): Das elektronhistochemische Verhalten der normalen und der arteriosklerotisch-veränderten Aortenintima. Frankf. Z. Path. **71,** 221—231. — OHTA, G., COHEN, S., SINGER, E. J., ROSENFIELD R., STRAUSS, L. (1959): Demonstration of gamma globulin in vascular lesions of experimental necrotizing arteritis in the rat. Proc. Soc. exp. Biol. (N.Y.) **102,** 187—189. — ORMOS, P. (1957): Rolle chronischer Hypoxie bei der Erkrankung der Gefäße. Zbl. allg. Path. Anat. **96,** 444—451. — ROTTER, W. (1949): Über die Bedeutung der Ernährungsstörung, insbesondere des Sauerstoffmangels für die Pathogenese der Gefäßwandänderungen mit besonderer Berücksichtigung der "Endarteritis obliterans" und der "Arteriosklerose", zugleich ein Beitrag zum Entzündungsproblem. Beitr. path. Anat. **110,** 46—102. — SCHWARTZ, S. M., BENDITT, E. P. (1972): Structure and permeability of rat thoracic aortic intima. I. Amer. J. Path. **66,** 224—264. — TALBOTT, G. D., FRAYSER, R. (1963): Hyperlipidaemia: a cause of decreased oxygen saturation. Nature (Lond.) **200,** 684. — WEISS, P. (1968): Submikroskopische Charakteristik und Reaktionsformen der Glattmuskelzelle unter besonderer Berücksichtigung der Gefäßwandmuskelzellen. Z. mikr. Anat. Forsch. **78,** 305—331. — WHEREAT, A. F. (1967): Recent advances in experimental and molecular pathology. Atherosclerosis and metabolic disorder in the arterial wall. Exp. molec. Path. **7,** 235—247. — WISSLER, R. W. (1967): The arterial medial cell, smooth muscle or multifunctional mesenchyme. Circulation **36,** 1—4. — YARDLEY, J. H., BROWN, G. D. (1965): Fibroblasts in tissue culture. Use of colloidal iron for ultrastructural localisation of acid mucopolysaccharides. Lab. Invest. **14,** 501—513.

PERMEABILITÄTSUNTERSUCHUNGEN AN DER POSTISCHÄMISCHEN RATTENAORTA

T. KERÉNYI, G. HORVÁTH, Z. DETRE, S. KURUNCZI und H. JELLINEK

Untersuchungen über den zeitlichen Verlauf und die quantitativen Verhältnisse der Permeabilitätsstörungen nach akuter Gefässwandhypoxie sowie über das Schicksal der in die Gefässwand gelangten Plasmaanteile.

Nach lokaler Hypoxie durch eine Doppelligatur der Aorta abdominalis der Ratte wurde in der nachfolgenden Rezirkulationsperiode von 1 Stunde bis zu 30 Tagen mit histochemischen und mikrochemischen Methoden in die Gefässwand gelangtes markiertes Plasma bestimmt. Das eine Stunde von den Herausnahme der Aorta zur Bestimmung der aktuellen Permeabilität injizierte kolloidale Eisen war am stärksten nach 24—48 stündiger Rezirkulation. Am Gipfelpunkt der Permeabilitätsstörung, eingegebene kolloidale Eisen durchtränkte anfänglich die gesamte Gefässwand. Ab 7. Tag war es nur im äusseren Drittel der Media und in der Adventitia nachweisbar.

Hauptsächlich wird das Plasmaeindringen durch das Endothel verhindert. Für Plasmaanteile, die die Endothelbarriere überwunden haben, stellen nur die elastischen Membranen ein zeitweliges mechanisches Hindernis dar.

ИССЛЕДОВАНИЕ ПРОНИЦАЕМОСТИ ПОСТИШЕМИЧЕСКИХ АОРТ У КРЫС

КЕРЕНЬИ, Т., ХОРВАТ, Г., ДЕТРЕ, З., КУРУНЦИ, Ш. и ЙЕЛЛИНЕК, Х.

Исследования были проведены для выяснения временных и количественных отношений нарушения проницаемости после острой гипоксии стенки сосудов и изменения плазматических веществ, попавших в стенку сосудов.

Плазматическое индикаторное вещество, попадающее в стенку сосудов было определено с помощью гистохимических и микрохимических методов в периоде рециркуляции, охватывающем от одного часа до десяти дней, после гипоксии, вызванной двойной лигатурой, наложенной на один час на брюшную аорту крыс.

Коллоидное железо, введенное с целью определения временной проницаемости в течение одного часа перед удалением аорты, было обнаружено в самом большом количестве в стенках сосудов после 24—48 часовых рециркуляций.

На десятый день количество железа в стенках сосудов понизилось почти до нормального уровня.

С целью выяснения дальнейшего состояния плазматических веществ, попавших в стенку сосудов в наивысшем нарушении проницаемости, т. е. при введении коллоидного железа на второй день резиркуляции, оно пропитывало вначале всю стенку сосудов, а на седьмой день и позже, коллоидное железо было обнаружено только в наружной трети средней оболочки и в адвентиции.

Попадание в стенку сосудов плазматических веществ довольно значительно задерживается эндотелием. Для плазмы, проникающей через эндотелий, только эластические пластинки служат временной механической помехой.

Dr. Tibor Kerényi
Dr. Gábor Horváth
Dr. Zoltán Detre
Dr. Sándor Kurunczi
Dr. Harry Jellinek

Semmelweis Orvostudományi Egyetem
II. Kórbonctani Intézete,
1450 Budapest, Pf. 41, Hungary

RECENSIONES

Morphology of the Maxillo-Mandibular Apparatus

Ed.: G. H. SCHUMACHER. 9th International Congress of Anatomists. Thieme, Leipzig 1972, 204 pages

The 9th International Congress of Anatomists was held in Leningrad, between the 17th and 22nd August, 1972. Within the Congress a special Symposium, in which 41 authors presented 30 papers, was devoted to the morphology of the maxillo-mandibular apparatus.

The book at issue contains their full text including figures and references. Among the papers we find reports on comparative and functional anatomical, osteological, histological and electron microscopic studies and experimental observations; this makes impossible to review the book as a whole, thus only some papers shall be mentioned to illustrate the multiplicity of the subjects. The introductory lecture was held by G. G. Schumacher (GDR) who, among others, described the lesion of Gasserian ganglia caused by tooth extraction and local anaesthesia. R. Barghausen (USA) dealt with the comparative anatomy of the masticatory organs of mammals. L. Du Brul (USA) spoke about the evolution of the oral apparatus of hominoideae. N. Mosolov (USSR) reported on the anatomy of the masseter muscle, A. Aronov (USSR) on the development of the soft and hard palate, M. Strassburg and S. Peters (FRG) on experiments causing abnormal development of the palate, H. Fromme and H. Riedel (FRG) on observations concerning the ultrastructure in the initial stages of dentin formation, E. Lautenbach (FRG) on scanning electron microscopic tests of the fine structure of the mandible. Some of the lectures included classical morphological tests e.g. of the neurohistological type (for instance that by J. Griffin and H. Spain [Australia] and H. Lau [GDR]). L. L. Kolesnikov (USSR) summed up his X-ray anatomical findings, while L. V. Kutnetsova et al. (USSR) and also K. Balogh and A. Csiba (Hungary) dealt with the gross examination of the mandible.

This representative volume with its excellent figures helps the interested specialist to become acquainted with the papers presented at this conference of a high scientific standard.

L. HARSÁNYI

Nomina Anatomica Veterinaria

Second Edition, with Index (International Committee on the Veterinary Anatomical Nomenclature). Vienna 1973, 218 + XIII pages.
Price: U.S. $ 8.50

Available at: Anatomisches Institut der Tierärztlichen Hochschule in Wien. Linke Bahngasse 11, A-1030 Wien, Österreich

The Anatomical Nomenclature of Paris (A.N.P.) brought the veterinary anatomists to subject the Veterinary Anatomical Nomenclature (V.A.N.) to revision. The acceptance of the A.N.P. produced a considerable gap between human and veterinary anatomists and to arrive at an understanding was considered necessary also by the human anatomists. Consideration of erect posture as the standard position was the main difference between the A.N.P. and V.A.N., which rather overshadowed the aspects of comparative anatomy. The principles of the V.A.N. agree mostly with those of the A.N.P., especially in the adoption of Anglosaxon words and the sometimes strange spelling of the original Latin terms, e.g. esophagus instead of oesophagus and cecum instead of caecum. As to general principles, the Nomenclature prescribes the use of contrasting epithets, but as regards the indication of the head's direction (rostralcaudal), this has not been attained in the new edition.

Since the first edition, the thorough consideration of technical terms and of the requirements of comparative anatomy has caused certain changes. The Nomenclature suggests the use of new, short, easily intelligible technical terms to serve standardization. At the same time the effort at standardization is confined not only to the common use of words but also to the similar way of application of the technical terms used only for domestic animals.

The system of technical terms follows that of descriptive anatomy, which is preceded by the Termini Generales and regions as well as by the chapters of Henle's technical terms and by the names of the body parts. Within this system the differences among the individual species, especially in angiology and to a lesser extent in splanchnology, are discussed in subchapters. These highly facilitate the use of the book. Interpretation of the text and the alphabetical index which is newly presented in the second edition are also helpful in this respect. Beyond that, the old Veterinary Nomenclature of Jena (V.N.J.) would also be of help and so would be the comparative list of words in following the changes. In the second edition, the changes accepted by the World Congress in Mexico have made the principles of comparative anatomy more universal. The comprehensive work of the Nomenclature Committee, which takes into consideration the proposals of veterinary anatomists of all over the world, endeavouring at the same time to elaborate a uniform nomenclature, needs to be emphasized. Fine examples of the above mentioned principles are to be seen in the fields of splanchnology and nervous system and also among the technical terms of osteology and myology.The Nomenclature Committee will be always glad to consider the opinion and proposals of both veterinary and human anatomists.

The book gives useful information for medical doctors performing animal experiments, for zoologists, biologists and for investigators of comparative physiology. Use of the Nomenclature has became practically universal in comparative anatomy, and it would be welcomed if this common language were also used by medical doctors.

G. Fehér

Blyuger, A. F., Vexler, H. M., Novitsky, I. N., Sinelnikova, M. P., Terenteva, L. A.: **Small Intestinal Biopsy in Acute Enteral Infections**

Publication of the Academy of Sciences of the Latvian Socialist Republic, Zinatne Publishing House, Riga 1973

The authors are the first to present modern intravital morphological findings in acute enteral infections. They describe the method and report on its usefulness in cholera, acute dysentery, E. coli enteritis, salmonellosis and virus hepatitis. They recommend their monograph to scientists dealing with the problem of enteral infections and also to practitioners. The rich illustration material (102 figures, 300 bibliographical data) as well as the detailed presentation of modern morphological methods make this bibliography highly interesting also for the morphologist.

The book consists of seven chapters. The first gives a general review of the history of small intestinal biopsy technique. It is pointed out that even the most up-to-date instruments are variants of those recommended by Wood and coworkers in 1949; the basic principle has not changed. The first instrument of Soviet design has been used since 1961. The authors performed their examinations with an instrument constructed by M. I. Dumes, which offers the possibility of taking several specimens with a single intubation. The tube giving a radiographic shadow is provided with distance marks, thus the distance from the teeth can be controlled. The method is considered harmless as repeated examinations revealed that the 3 to 10 mm large mucosal defects healed in 72 hours. An average loss of blood of 2 to 3 ml must be taken into consideration. Because of eventual haemorrhagic complications (which occur very rarely) the blood group should previously be typed. The authors discuss in detail the fields of application of the technique. They use it for diagnostic and differential diagnostic purposes, for controlling therapeutic effects, to study the pathogenesis of intestinal diseases as well as for the evaluation of the effect of new drugs and new methods. Haemorrhagic disorders, oesophageal stricture, phlebectasy, aortic aneurysm, bleeding stomach tumour and significant hypertension are regarded as contraindications. Haemorrhages of different intensity, peritonitis without perforation, and the gravest among them, intestinal perforation, are rare complications of biopsy. These are, however, the reasons, why the authors are against the ambulatory application of the method. They point out also the errors, emphasizing especially that the exact localization cannot always be determined and thus the absence of morphological alterations may only refer to a given region.

The second chapter gives a brief review on the techniques of evaluation (stereomicroscope, electron microscope, light microscope).

In the third chapter the knowledge obtained from morphological investigations is discussed on the basis of Soviet and international literature. The authors point out the deficiencies and eventual errors, and give practical advice to the investigators.

The comprehensive fourth chapter deals with the intact intestinal mucosa. It is important to know its normal histological picture, considering the large number of "physiological variants". Our conventional morphological knowledge obtained post mortem has to be completed by modern intravital morphological examinations. For this we must, however, know the basic principles of general histology, histochemistry, cytochemistry, microchemistry and electron microscopy. These problems are dealt with in detail. The different cell types too are thoroughly discussed.

The authors are the first in the international literature to present results of biopsy performed in acute enteral infections. These findings are included in the fifth chapter. The post mortem data concerning cholera, which has ceased to be an "exotic" disease, must be subjected to a thorough revision. Light and electron microscopic alterations are also discussed. In acute dysentery, significant structural histochemical and cytochemical changes have been found. E. coli enterocolitis belongs to the third group studied by the authors. They found that the light and electron microscopic as well as the histochemical and cytochemical changes are usually less severe in this disease than in acute dysentery. In gastrointestinal salmonellosis, proliferative changes of the mucosa dominate. Biopsy allows the recognition and separation of latent and clinically mild cases of this disease. The chapter which deals with the problem of viral hepatitis is of great importance. It describes the complex epithelial and stromal changes occurring in the small intestinal mucosa. The possible connection between the reduction of Kultschitzky-cells in viral hepatitis and the decrease of the serotonin level is also discussed.

In the sixth chapter certain basic but non-specific morphological changes are treated. Regeneration of the intestinal epithelium under physiological and pathological conditions is also dealt with and the physiological regeneration of the epithelial cells in different intestinal sections is presented in Tables. The intestinal epithelium is usually renewed every 1.6—3 days. Some syndromes are also discussed: hyperogeneration syndrome, sprue-like syndrome, subtotal atrophy etc. The authors throw light upon the concept of "physiological inflammation" and on the inflammatory syndrome.

In the last chapter the results obtained are analyzed from the clinico-pathological point of view. It is pointed out that the response of the intestinal mucosa to pathological effects is to a certain extent stereotyped; the reaction is a hyperogeneration or a sprue-like syndrome. At the end of the book, pathogenesis and morphogenesis of certain acute enteral infections are briefly summarized; among these the up-to-date information concerning the problem of viral hepatitis is outstanding.

The light and electron microscopic photographs add new information to our experience based so far chiefly on post mortem examinations. Small intestinal biopsy is an important method for recognizing subclinical and latent enteral infections, considering that the clinical picture and the structural functional changes do not always develop in parallel. The monograph provides useful information especially for clinicians interested in gastroenterology, for experts who perform endoscopic examinations and also for pathologists.

J. JUHÁSZ

Research in Medical Care
British Medical Bulletin, Vol. 30, No. 3, September 1974

The recent number of the British Medical Bulletin edited by the British Council summarizes various fields of research in a theoretical as well as practical way. The general concept of Medical Care includes a broad sphere. The actuality of the discussion of pertinent research is offered by the recently performed considerable reorganization of the National Health Service (NHS). The predecessor of the NHS, the National Insurance, introduced by Lloyd George in 1911, provided medical care and emergency aid only for those of low income without providing for hospital care. The NHS, following the legislation of 1946 has been enacted in 1948. Since that time, in the course of 26 years, a great number of difficulties manifested themselves and it has become obvious that the care of the aged, of mentally retarded and mental patients failed to receive satisfactory attention. Following long disputes, the triple organizational division existing until that time (district general practitioner, hospital, local public health) was integrated into one single unit. It seems remarkable that the organization within the United Kingdom is different in England from that in Scotland and there are

further differences in Wales and Northern Ireland. The scientific material, knowledge and research techniques collected in the course of the discussions have made it clear that the science of health organization has not been sufficiently appreciated in the past and the true requirements were not appropriately coordinated with the possibilities. On the basis of this recognition the cost of research spent for this purpose has been increased between the years 1962 and 1972 from the original several hundred thousands to 10 million pounds.

The present number of the British Medical Bulletin contains the papers of those, who had outstandingly participated in the research. It is thought in general that the efficacious plan would be of reactive (circular) and mostly of self-regulating effect.

1. After the clearly outlined program follows 2. the drafting of alternative solutions; 3. the most suitable should be chosen and 4. has to be realized; 5. the result has to be evaluated and, finally 6. on this basis the purpose, method and realization have to be modified. The multilateral requirements are to be considered; it should be decided that on various levels which ones are entitled to priority, in accordance with the requirements and the available — obviously restricted — possibilities.

The present number, which of course is of importance in the first place for the actual development of medical care of the United Kingdom, but at the same time enlightening for all health services, has been assembled by a special committee directed by Professor W. W. Holland. The themes discussed, each deserving special attention within its sphere, are as follows. *Organisation of Health Services Research* (Sir Douglas Black); *Evaluation of Health Information Systems* (M. R. Alderson); *Demand, Need and Quality in General Practice* (J. D. E. Knox and D. C. Morrel); *Sociology of Prescriptions* (P. A. Parish); *Studies of Patients* (Ann Cartwright); *Evaluation of the Role of the Community Hospital* (A. E. Bennet); *The Hospital: Towards a Rational Use* (Vera Carstairs and M. A. Heasman); *Demand for Laboratory Services* (J. S. A. Ashley); *Techniques for Quality Control of Laboratory Services* (T. P. Whitehead); *Experiments in Medical Care* (M. W. Adler, K. Dunnell and J. M. Weddell); *Research into Nursing Services* (Lisbeth Hockey); *Cost-Benefit Approach* (A. Williams); *Operational Research Approach to Problem Solving* (J. Luckmann and J. Stringer); *Mathematical Models of the Balance of Care* (A. G. McDonald, G. C. Cuddeford, E. M. L. Beale); *Studies of Health Services Organization* (Maureen Dixon and J. Jacques); *Research in Health Education* (W. T. Jones, Helene Grahame).

No matter how different are the circumstances of the United Kingdom from those of e.g. the socialist countries, many useful lessons may be obtained from these studies and still more from the extensive literature quoted in them. The increased possibilities of demand and supply for the health services is enormus. Whichever should be the direction of research in the medical sciences, sooner or later any valuable result will assert itself in both diagnostics and therapy. Apart from the difficulties to predict the future these should be taken also into account by the research of Health Services organization.

The costs are ever increasing, characterized among others by the brief framed note, which announces that from January 1, 1975, one number of the British Medical Bulletin itself will be sold abroad for £ 3.5 instead of £ 2.5 as before.

It follows clearly from the theme that this group of studies will interest in the first place the health service organisers; however, health service research will not be restricted to the interest of those who are strictly occupied in this territory, but may become a profitable reading for all those, who are interested in the future of public health and the applied medical sciences. It seems objectionable that in addition to provision and care, prevention has been omitted. The excellent papers in this issue of the B.M.B. are completed by critical reviews on some recently published books of pertinent themes. The whole issue will offer an opportunity for profound study for all interested persons.

Prof. Gy. PETRÁNYI

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Proceedings of the lectures held at the Congress

Edited by I. KÖRNYEY, I. TARISKA, G. GOSZTONYI

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Institute of Pathology, University Medical School, Debrecen, Hungary,
and
Electron Microscopic Laboratory of the Institute of Pathology of Humboldt-University (Charité), Berlin, GDR

THE EFFECT OF VASCULAR PERFUSION FIXATION ON THE ULTRASTRUCTURE OF THE JUXTAGLOMERULAR APPARATUS OF THE RAT

J. Szabó, Gy. Lustyik and R. Dreher

(Received December 21, 1974)

After fixation by low pressure retrograde perfusion the electron microscopic picture of the rat JGA was studied and compared with the picture obtained after fixation by dripping and immersion. The most conspicuous difference was the presence of the basal compartments of the macula densa cells, which might land electron microscopic support to the tubulo-glomerular feedback theory. Among the JG granular cells, desmosome-like connected structures were seen. Crystalline and granule in granules specific secretion forms were frequent in the epitheloid cells.

Introduction

The electron microscopic picture of the JGA has been discussed in numerous publications [1—8, 12—14, 18—21), but few data are available concerning the electron microscopic picture of the JGA after perfusion fixation. Gomba et al. and Riedel et al. [10, 21] discussed the characteristics of the macula densa subsequent to perfusion fixation. In the present work we processed the JGA of kidneys fixed by the retrograde low pressure aortic perfusion method elaborated by us [25]. In addition to a detailed analysis of the macula densa, the electron microscopic picture of the JG granular cells and of Goormaghtigh's cells is also described.

Materials and methods

Male Wistar rats of 160—180 g body weight were used in the experiments. The animals were kept on a standard normal diet and received tap water ad libitum. In 10 animals under light ether anaesthesia the abdominal aorta was prepared. Immediately over the bifurcation through a cannule inserted into the aorta, perfusion at 50 cm water pressure was carried out for 3 minutes [25]; the fixing solution was 1% glutaraldehyde [16] dissolved in a modified Tyrode's solution. The final pH was adjusted to 7.2. Before perfusion the aorta was ligated below the diaphragma and the left renal vein was cut. After the perfusion, about 1 cumm specimens were obtained from the renal cortex; these were fixed in the same solution for further 2 hours then after fixed in 1% OsO_4 solved in modified Tyrode's solution for 90 min. After alcoholic dehydration the material was embedded in Mikropal (FERAK, Berlin). In 1 μ thick sections stained with toluidine blue the presence of JGA was ascertained then after orientation and reduction of the block surface ultrathin sections were prepared with a Reichert OM U2 ultramicrotome. After uranyl-acetate and lead-citrate staining sections were examined with the Siemens 1A and TESLA BS 513A electron microscope. In each animal, two JGA were studied.

Results

Macula densa. The compartments observed by RIEDEL et al. and GOMBA et al. on the basal part of the macula densa cells could be detected in each examined apparatus. It is clearly seen also on the light microscopic picture that the compartments are confined to the area of the macula densa, whereas on the opposite side of the distal tubular wall none can be found (Figure 1). In the electron microscopic pictures the compartments proved to be dilated intercellular spaces (Figures 2, 3, 4). In the cytoplasm surrounding these spaces mitochondria, ribosomal groups and endoplasmic reticulum portions could be seen. The basal membrane is covered by the processes of the macula densa cells including gaps varying in width. Through these gaps direct contact is ensured between the compartments and the basal membrane. On the hilar side of the basal membrane no granular cells can be seen. There is a border consisting of 1 or 2 Goormaghtigh cells. The intercellular compartments do not reach the lumen, the bordering cell membranes adhere to each other; in these areas regular terminal bars could be observed (Fig. 3). Laterally the compartments are everywhere respecting the margin of basal membrane forming the base of the macula densa. In the area of the division of the common basal membrane the compartments gradually decrease in size then cease to the present (Fig. 4).

Granular cells: In the section of the afferent arteriole close to the hilus there is a basal membrane-like network among the epitheloid cells which form the media and are arranged in several layers. In some sections this network is thin, thus the cytoplasmic membranes of the adjacent cells are closely

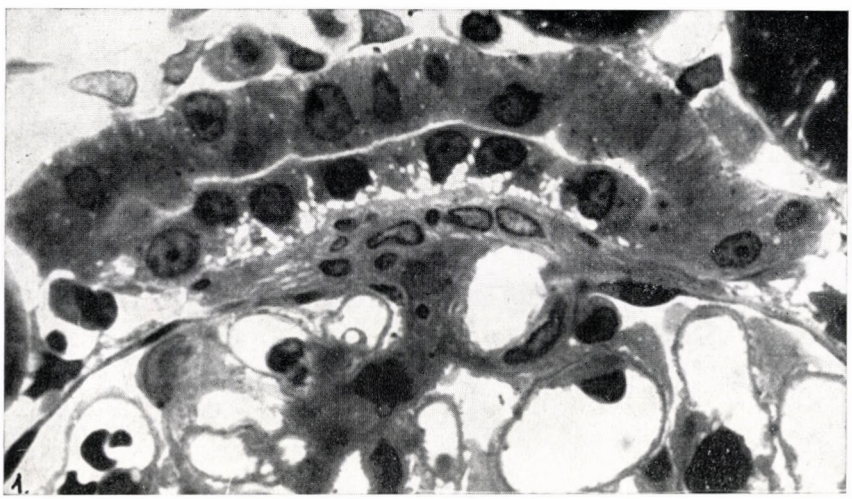

Fig. 1. Light microscopic picture. Marked vacuolization in the basal part of macula densa; this cannot be seen in the distal tubule on the opposite side of the macula. 1 μ thick section stained with toluidine blue. $\times 625$

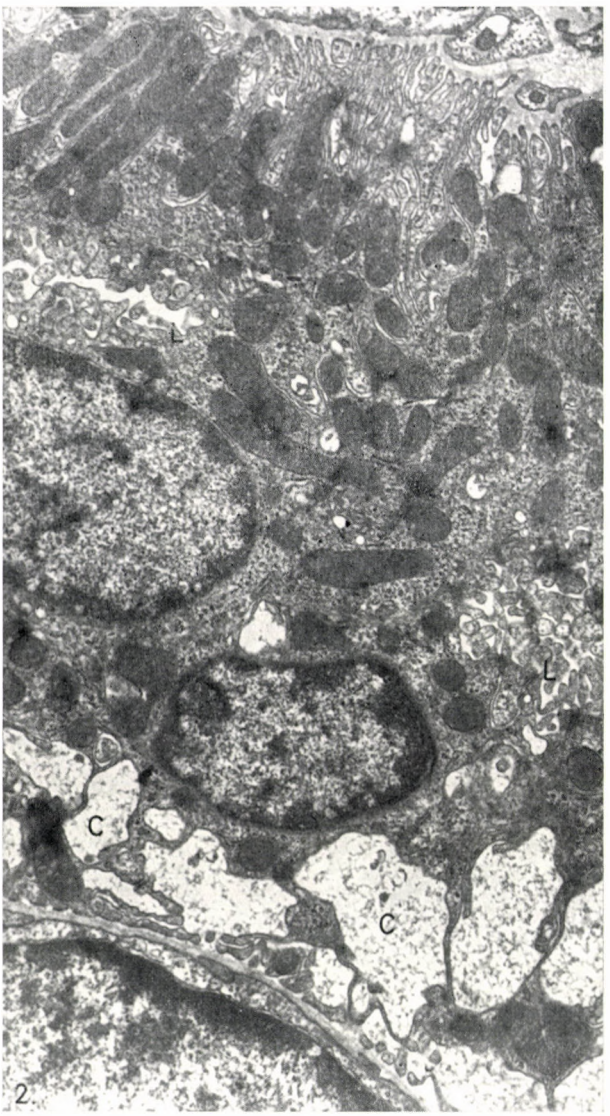

Fig. 2. Electron microscopic picture. Wide intercellular compartments; in the basal part of the macula densa on the opposite side of the tubular wall these are lacking. In the cytoplasmic processes bordering the compartments ribosomes and mitochondria are present. C = compartment, L = tubular lumen. ×8000

connected (Fig. 5). We have failed to observe any tight junctions. Infrequently, desmosome-like connected structures were seen among the epitheloid cells (Fig. 6). The cells have processes and are intertwined by means of these. The specific granules vary in shape and size; part are spherical, part ovoid or have

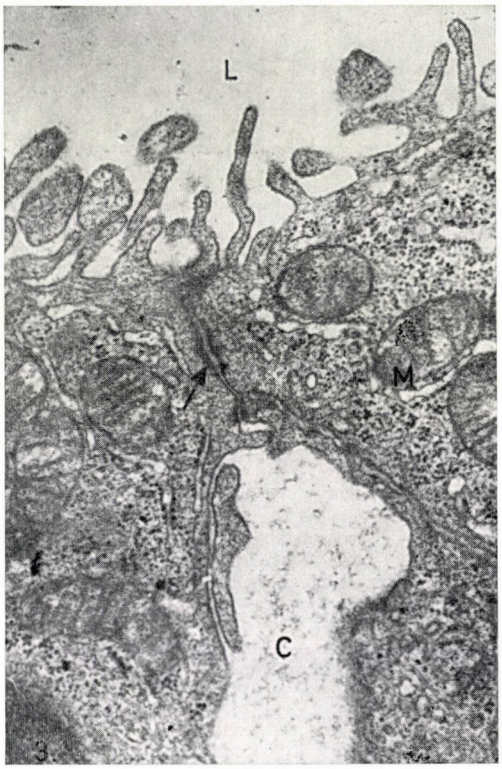

Fig. 3. Electron microscopic picture. NO intercellular compartments (C) near the lumen, the cell membranes bordering the compartment touch each other. Connected structures (arrows) corresponding to terminal bars. L = distal tubular lumen, M = mitochondria. ×40 000

a rod-like elongated cross-section. Forms with lobular margins too may be detected as if they would have developed by the fusion of several ovoid granules. The difference in density is also marked (Fig. 5). Rather infrequently crystalline and granula in granulis forms can be seen (Fig. 7). The granules are surrounded by a single membrane. The Golgi apparatus is localized near the nucleus and has a sacculo-vesicular structure (Fig. 5). The cytoplasm contains endoplasmic reticular cisterns, lamellar mitochondria and ribosomes; near the cell membrane there is a narrow myofilament border.

Goormaghtigh's cells. Among the cells there is a network of basal membrane-like material which in some places is growing thin. The surface membranes of the cells are densely layered; connected structures could not be detected (Fig. 8). The cells have processes and are interlocked. In the juxtanuclear region sacculo-vesicular Golgi-apparatus may be observed, the cytoplasm is poor in organelles and contains mitochondria, ribosomes and some wide endoplasmic reticular cisterns (Fig. 8).

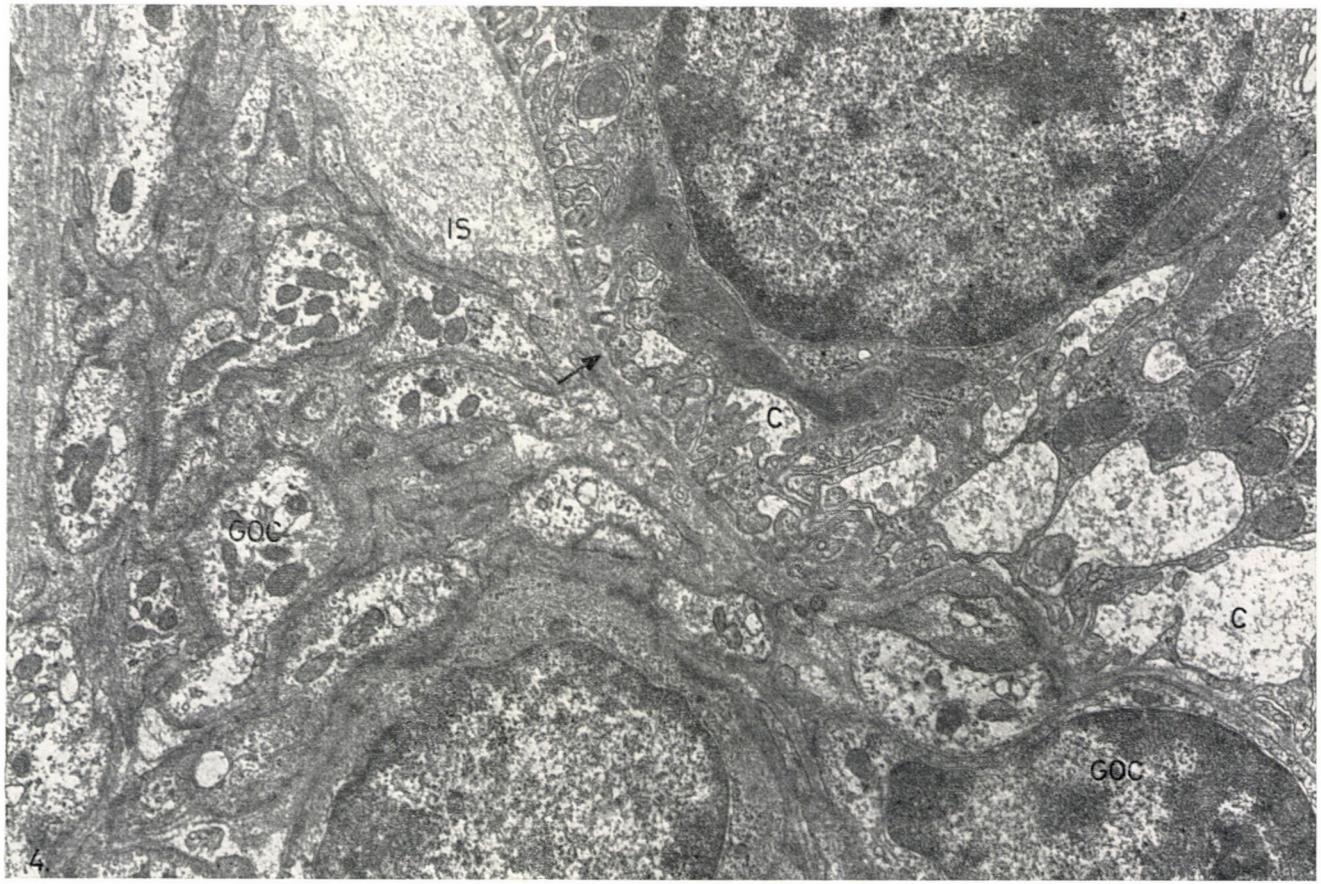

Fig. 4. Electron microscopic picture. In the region of the division of the hilar — tubular common basal membrane (arrow) the compartments decrease in size than cease to be present. C = compartment; GoC = Goormaghtigh's cell; IS = intertissular space. ×24 000

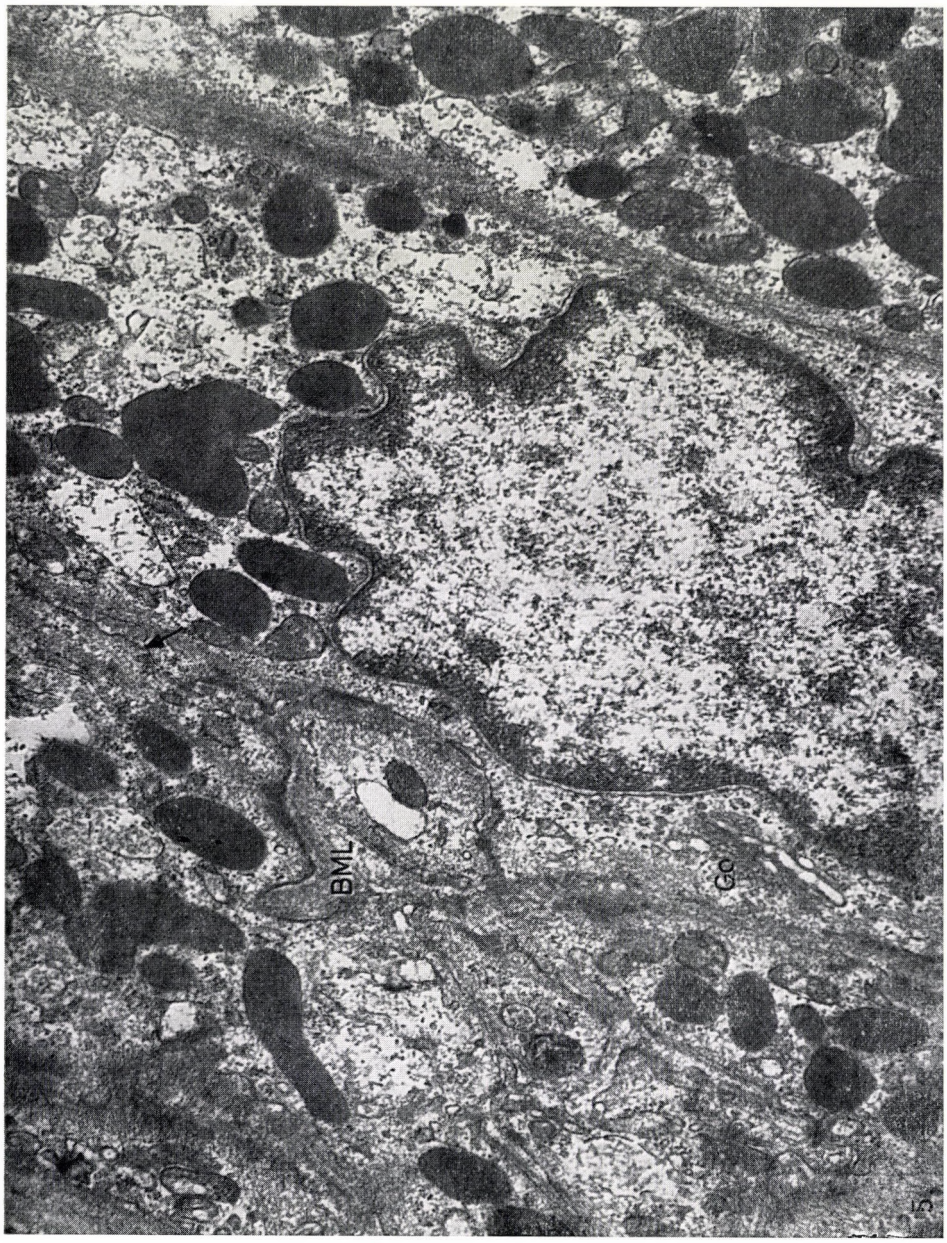

Fig. 5. Electron microscopic picture. Granular cells in the media of the afferent arteriole. Thinning of the intercellular basal-membrane-like network; closely adhering cell membranes (arrow). The renin granules are different in shape and density. G = Golgi apparatus; BML = basal membrane-like material among the granular cells. ×20 000

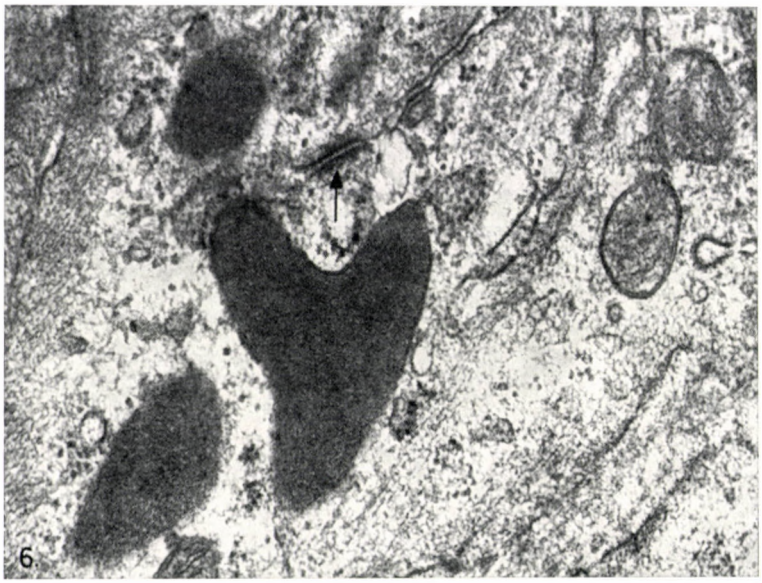

Fig. 6. Electron microscopic picture. Desmosome-like connecting structure among the juxtaglomerular granular cells (arrow). ×52 000

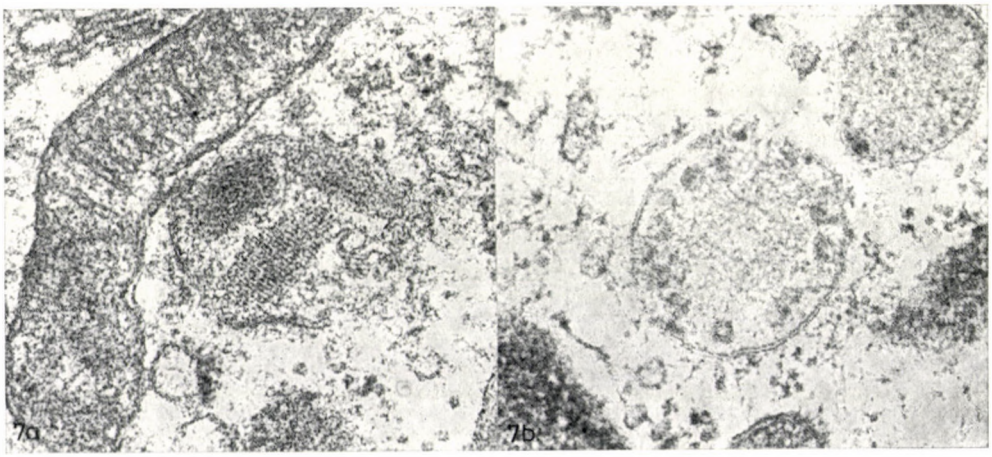

Fig. 7. Electron microscopic picture. a. crystalline granule form; b. granula in granulis form. ×73 000

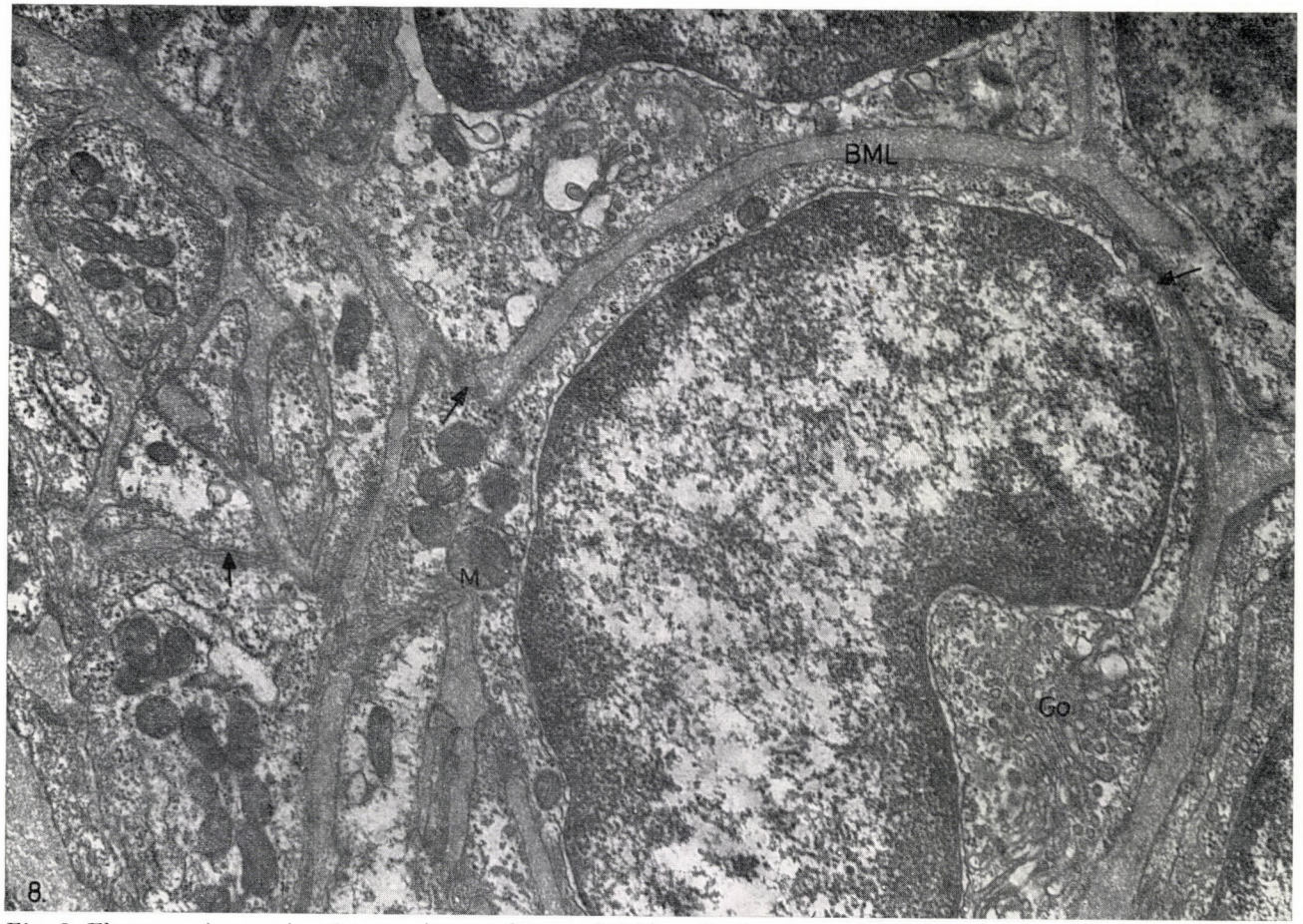

Fig. 8. Electron microscopic picture. Among Goormaghtigh's cells, basal membrane-like (BML) basic substance which disappears at sites (arrow). Closely adhering. Go = Golgie apparatus; M= mitochondrium. ×20 000

Discussion

The regulatory mechanism of renin synthesis occurring in the juxtaglomerular granular cells is a much disputed question. Essentially two opinions keep themselves in the literature. According to the first renin synthesis and secretion would be regulated by the pressure in the afferent arteriole and the stretch acting on the JGC-s of the media (baroreceptor theory, stretch receptor theory, [24, 28]). According to the second view the primary regulatory factor would be the ionic composition of the distal tubular fluid (macula densa theory, [17, 26, 27, 29, 30]). In the latter case a feedback through the macula densa towards the epitheloid cells should be supposed. This theory is supported by the enzyme histochemical reactions of the macula densa cells [10] and by the presence of the intercellular compartments described by GOMBA et al. and RIEDEL et al., and observed also in the present study. This would refer to an increased absorption in this region. It seems noteworthy that in the guinea pig the macula densa compartments are found also in normal animals after immersion fixation [20]. In rats rendered polyuric by glucose administration [15], observed these wide intercellular spaces in the proximal and distal tubular sections.

Although the supposed feedback of the ionic state of the distal tubular fluid would be directed in the first place towards the renin-producing JGC-s, we have failed to observe any direct connection between the macula densa cells and the JGC. The macula densa cells were directly connected only with Goormaghtigh's cells. Our findings agree with the results of the three-dimensional examinations of BARAJAS [2, 3]. COSSEL found by the ruthenium red method [9], GOMBA et al. by potassium pyroantimonate fixation [11] a continous intercellular space encircling the granular cells from the base of the macula densa to the lumen of the afferent arteriole, which may ensure free electrolyte transport between the macula densa and the granular cells. In agreement with other authors [2, 3, 6, 9, 11, 12] we observed the basal membrane-like continuous basic substance connecting the macula densa cells with Goormaghtigh's cells and granular cells.

Studies concerning the innervation of the JGA [5, 6, 31] agree in that only part of the JGC-s are directly connected with nerve endings. Among the JGC-s, similar as with the myocardial fibrils, there exists a syncytium-like stimulation transfer or connection. This hypothesis is supported by the nexal junctions by BIAVA and WEST [6]. These quintuple layered junctions are visible only after $KMnO_4$ fixation, thus they could not be studied by our glutaraldehyde perfusion method. In our material we have, however, observed structures with desmosome-like junctions among the JGC-s. Our results and the nexal junctions described by BIAVA and WEST support the existence of syncytium-like connections of the JGC-s at the electron microscopic level.

The ultrastructure of the granular cells is different in preparations fixed by other methods. We have frequently observed crystalline granules and granula in granulis forms described before to occur in hyperfunctional states [1, 19, 22, 23].

Our findings differed from those obtained in material fixed by conventional methods. This may have been done to the effect of perfusion fixation being closer to the physiological conditions. Thus, the procedure might offer more useful information concerning the JGA than that supplied by other methods.

REFERENCES

1. Barajas, L. (1966): The Development and ultrastructure of the juxtaglomerular cell granule. J. Ultrastruct. Res. **15.** 400—413. — 2. Barajas, L. (1970): The ultrastructure of the juxtaglomerular apparatus as disclosed by three-dimensional reconstructions from serial sections. J. Ultrastruct. Res. **33.** 116—117. — 3. Barajas, L., Latta, H. (1963): A three-dimensional study of the juxtaglomerular apparatus in the Rat. Lab. Invest. **12.** 257—269. — 4. Barajas, L., Latta, H. (1967): Structure of the juxtaglomerular apparatus. Circulat. Res. 21. Suppl. **2.** 15—28. — 5. Barajas, L. Müller, J. (1973): The innervation of the juxtaglomerular apparatus and surrounding tubules: A quantitative analysis by serial section electron microscopy. J. Ultrastruct. Res. **43.** 107—132. — 6. Biava, C. G., West, N. (1966): Fine structure of normal human juxtaglomerular cells. I. General structure and intercellular relationships. Amer. J. Path. **49.** 679—721. — 7. Biava, C. G., West, N. (1966): Fine structure of normal human juxtaglomerular cells. II. Specific and nonspecific cytoplasmic granules. Amer. J. Path. **49.** 955—979. — 8. Bucher, O., Riedel, B. (1965): L'appareil Juxtaglomerulaire du Rein. Bull. Ass. Anat. (Nancy) **130.** 43—77. — 9. Cossel, L. (1971): Elektronenmikroskopische Befunde mit Rutheniumrot am juxtaglomerulären Apparat. Virchows Arch. path. Anat. Abt. A. **353.** 289—301. — 10. Gomba, Sz., Benkő, K., Soltész, B. M. (1968): Extracellular compartmentation of the perfusion fixed renal macula densa. Acta anat. (Basel) **70.** 554—558. — 11. Gomba, Sz., Soltész, B. M., Szabó, J., Endes, P. (1972): An electronhistochemical study on the distribution of sodium ions in the juxtaglomerular apparatus. Virchows Arch. path. Anat. Abt. Zellpath. **11.** 284—288. — 12. Hartroft, P. M., Newmark, L. N. (1961): Electron microscopy of renal juxtaglomerular cells. Anat. Rec. **139.** 185—199. — 13. Hatt, P. Y. (1967): The juxtaglomerular apparatus. In: Ultrastructure of the Kidney. Eds.: Dalton, A. J., Haguenau, F., Academic Press, New York, pp. 101—129. 14. Latta, H., Maunsbach, A. B. (1962): The juxtaglomerular apparatus as studied electron microscopically. J. Ultrastruct. Res. **6.** 547—561. — 15. Latta, H., Bencosme, S. A., Knigge, K. M., Madden, S. C. (1962): Extracellular compartments in renal tubules associated with polyuria from glucose imbibition. Lab. Invest. **11.** 569—579. — 16. Maunsbach, A. B. (1966): The influence of different fixatives and fixation methods on the ultrastructure of rat kidney proximal tubule cells. J. Ultrastruct. Res. **15.** 242—282. 17. Meyer, P., Menard, J., Papanicolaou, N., Alexandre, J. M., Devaux, C., Milliez, P. (1968): Mechanism of renin release following fluorosemide diuresis in rabbit. Amer. J. Physiol. **215.** 908—915. 18. Oberling, C., Hatt, P. Y. (1960): Study of the juxtaglomerular apparatus of the rat using the electron microscope. Ann. Anat. path. **5.** 441—474. — 19. Reale, E., Marinozzi, V., Bucher, O. (1963): A propos de l'ultrastructure de l'appareil Juxtaglomerulaire du Rein. Acta. Anat. (Basel) **52.** 22—33. — 20. Riedel, B., Bucher, O. (1967): Die Ultrastruktur des Juxtaglomerulären Apparates des Meerschweinchens. Z. Zellforsch. **79.** 244—258. — 21. Riedel, B., Bucher, O., Petrik, P. (1967): Beitrag zur Ultrastruktur der Macula densa von Ratten - und Meerschweinchennieren. Verh. anat. Ges. (Jena) **62.** 505—510. — 22. Shimura, T., Shaw, E. H., Jr. (1968): Crystalline structure in juxtaglomerular Granules of the Rat. S. Dak. J. Med. **21.** 33—35. — 23. Simpson, F. O. (1963): crystalline structure of juxtaglomerular granules, as shown by electron microscopy. Proc. Univ. Otago med. Sch. **41.** 324. — 24. Skinner, S. L., McCubin, J. W. Page, I. H. (1964): Control of renin secretion. circulat. Res. **15.** 64—76. — 25. Szabó, J., Lustyik, Gy., Dreher, R. (1974): A simple vascular perfusion method for electron microscopic investigation of the juxtaglomerular apparatus. Exp. Path. **9.** 128—130. — 26. Thurau, K. Schnermann, J. (1965): Die Natriumkonzentration an den Macula densa-Zellen als regulierender Faktor für

das Glomerulumfiltrat. Klin. Wschr. **43.** 410—413. — 27. THURAU, K., SCHNERMANN, J., NAGEL, W. HORSTER, M. WOHL, M., (1967): Composition of tubular fluid in the macula densa segment as a factor regulating the function of the juxtaglomerular apparatus. Circulat. Res. 21. Suppl. **2.** 79—90. — 28. TOBIAN, L., TOBOULIAN, A., JANACEK, J. (1959): The effect of high perfusion pressures on the granulation of juxtaglomerular cells in an isolated kidney. J. clin. Invest. **38.** 605—610. — 29. VANDER, A. J., (1967): Control of renin release. Physiol. Rev. **47.** 359—382. — 30. VANDER, A. J. MILLER, R. (1964): Control of J., renin secretion in the anaesthetized dog. Amer. J. Physiol. **207.** 537—546. 31. WAGERMARK, UNGERSTEDT, U., LJUNGVIST, A. (1968): Sympathetic innervation of the juxtaglomerular cells of the kidney. Circulat. Res. **22.** 149—153.

WIRKUNG DER VASKULÄREN PERFUSIONSFIXIERUNG AUF DIE ULTRASTRUKTUR DES JUXTAGLOMERULÄREN APPARATES VON RATTEN

J. SZABÓ, GY. LUSTYIK und R. DREHER

Das elektronenmikroskopische Bild der JGA von Rattennieren nach retrograder Perfusionsfixierung auf niedrigen Drücken wurde untersucht und mit dem Bild der IGA, fixiert mit dem Tropf- und Tauchverfahren, verglichen. Der augenfälligste Unterschied bestand in der Gegenwart der basalen Compartemente der Macula densa-Zellen, die eine elektronenmikroskopische Bestätigung der »Tubulo-glomerular«-Theorie sein können. Zwischen den gekörnten JG-Zellen werden desmosomaartig angeschlossene Strukturen beschrieben. In den epitheloiden Zellen konnten auch verhältnismässig häufig kristalline und granula in granulis spezifische Sekretumgranula-Formen beobachtet werden.

ВЛИЯНИЕ ПЕРФУЗИОННОЙ ФИКСАЦИИ СОСУДОВ НА УЛЬТРАСТРУКТУР ПАРАГЛОМЕРУЛЯРНОГО АППАРАТА У КРЫС

Й. САБО, Д. ЛЮСТОК и Р. ДРЕХЕР

Электронномикроскопически исследовалось парагломерулярный аппарат почки крысы после ретроградной перфузионной фиксации при низком давлении и сравнивалось со структурой ПГА-а после капельной и погруженной фиксации. Наяичие базальных частей клеток плотного пятна электронномикроскопически доказательных »тубулогломерулярной« теории обнаруживалось самым очевидным. Обнаруживались десмосомовидные соединительные структуры между зернистыми клетками и сравнительно часто кристаллические и граннулярные формы специфических секреторных зернышек в эпителоидных клетках.

Dr. Jenő SZABÓ, György LUSTYIK — DOTE Kórbonctani Intézet, 4012-Debrecen, Pf. 22., Hungary

R. DREHER — Institute of Pathology of Humboldt-University (Charité), Berlin, GDR

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Institute of Anatomy, Histology and Embryology, University Medical School, Debrecen

ANTIGENICITY OF BONE TISSUE. I. IMMUNOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF NON-COLLAGENOUS PROTEINS OF THE BOVINE CORTICAL BONE

T. Glant, Cs. Hadházy, L. Bordán and S. Harmati

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From powdered bovine bone cortex an extract has been prepared with 0.15 M NaCl and its collagen-free fraction obtained on DEAE-cellulose column was used for the immunization of rabbits. The non-collagenous protein fraction *(NCP)* produced against immune serum two precipitation arcs both on agarose double gel-diffusion and with immune electrophoresis.

After decalcination in 10% EDTA pH 7.4, direct and indirect immune histochemical examinations were performed with fresh, freshly lyophilized, as well as with bones kept for three and six months at 20°C, and the antigens were localized. Prolonged storage increased the antibody-binding capacity of the bones, whereas lyophilization decreased it. Fixation in cold acetone was suited best for immunohistochemical study of the cortical bones.

Chalmers [12] in his studies of bone grafts was the first to use immunological methods. Subsequently, the antigenicity of various kinds of bone was examined by anaphylactic reactions [32], skin sensitivity test [8], precipitation reactions [5, 14, 27, 32], haemagglutination [5, 14, 32] as on the basis of morphological changes induced in lymph nodes [6, 7, 9, 23, 29]. The majority of the authors considered the red bone marrow responsible for the antigenicity, since when washed spongiosa or cortical substance was used reaction failed to appear, or was rather weak [1, 7, 8].

The composition of the bone extracts utilized for immunization was not studied [5, 27, 32] or where it has been examined it was found to be of collagenous nature [35] or of serous origin [14] in almost every case. Several papers discussed the composition of bone tissue [10, 11, 19—21] but the antigenicity of the components has not been studied. In the literature no data whatsoever could be found concerning the antigenicity of the non-collagenous bone proteins or their tissular localization. The present study aimed at clarifying these questions by immunological methods.

Materials and methods

Preparation of the antigen. Bovine cortical bone pieces were cleaned from the soft parts, then washed with distilled water, refrigerated to —22°C and from these bone-meal was prepared by a motor-driven rasp in such a way that its temperature would not exceed 0°C. For preparation of the extract such meal was used which fell through a 26 mesh sieve. Extraction was carried out with 0.15 M NaCl (1 : 2=w:v) in the cold under constant mixing for 48 hours. The mixture was centrifuted (30 min, 6000 g, 4°C), the supernatant was desalted on a Sephadex G-25 column (2.5 × 70 cm), then concentrated by lyophilization.

The collagen of the extract was removed on DEAE-cellulose column (Serva, Heidelberg; Type: DEAE-SS, capacity 0.72 mEq/g) according to MILLER [26]. The collagen-free fraction (NCP) was desalted, then lyophilized. For further examinations and immunization this latter fraction has been used.

From both the complete bone extract and the collagen-free fraction, N was estimated according to KJELDAHL, and protein by the biuret reactionas wellas hydroxyprolin [33], uronic acid [4] and hexose [24] were determined.

Immune sera were produced in rabbits. "Hungarian giant" rabbits of both sexes and 1500 to 2000 g body weight a mixture of 1 ml of complete Freund adjuvant (Difco Lab., Detroit, Mich) and 10 mg protein (1 ml in 0.15 M NaCl) was administered. Every 3 weeks the mixture was injected into the paws and the gluteal muscles until the sera gave perceptible precipitation arcs with the diluted antigen solution used for immunization. With the bone extract originating from one single cattle always the same rabbit was immunized. The immuno sera were inactivated at 56°C for 30 min.

Immune diffusion was carried out in 0.5% agarose gel in 0.15 M NaCl (Serva, Heidelberg).

Immunoelectrophoresis was performed according to the micro-method of SCHEIDEGGER [31] in 0.5% agarose (barbital-HCl buffer, u: 0.05, pH 8.2) at 4°C. For immunoelectrophoresis the non-collagenous fraction was digested with hyaluronidase (From bovine testicles; Reanal, Budapest; Activity: min. 300 USP Units/mg; — 10 mg protein +2 mg enzyme in 1 ml 0.1 M Na-acetate buffer, pH 5.6, 37°C, for 1 hour) or with collagenase (Serva, Heidelberg; 387 Mandl U/mg; 10 mg protein + 1 mg enzyme in 1 ml 0.1 M Phosphate buffer, pH 7.4, 37°C, for 30 min).

Staining of the plates was performed with Coomassie-Brillant Blue R-250 (Serva Heidelberg).

For the immunohistological examinations IgG was prepared from the immune sera [3] and conjugated with fluorescein-isothiocyanate (FITC) (BDH, England) [13]. Unbound dye was removed by gel filtration. (Fluorescein to protein ratio, 2.2; protein content, 5.4 mg/ml). In order to avoid unspecific conjugation, the conjugate was adsorbed before use on bovine hepatic powder treated with acetone. For indirect immunofluorescent examinations IgG obtained from immuno sera and adsorbed to powdered bovine liver as well as fluorescent-labelled goat gamma-globulin (Sevac, Praha) produced against rabbit gamma-globulin was used.

For direct and indirect immunohistological reactions two to three mm thick bovine cortical bone slices of bone were used, either fresh or freshly lyophilized, or stored at —20°C for 3 and 6 month. They were fixed for 72 hours in alcohol, acetone, 10% neutral formaldehyde solution or 2.5% glutaraldehyde, or were kept without fixation in PBS (salt solution buffered with phosphate: 0.15 M NaCl in 0.01 M phosphate buffer pH 7.4). Subsequently they were washed in PBS changed several times for 72 hours then kept for 4 to 7 days in 10% EDTA pH 7.4, changed twice daily. Decalcination was followed by 24 hours washing in PBS. These procedures were performed under constant mixing at a temperature between 0 and 2°C. From the decalcinated bones 4 to 6 mm thick cryostat sections were prepared at —22°C, or they were dehydrated in acetone at 4°C and embedded into paraffin according to SAINTE-MARIE [30].

The sections were studied without digestion or after digested with hyaluronidase for 30 min or with collagenase for 22 min, by direct or indirect immunohistochemical methods. (The conditions of enzymatic digestion have been described previously. The intervals were chosen so that no disruption of the sections should result.) The sections were covered with 3 to 7 mixture of glycerin and PBS.

The following controls were used:

For direct immunohistochemical reaction:

a: unlabelled anti-bone rabbit IgG → FITC-labelled anti-bone rabbit IgG;

b: unlabelled normal rabbit IgG → FITC-labelled anti-bone rabbit IgG;

c: FITC-labelled normal rabbit IgG;

d: Without treatment (except PBS covered sections).

For indirect immunohistochemical reactions:

a: unlabelled anti-bone rabbit IgG → unlabelled normal rabbit IgG → FITC-labelled anti-rabbit goat IgG;

b: unlabelled anti-bone rabbit IgG → unlabelled anti-rabbit goat IgG → FITC-labelled anti-rabbit goat IgG (indirect blocking);

c: unlabelled normal rabbit IgG → FITC-labelled anti-rabbit goat IgG;

d: FITC-labelled anti-rabbit goat IgG (in itself).

After performance of the immune reaction and fluorescent examination, part of the sections was stained with haematoxylin-eosin and azan.

Results

Table I demonstrates the composition of the bovine cortical bone extract as well as of its collagen-free fraction obtained on DEAE-cellulose column. Twelve per cent of the "complete" bone extract obtained with 0.15 M NaCl was non-collagenous protein. After removal of the collagen, the fraction displayed an altered chemical composition. This chemical fraction, in view of its high uronic and hexosamine content was partly proteoglycan and on the basis of its neutral carbohydrate content partly glycoprotein. In immunodiffusion the non-collagenous (NCP) fractions gave two precipitation arcs (Fig. 1), but reacted neither with anti-bovine rabbit serum (Fig. 1: central and fifth well) nor with the serum of the control rabbit treated only with complete Freund adjuvant.

In immunoelectrophoresis the antigen solution gave two distinct precipitation arcs in the alpha-beta regions (Fig. 2). The anti-bone rabbit IgG labelled with FITC formed precipitation arcs identical to these of the original immune serum (Fig. 2: lower well). The anti-NCP-fraction serum reacted to the original extract containing large amounts of collagen with two

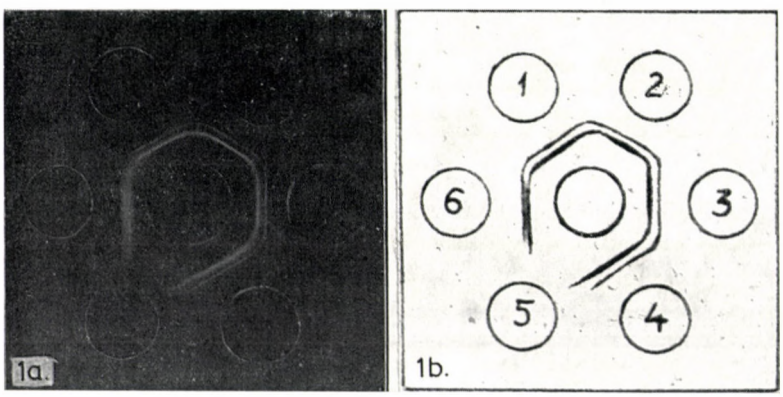


Fig. 1. Two-dimensional double gel-diffusion of the non-collagenous fraction of bovine cortical bone extract in 0.5% agarose. Central well: non-collagenous (NCP) fraction obtained from bone extract on DEAE-cellulose column. Wells 1, 2, 3, 4: anti-NCP fraction rabbit sera. Well 5: anti-bovine-serum (normal) rabbit serum. Well 6: fluorescein-isothiocyanate (FITC)-labelled anti-bone-NCP-fraction rabbit IgG

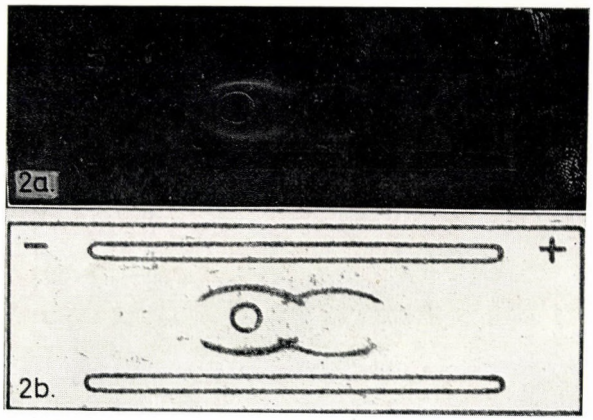


Fig. 2. Immunoelectrophoresis of NCP fraction of bovine cortical bone in agarose. Middle: NCP fraction. Upper trough: FITC-conjugated anti-bone-NCP-fraction rabbit IgG. Lower trough: anti-bone-NCP-fraction rabbit serum

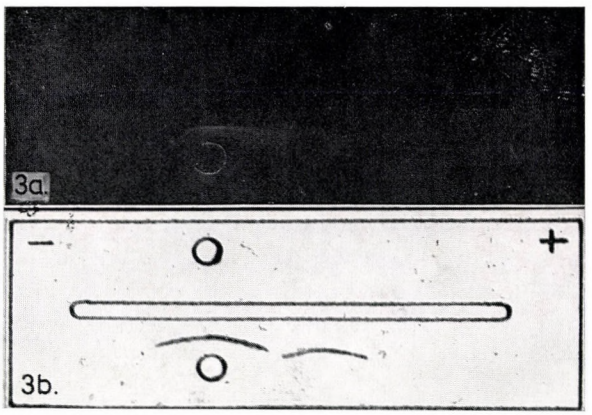


Fig. 3. Agarose immunoelectrophoresis of unfractioned extracts of bovine serum and bovine cortical bone. Upper well: bovine serum. Lower well: unfractioned bone extract. Middle trough: anti-bone-NCP-fraction rabbit serum

precipitation arcs, but failed to react with either of the components of bovine serum (Fig. 3: upper well). When extracts digested with hyaluronidase or collagenase were used, the number of precipitation arcs was unchanged (Fig. 4).

Since FITC was bound to the complete IgG fraction of the antiserum, the two antigens were represented together in the immunohistological investigations.

In cyrostat sections the fresh, the freshly lyophilized and the bones stored for 3 and 6 months at 20°C gave a slight immunofluorescence. When the indirect method was used the degree of fluorescence increased and when hyaluronidase digestion was performed before the indirect reaction, then a

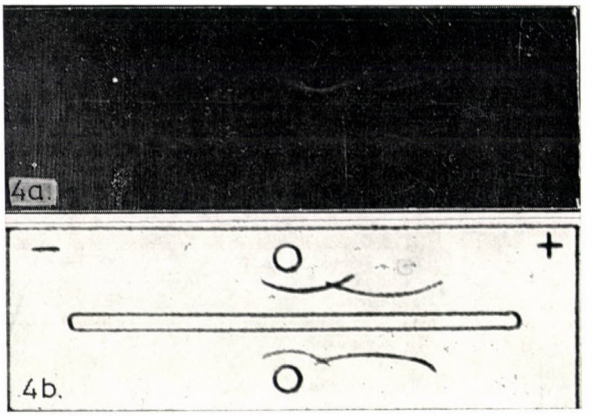


Fig. 4. Agarose immunoelectrophoresis of digested bovine cortical bone NCP-fractions. Upper well: NCP-fraction digested with hyaluronidase. Lower well: NCP-fraction digested with collagenase. Middle trough: anti-bone-NCP-fraction rabbit serum

Table I

Chemical composition of bovine cortical bone extract prepared with 0.15 M NaCl and the collagen-free (NCP) fraction obtained from it on DEAE-cellulose column. Percentual values calculated for dry substance

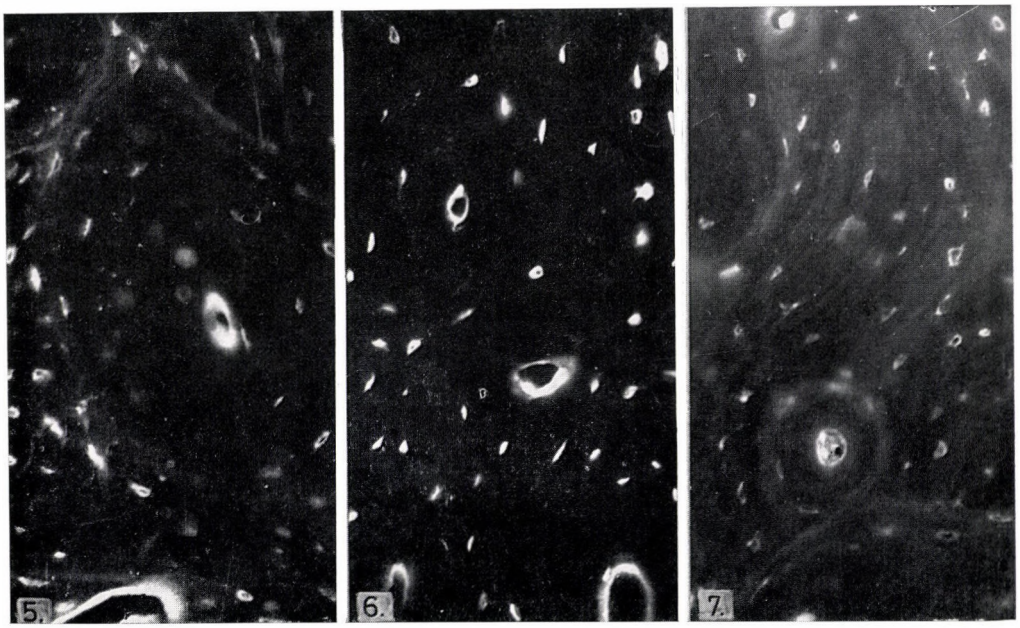
Composition	"Complete" bone extract	NCP fraction
Total protein	87.52	25.92
Glycosaminoglycan	2.07	65.12
Hexose	1.50	7.46
Total	91.09	98.50
Hydroxiprolin	10.58	0.01
Collagen	75.54	0.07
NCP	11.98	25.85
Total N	15.22	6.03
Uronic acid	0.30	11.72
Hexosamine	0.77	24.21
Chondroitin sulphate	0.80	31.41

The individual values were calculated by multiplying with the appropriate index factor [10, 28] as follows:

collagen = hydroxiprolin × 7.14
non-collagenous protein = total protein-collagen
hexosamine = hexosamine N/0.078
hexosamine N = total N − (collagen/5.7 + NCP/6.25)
glycosaminoglycan = hexosamine ×2.69
chondroitinsulphate = uronic acid ×2.68

strong positivity was observed (Figs 5—7). In these cases the specific label was localized in the osteocyte capsules, in the osteocytes and especially in the proximity of Haversian and Volkmann's canals. Their connective tissue lining displayed some autoflorescence both with and without digestion (Fig. 9).

The fixing solutions also influenced the immunohistochemical picture. With neural formaldehyde the control sections showed such a strong autofluorescence that no evaluation of the reaction was possible. After fixation in 2.5% glutaraldehyde the time required for decalcination was prolonged; it always took more than 3 to 4 weeks. Considerably better results were obtained with alcoholic fixation, but after this the fluorescence appeared in spot in both the direct and the indirect reaction. Fixation in cold acetone was the most suitable; in such materials, even if undigested the reaction was as strong or even stronger than the indirect immunofluorescence of unfixed material (Fig. 11) and the indirect reaction (Fig. 12) localized the antigens not only to the proximity of the osteocytes and canals, but also to the junction lines between the osteocyte processes and laminae. Hyaluronidase treatment



Figs 5—7. Bovine cortical bone. Indirect immunohistochemical method. Each of the three preparations was prepared without fixing after EDTA decalcination and hyaluronidase digestion. ×220. In freshly lyophilized bone (Fig. 6) antibody binding can be observed only in the osteocyte capsules as well as in the wall of the Haversian canals. In fresh bones (Fig. 5.) it is seen also in the periphery of osteocytes, in stored bones (Fig. 7) also in the region of the special intercalar laminae

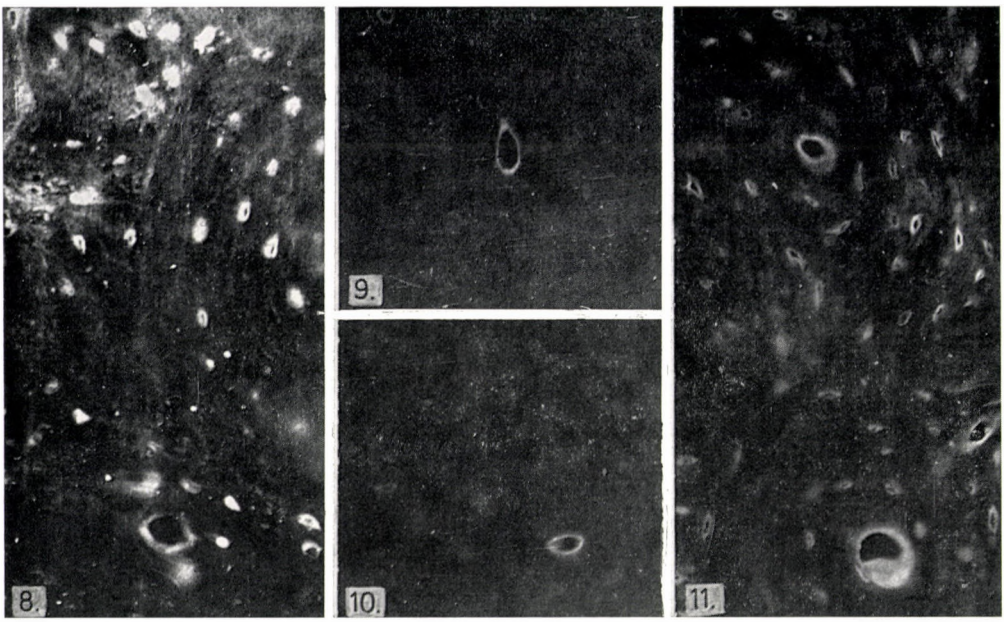


Fig. 8. Fresh bovine cortical bone fixed in cold alcohol, decalcinated with EDTA, digested with hyaluronidase. Indirect immunohistochemical method. Uneven distribution of the positivity. ×220

Fig. 9. Fresh bovine cortical bone without fixation. EDTA decalcination, hyaluronidase digestion. Autofluorescent control. Outlines of the Haversian canals can only be seen. ×220

Fig. 10. Fresh bovine cortical bone fixed in acetone decalcinated with EDTA. Indirect blocking. Positivity around the Haversian canals, weak binding in the osteocyte capsules. ×220

Fig. 11. Fresh undigested bovine cortical bone; fixation in cold acetone, decalcination in EDTA, direct immunohistochemical method. The fluorescence is as intensive as the positivity of the unfixed material (Fig. 5) observed after hyalouronidase digestion by indirect reaction ×220

(Figs 13, 14) increased the angiten-binding capacity of the intercellular spaces of the intercalated lamine and of the osteocyte processes. The control of the latter examinations (indirect blocking, Fig. 10) was only slightly different from the autofluorescence control (Fig. 9); the intercellular substance was absolutely negative.

The immunofluorescent picture of lyophilized bone embedded in paraffin (Fig. 15) was similar to that of the unfixed predigested and lyophilized cryostat section (Fig. 6). In the embedded material, predigested with hyaluronidase (Fig. 16) or collagenase (Fig. 17) the positivity increased chiefly in the intercellular substance and among the laminae, particularly after collagenase treatment. Azan staining showed the absence of collagen in their sections.

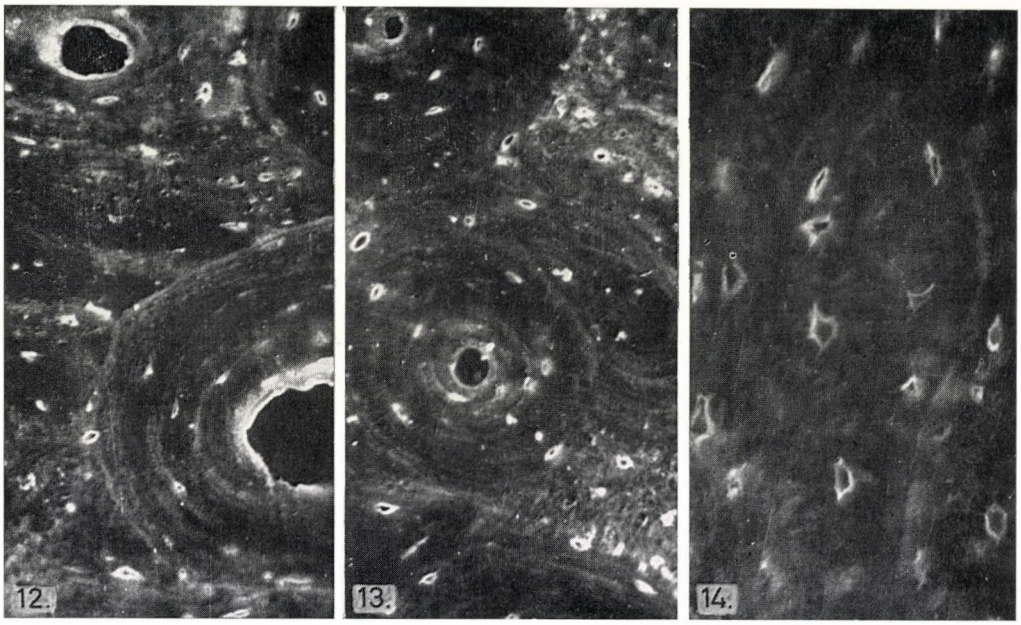
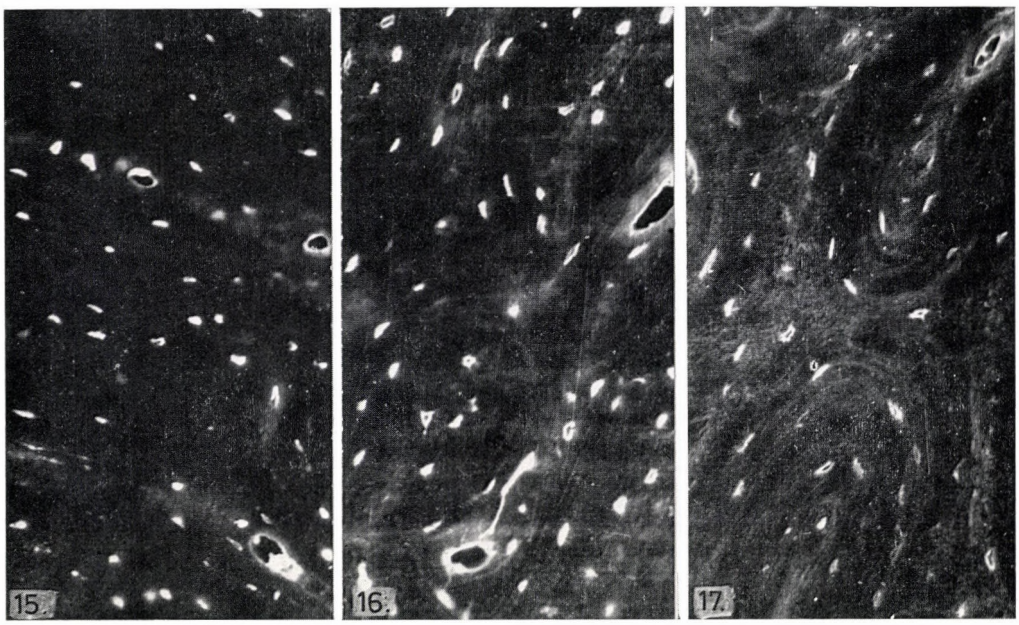


Fig. 12. Fresh undigested bovine cortical bone; fixed in cold acetone, decalcinated in EDTA. Indirect immunohistochemical method. The surroundings, the canals and the osteocyte capsules are more positive, the osteocyte processes are discernible, the junction lines and laminae bind more antibodies. ×220

Fig. 13. Fresh bovine cortical bone; fixed in cold acetone, decalcinated in EDTA, digested with hyaluronidase. Indirect immunohistochemical reaction. Compared with Fig. 12, positivity is particularly increased in the region of the laminae intercalares. ×220

Fig. 14. Detail of Fig. 13. Region of the intercalated laminae on Fig. 13. ×450



Figs 15—17. Bovine cortical bone, lyophilized, decalcinated in EDTA, dehydrated in acetone, embedded in paraffin. Indirect immunohistochemical method. ×220

Discussion

According to earlier data, bone tissue has weak antigenic properties; the extracts point to the presence of one [35] or two [27] substances of antigenic nature. The composition of the extracts used in these studies was not studied; they were made to react to immune sera produced against complete bone extracts. In spite of this the arcs obtained after exhaustion with homologous serum were presumed to be due to soluble collagen. The studies of ENGEL and CATCHPOLE [15] demonstrated that an antigen effect of collagenous origin should in fact be taken into consideration. They labelled with FITC the gamma-globulin fraction of anti-"collagen-D" rabbit serum and localized the collagen in different organs of the rat, at the periphery of newly formed bone trabecules and around osteocytes, among others.

According to the above results, the proteins of non-collagenous nature of the bovine cortical bone have antigenic properties, on both immunodiffusion and immunoelectrophoresis two well distinguishable precipitation arcs were formed. This circumstance refers by all probability to the presence of at least two non-collagenous proteins (proteoglycans? glycoprotein?) of antigenic properties. Our present methods are not yet suited for their more exact definition. Taking into consideration that these can be eluted from the Sephadex-G-200 column (1.9×85 cm; Tris-HCL 0.1 M pH 8) by exclusion volume, still they could be separated into different fractions on Sepharose 6-B column they seem to be considerable in size. This may explain why on agar the two antigens can be separated only with difficulty or not at all whereas on agarose they easily separate. In addition to these antigens the bone tissue seems to possess antigenicity of collagenous origin.

Our NCP fraction displayed an immunoelectrophoretic pattern similar to that of DICKSON's fraction II [14]. Extraction with EDTA was avoided because it would have removed those proteins too [11, 19, 20] which otherwise could act as antigens [18], and induce a negative reaction. This supposition was justified by the picture of the unfixed bones decalcinated with EDTA (Figs 5—7).

Cold alcohol and acetone were found suitable as fixing solutions. Despite the fact that the degree of autofluorescence was almost idential with alcohol and acetone the result was slightly different. After fixation in alcohol, "mute regions "were obtained, antibody-binding was slight even with the indirect reaction. These regions were located alternately nearer or farther to the surface. This may have been due to incomplete diffusion or fixation, but there is a

Fig. 15. No digestion. Strong positivity of cells and canals. × 450
Fig. 16. Hyaluronidase digestion. In some regions intercellular specific binding. ×450
Fig. 17. Collagenase digestion. Positivity in the laminar region. × 450

possibility that the solubility of proteoglycans was influenced, was attacked by the alcohol as indicated by SEFER et al. [32], who after decalcination with EDTA, obtained a separate antigen fraction from human bone treated with alcohol. After fixation in acetone no similar phenomenon occurred.

In the bones stored at −20°C for 3 and 6 months no decrease of the binding of labelled specific antibodies could be observed; moreover, on immunohistochemical positivity had increased in extent. The phenomenon may be explained by the action of lysosomal enzymes, which rendered numerous determinants of the NCP antigens accessible, as demonstrated in cartilage by BARLAND et al. [2]. Hyaluronidase digested cartilage [2, 16, 17, 25] displays a similar phenomenon.

In lypophilized material the degree of antibody binding was less pronounced; positivity of similar intensity and localization as in fresh and stored bones appeared only subsequent to collagenase digestion. This may be connected with the decrease of antigenicity [22, 34], but freeze-drying and decalcination may have induced such changes in the collagen which could partly camouflage some of the determinants of the NCP antigen.

REFERENCES

1. ANDERSON, K. J.: (1961) The behaviour of autogenous and homogenous bone transplants in the anterior chamber of the rat's eye. A histological study of the size of the implant. J. Bone Jt Surg. **43-A,** 980—995. — 2. BARLAND, P., JANIS, R., SANDSON, J.: (1966) Immunofluorescent studies of human articular cartilage. Ann. rheum. Dis. **25,** 156—164. — 3. BAUMSTARK, J. S., LAFFIN, R. J., BARDAWIL, W. A.: (1964) A preparative method for the separation of 7S gammaglobulin from human sera. Arch. Biochem. **108,** 514—522. — 4. BITTER, T., MUIR, H. M.: (1962) A modified uronic acid carbasole reaction. Anal. Biochem. **4,** 330—334. — 5. BURWELL, R. G., GOWLAND, G.: (1961) Studies in the transplantation of bone. Assessment of antigenicity. Serological studies. J. Bone Jt Surg. **43-B**, 814—819. — 6. BURWELL, R. G., GOWLAND, G.: (1961) Studies in the transplantation of bone. II. The change occurring in the lymphoid tissue after homografts and autografts of fresh cancellous bone. J. Bone Jt Surg. **43-B,** 820—843. — 7. BURWELL, R. G., GOWLAND, G.: (1962) Studies in the transplantation of bone. III. The immune responses of lymph nodes draining components of fresh homologous cancellous bone and homologous bone treated by different methods. J. Bone Jt Surg. **44-B,** 131—148. — 8. BURWELL, R. G.: (1963) Studies in the transplantation of bone. V. The capacity of fresh and treated homografts of bone to evoke transplantation immunity. J. Bone Jt Surg. **45-B,** 386—401. — 9. BURWELL, R. G., GOWLAND, G., DEXTER, F.: (1963) Studies in the transplantation of bone. VI. Further observations concerning the antigenicity of homologous cortical and cancellous bone. J. Bone Jt Surg. **45-B,** 597—608. — 10. CAMPO, R. D.; TOURTELLOTTE, C. D.: (1967) The composition of bovine cartilage and bone. Biochim. Biophys. Acta (Amst) **141,** 614—624. — 11. CANDLISH, J. K., HOLT, F. J.: (1971) The proteoglycans of fowl cortical and medullary bone. Comp. Biochem. Physiol. **408,** 283—293. — 12. CHALMERS, J.: (1959) Transplantation immunity in bone homografting. J. Bone Jt Surg. **41-B,** 160—179. — 13. COONS, A. H., KAPLAN, M. A.: (1950) Localisation of antigens in tissue cells. II. Improvements in a method for the detection of antigen by fluorescent antibody. J. exp. Med. **91.** 1—13. — 14. DICKSON, I. R.: (1971) Antigenicity of sheep cortical-bone matrix proteins. Biochem. J. **124,** 53P—54P. — 15. ENGEL, M. B., CATCHPOLE, H. R.: (1972) Collagen distribution in the rat demonstrated by a specific anti-collagen antiserum. Amer. Anat. **134,** 23—40. —16. GLANT, T. T., HADHÁZY, Cs.: (1973) Comparative investigation on cartilage antigens. Abstr. 3rd Congress of the Hungarian Association of Anatomists, Histologists and Embryologists. Akadémiai Kiadó, Budapest, P. 8. — 17. HADHÁZY, Cs., GLANT, T., MÁNDI, B., MILTÉNYI, L.: (1972) On the antigenicity of cartilage tissue. Acta biol. Acad. Sci. Hung. **23,** 277—288. — 18. HANCOX,

N. M., OWEN, R., SINGLETON, A.: (1961) Cross-species grafts of deproteinised bone. J. Bone Surg. **43-B,** 152—161. — 19. HERRING, G. M.: Mucosubstances and ion-binding in bone. In: Nutritional aspects of the development of bone and connective tissue. Nutr. Dieta **13,** 147—154 (Karger, Basel). — 20. HERRING, G. M., ASHTON, B. A., CHIPPERFIELD, A. R.: (1974) The isolation of soluble proteins, glycoproteins and proteoglycans from bone. Prepar. Biochem. **4,** 179—193. — 21. IWATA, H., URIST, M. R.: (1972) Protein polysaccharide of bone morphogenetic matrix. Clin. Orthop. **87,** 257—274. — 22. JACOB, S. W., GOWING, D., DUNPHY, J. E.: (1959) Transplantation of tissues. Amer. J. Surg. **98,** 55—90. — 23. KOSSOWSKA-PAUL, B.: (1966) Studies on the regional lymph node blastic reaction evoked by allogeneic grafts of fresh and preserved bone tissue. Bull. Acad. pol. Sci. Cl. 2. **14,** 651—657. — 24. LAURENT, T. C., ANSETH, A.: (1961) Studies on corneal polysaccharides. II. Characterization. Exp. Eye Res. **1,** 99—105. — 25. LOEWI, G.: (1965) Localization of chondromucoprotein in cartilage. Ann. rheum. Dis. **24,** 528—535. — 26. MILLER, E. J.: (1971) Isolation and characterization of a collagen from chick cartilage containing three identical chains. Biochemistry **10,** 1652—1659. — 27. PANDEY, R., PATHAK, R. C.: (1966) Agar diffusion studies on the species specificity of cow and buffalo bones. Indian J. exp. Biol. **4,** 20—22. — 28. PUGLIARELLO, M. C., VITTUR, F., DE BERNARD, B., BONUCCI, A., ASCENZI, A.: (1973) Analysis of bone composition at the microscopic level. Calc. Tiss. Res. **12,** 209—216. — 29. RYMASZEWSKA, T., WLODARSKI, K., JELINSKA, S., KOSSOWSKA-PAUL, B., ZALESKI, M.: (1965) Blastic reaction in the regional lymph nodes evoked by xenogeneic second-set grafts. Bull. Acad. pol. Sci. Cl. 2. **13,** 679—682. 30. SAINTE-MARIE, G.: (1962) A paraffin embedding technique for studies employing immunofluorescence. J. Histochem. Cytochem. **10,** 250—256. — 31. SCHEIDEGGER, J. J.: (1955) Une microméthode de l'immunoélectrophorèse. Int. Arch. Allergy **7,** 103—110. — 32. SEFER, M., POZSGI, N., SULICA, A., DENISCHI, A., TEMELIE, A.: (1968) Étude des propriétés antigéniques du tissu osseux humain. Arch. roum. Path. exp. **27,** 619—630. — 33. STEGEMANN, H., STALDER, K.: (1967) Determination of hydroxyproline. Clin. Chim. Acta **18,** 267—273. — 34. STEINMÜLLER, D.: (1964) Failure of lyophilized tissues to evoke a secondary response in skin homografts. Transplantation **2,** 383—386. — 35. WELLMITZ, G., SCHUMANN, J.: (1969) Results of series of immunological Ouchterlony tests in transplantations of bones. Wiss. Z. Humbold-Univ. Berlin Math.-Nath. R. **18,** 443—448.

DIE ANTIGENEIGENSCHAFT DES KNOCHENGEWEBES. I. IMMUNOLOGISCHE UND IMMUNHISTOCHEMISCHE UNTERSUCHUNG DER NICHT-KOLLAGENEN PROTEINE IN DER KORTIKALEN KNOCHENSUBSTANZ DES RINDES

T. GLANT, CS. HADHÁZY, L. BORDÁN und S. HARMATI

Aus dem Mehl der kortikalen Knochensubstanz von Rindern wurde mit Kochsalzlösung von 0,15 M ein Extrakt hergestellt und daraus auf DEAE-Zellulosesäule eine kollagenfreie Fraktion gewonnen. Mit dieser Fraktion wurden Kaninchen immunisiert. Die nicht-kollagene Proteinfraktion (NCP) hatte mit dem ihr gegenüber erzeugten Immunserum bei dem Doppeldiffusionsverfahren auf Agarose und bei der Immunelektrophorese zwei Präzipitationsbögen ergeben.

Nach Dekalzinierung mit 10%iger EDTA-Lösung (bei pH 7,4) wurden an frischen, frisch lyophilisierten sowie an drei und sechs Monate bei —20°C gelagerten Knochen direkte und indirekte immunhistochemische Untersuchungen durchgeführt und die Antigene lokalisiert. Den Ergebnissen nach wird durch längere Lagerung die Antikörper-Bindungsfähigkeit der Knochenstücke etwas gesteigert, während sie durch Lyophilisierung herabgesetzt wird. Zur immunhistochemischen Untersuchung der kortikalen Knochenstücke ist die kalte Aceton-Fixierung am meisten geeignet.

АНТИГЕННОЕ СВОЙСТВО КОСТНОЙ ТКАНИ I. ИММУНОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ НЕ-КОЛЛАГЕННЫХ БЕЛКОВ КОРКОВОГО ВЕЩЕСТВА КОСТЕЙ КРУПНОГО РОГАТОГО СКОТА

Т. ГЛАНТ, Ч. ХАДПАЗИ, Л. БОРДАН и Ш. ХАРМАТИ

Из муки коркового вещества костей крупного рогатого скота авторы приготовили при помощи раствора поваренной соли в 0,15 М вытяжку, из которой на столбе ДЕАЕ-целлюлозы была получена фракция без коллагена. С этой фракцией они иммунизировали кроликов. Не-коллагеновая белковая фракция (NCP) дала с иммунной сывороткой (образованной против нее) при двойной диффузии на агарозе и при иммуноэлетрофорезе две дуги преципитации.

После декальцинации в 10%-ом растворе ЭДТА (при pH 7,4) на свежих, на лиофилизированных в свежем виде костях, а также на костях, хранявшихся в течение трех или шести месяцев при температуре в —20 °C, проводили непосредственные и косвенные иммуногистохимические исследования и локализовали антигены. На основе наблюдений авторов более длительным хранением до некоторой степени повышается способность костей к связыванию противотел, в то время как лиофилизация понижает эту способность. Для иммуногистохимического исследования корковых кусков костей наиболее подходящим методом является холодная фиксация в ацетоне.

Dr. Tibor GLANT
Dr. Csaba HADHÁZY
Dr. László BORDÁN
Dr. Sándor HARMATI

DOTE Anatómiai, Szövet- és Fejlődéstani Intézet
4012-Debrecen, Pf. 14., Hungary

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Institute of Histology and Embryology, University of Lausanne, Lausanne, Switzerland

MORPHOLOGICAL AND HISTOCHEMICAL CHANGES OF ULTIMOBRANCHIAL FOLLICLES OF THE RAT THYROID IN THE COURSE OF POSTNATAL LIFE

M. PETKÓ*

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Morphological and histochemical changes of ultimobranchial follicles of the thyroid have been investigated in rats from newborn to 18 months of age.

The first well-delimited ultimobranchial follicles, though with no lumen, were detected in the thyroid gland of 10-day-old rats. At 30 days of age, follicles possessing regular lumina were present in the thyroid. With age, the follicles gradually increased in volume assuming extreme dimensions in adult age. The follicles displayed varying shapes from simple cysts to bizarre forms. From the age of 50 days the cells of the follicular wall are separated from the cell debris contained in the lumen. The latter gave a PAS positive reaction. The cells of the ultimobranchial follicles did not exhibit argyrophilia and metachromasia showing that they differ considerably from the C-cells likewise of ultimobranchial origin, which are known to give marked argyrophilic and metachromatic reactions.

Introduction

In mammals, the thyroid gland has a double embryonic origin.

One part of the gland is derived from a ventral outgrowth of the floor of the pharynx, while the other part develops from the ultimobranchial body [15, 18, 23]. Though this has long been known, the fact that the histological structure of the thyroid is not uniform, has only recently been recognized. NONIDEZ [11] demonstrated that in addition to the follicular epithelium the thyroid possesses a "second follicular epithelium". It has been shown that this epithelium produces calcitonin, a hormone influencing calcium homeostasis [1, 7, 9]. On the basis of their calcitonin producing capacity, these cells were termed C-cells [4, 12]. A second kind of follicle has also been found in the thyroid. This kind has a different structure and has been termed postnatal ultimobranchial follicle by VAN DYKE [19, 20] who was the first to describe it and assumed its ultimobranchial origin. Numerous studies were carried out on the origin [22], morphology and ultrastructure [2, 3, 22], of the ultimobranchial follicle.

According to these studies, the ultimobranchial (UB) follicle is a cyst-like or tube-like formation varying in shape, composed of ultimobranchial (U)

* Present address: Institute of Anatomy, Histology and Embryology, University Medical School, Debrecen (Hungary).

cells [10]. In the lumen of the tube or cyst, cell debris and an acellular amorphous substance can be found, while the wall is made up of U-cells of two different types. The outer wall of the follicle consists of several layers of flattened "basal U-cells" characterized by numerous desmosomes and dense clusters of tonofibrils. The cells cover each other onion-like. The "apical U-cells" lining the follicular lumen in a single layer, are usually flat, sometimes cuboidal or columnar in form, having short microvilli in contact with the lumen. According to NÈVE and WOLLMAN [10] the general properties and cellular structure of the ultimobranchial follicles are similar at all ages, though different in size. The number and volume of follicles within the lobes increase with age.

The aim of the present work was to study the morphological and histochemical changes of the postnatal ultimobranchial follicles, and to investigate the histochemical differences existing between the U-cells and C-cells also of ultimobranchial origin.

Material and methods

Wistar rats of both sexes were used in the study. Thyroid glands were obtained from newborn, 10, 30, 50, 70, and 120-day-old rats. One age group comprised 5 rats of either sex from the same litter. Under pentobarbital anaesthesia, the thyroid was excised, one of the lobes was removed and fixed in a mixture of glutaraldehyde-picric acid-acetic acid (GPA) for 24 hours. This was followed by embedding in paraffin and serial sectioning (7 μ). The series of sections prepared from each lobe were divided in three groups so that each third section was included in a separate group. The sections were used for the following histochemical reactions:

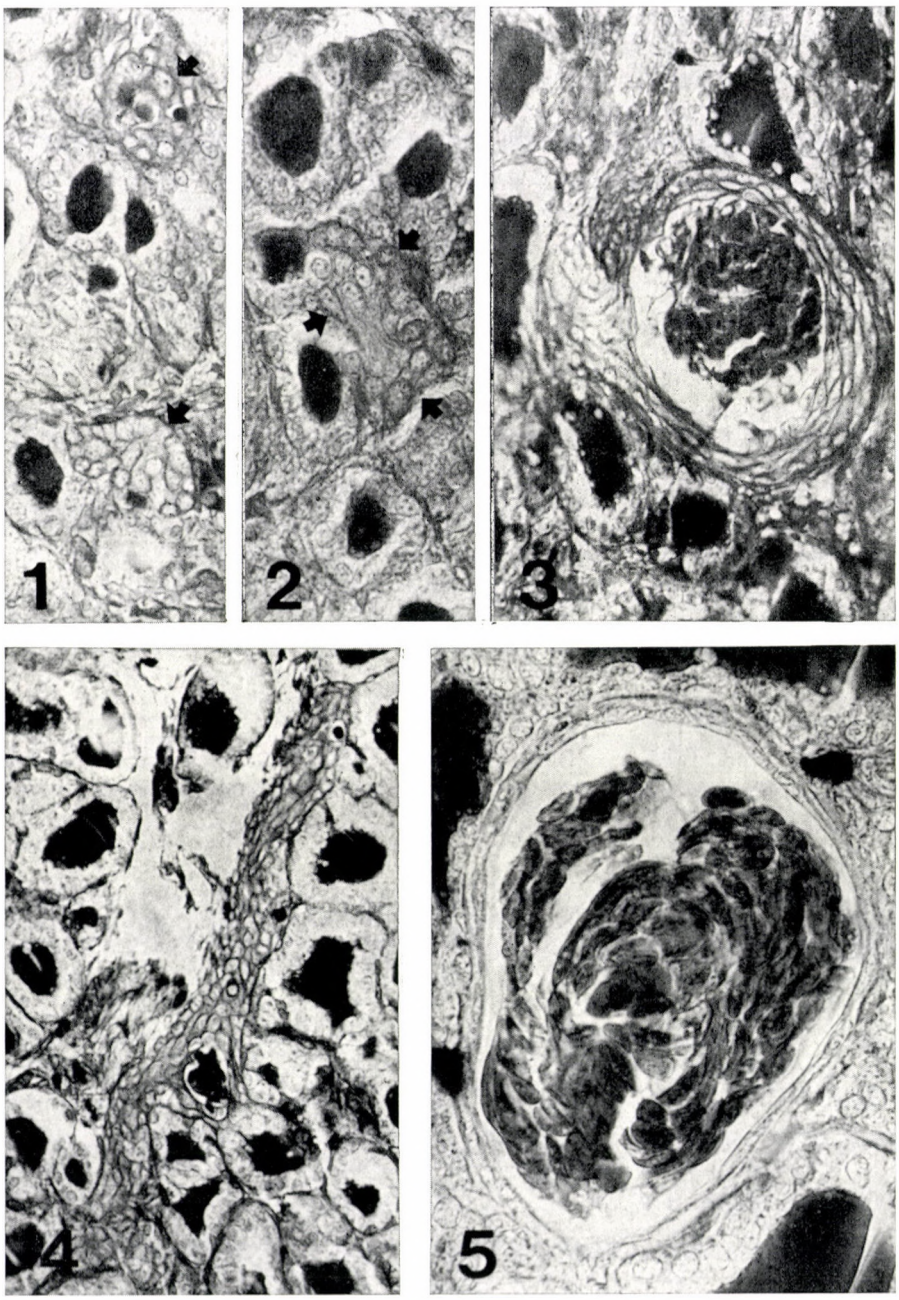
— Silver impregnation according to DE GRANDI [6].
— Hydrolysis with 1 *n* hydrochloric acid at 60°C for 6 hr according to SOLCIA and SAMPIETRO [16], followed by staining with 0,01% 1 : 9-dimethylmethylene blue at pH 5.4 in McIlwaine buffer.
— PAS reaction that in some cases was preceded by digestion with saliva.

Results

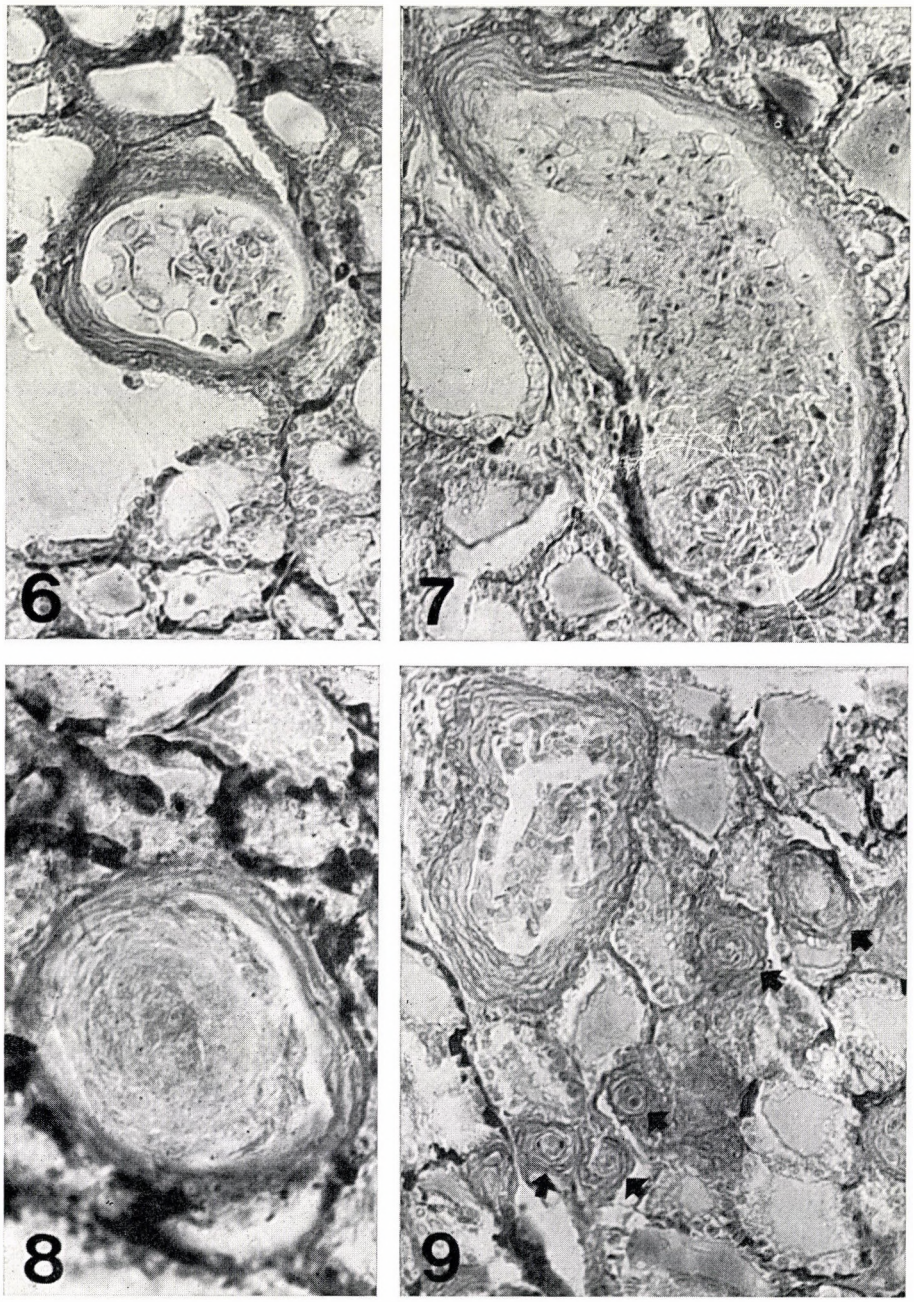
In the *newborn rat* no ultimobranchial cells could be differentiated from the thyroid tissue by any of the reactions employed.

At *10 days of age* well circumscribed ultimobranchial cell groups were detected in the central area of the lobe (Figs 1 and 2). The cells were oval in shape and localized in groups. No lumina surrounded by cells could be noted. The cells did not exhibit argyrophilia and metachromasia and their PAS reaction was negative.

At *30 days of age* the area occupied by U-cells was much larger than in the previous stage. The cells bounding a lumen were follicle-like in appearance (Fig. 10). Sometimes several follicles close to each other could be noted (Fig. 11). The lumina of the follicles contained degenerated cells and cell debris yielding orthochromasia and a positive PAS reaction. The wall of the follicle was made up of cubic or ovoid cells displaying no argyrophilia and meta-



Figs 1—5 Ultimobranchial follicles in the rat thyroid, 1 : 9-dimethyl-methylene blue staining
Fig. 1. 10 days of age. Islet-like accumulations of U-cells (marked with arrow) in the central area of the thyroid lobe. Magnification, 40 × 6.3
Fig. 2. 10-day stage. Rudimentary UB follicle (arrows). Magnification, 63 × 6.3
Fig. 3. 50-day stage. UB follicle. Magnification, 40 × 6.3
Fig. 4. 120-day stage. Ultimobranchial process in the thyroid. Magnification, 40 × 6,3
Fig. 5. 18-month stage. UB follicle with thin wall and large amounts of cell debris in the lumen. Magnification, 63 × 6.3



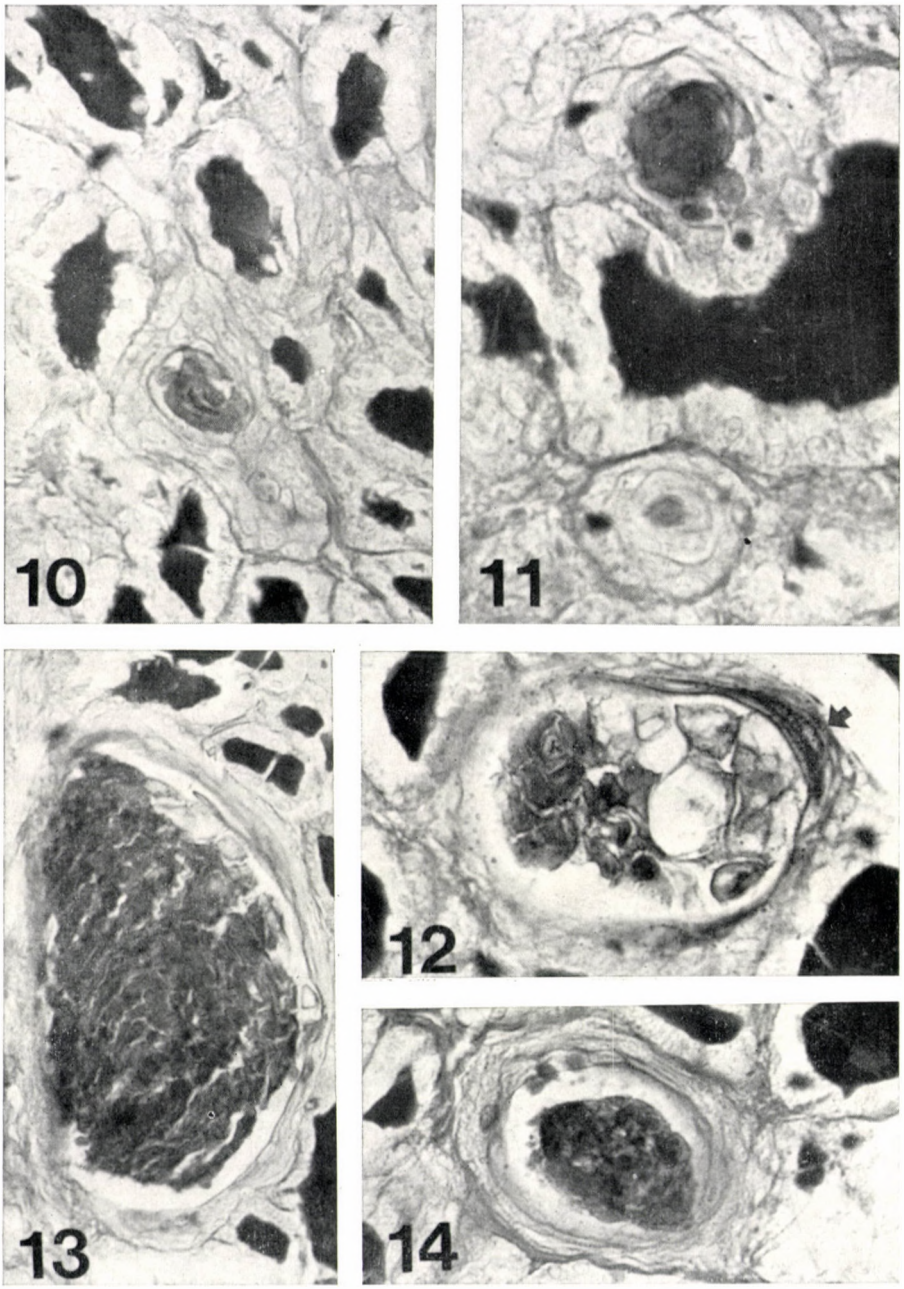
Figs 6—9 Ultimobranchial follicles of rat thyroid De Grandi's silver impregnation

Fig. 6. No argyrophilia in UB follicle of *50-day-old* rat. In the vicinity, a few argyrophilic C-cells are visible. Magnification, 25×6.3

Fig. 7. In the *70-day-old* rat the UB follicle is extremely large. No argyrophilia is present. Magnification, 10×6.3

Fig. 8. In the *120-day-old* rat the UB follicles exhibit no argyrophilia. In the vicinity, many argyrophilic C-cells. Magnification, 25×6.3

Fig. 9. Thyroid gland of *120-day-old* rat. One large UB follicle and many small ones (marked with arrows) in the environment. Magnification, 10×6.3



Figs 10—14 Ultimobranchial follicles of rat thyroid. PAS reaction

Fig. 10. At *30 days of age* the UB follicles contain lumina filled with PAS postitive cell debris. The cells of the follicular wall are PAS negative. Magnification, 40 × 6.3

Fig. 11. Thyroid gland of *30-day-old rat.* Two UB follicles with lumina containing colloid-like substance and PAS positive cell debris. The cells of the follicular wall are PAS negative. Magnification, 63 × 6.3

Fig. 12. At *50 days of age* the lumen of the UB follicle is filled with a PAS positive mass and cell fragments. Some of the cells in the follicular wall exhibit a moderate PAS positive reaction (arrows). Magnification, 40 × 6.3

Fig. 13. At *70 days of age* the lumen of the UB follicle contains PAS positive degenerating cells and cell fragments. No reaction in the follicular wall. Magnification, 25 × 6.3

Fig. 14. At *120 days of age* the mass in the lumen of the UB follicle is PAS positive. The cells of the wall are PAS negative. Magnification, 25 × 6.3

chromasia and no PAS reaction. In one section an UB follicle was detected on the surface of the thyroid lobe, in the immediate vicinity of the thyroid and parathyroid gland from which ultimobranchial processes penetrated into the parathyroid gland (Fig. 15).

At *50 days of age* large UB follicles were visible (Fig. 3). The cell debris in the lumen became sharply separated from the wall of the follicle. Sometimes a slit was formed between them. The cell debris in the lumen stained ortho-

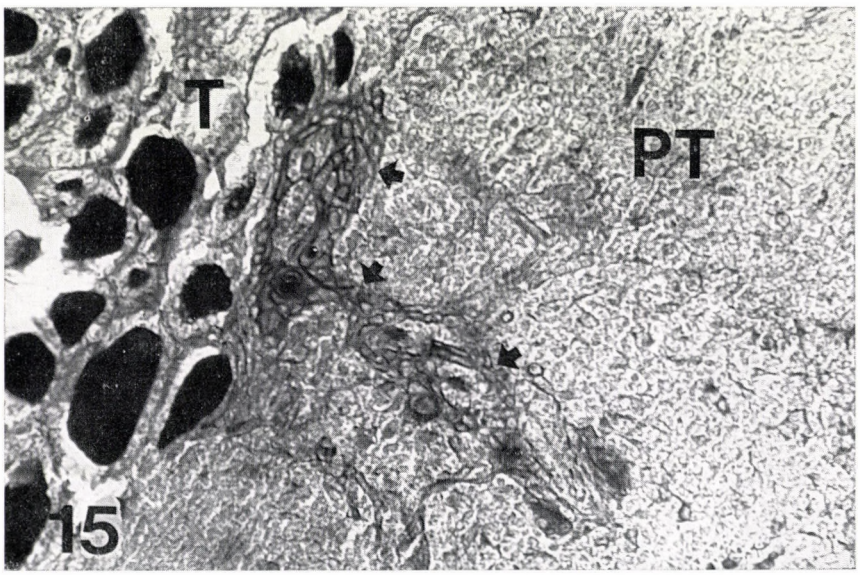


Fig. 15. Thyroid and parathyroid glands of *30-day-old* rat stained with 1 : 9-dimethylmethylene blue. Magnification, 25 × 6.3. At the margin of the thyroid gland (T) an ultimobranchial follicle is visible. The ultimobranchial process connected with the follicle (marked with arrow) penetrates into the substance of the parathyroid (PT).

chromatically, yielding a postive PAS reaction but no argyrophilia. Occasionally, the cells forming the follicular wall also gave a slight PAS positivity (Fig. 12). Most of the cells were flat, covering each other onion-like. In the vicinity of the follicles argyrophilic C-cells were often noted (Fig. 6). The follicles themselves never exhibited argyrophilia.

At *70 days of age* pattern was the similar as previously, but at this age the UB follicles assumed extreme sizes (Fig. 7). The central cell debris exhibited marked orthochromasia and PAS positivity (Fig. 13).

At *120 days of age*, in addition to large UB follicles similar as those seen at 70 days (Fig. 8), tube-like formations (Fig. 4) were also present. In their vicinity, numerous small follicles were noted (Fig. 9.); they were probably the sections of contorted tube-like or bizarre-shaped follicles.

At *18 month of age* the UB follicles were extremely large (Fig. 5) containing many desquamated cells, dead cells and fragments. These cells giving a positive PAS reaction and marked orthochromasia, were always well differentiable from the follicular wall from which they were often separated by a slit. No argyrophilia and metachromasia could be noted in these cells.

The PAS reaction performed after digestion with saliva was somewhat less positive in all age groups beyond 30 days.

Discussion

In rats the ultimobranchial body migrates to the anlage of the thyroid on the 16th day of embryonic life [17, 21]. According to our results on the 10th day of postnatal life, some of the U-cells migrated in the thyroid anlage are still located as a homogeneous islet in the central part of the lobe. These cells do not surround a lumen, display no differentiation into basal and apical types and do not exhibit either argyrophilia or metachromasia, or PAS positivity. Most probably, this cell islet is the site where the UB follicles arise. The cells of the UB follicles become more and more flattened showing an onion-like structure. In the interior, the cells undergo degeneration and desquamate into the lumen of the follicle. Other cells of the ultimobranchial body scatter in the thyroid to become there parafollicular, or C-cells [8, 13].

At 30 days of age the rat thyroid was found to contain regular UB follicles with lumina containing PAS positive cell debris. At this time the wall of the follicle is made up of basal (flattened) and apical (ovoidal) cells displaying no PAS positivity. In one case we have noted in a 30 days old rat that the UB follicle was located between the thyroid and the parathyroid extending processes into the latter gland (Fig. 15). This observation is consistent with the results of GOLDSTEIN and WHITE [5] that anlages migrate from the ultimobranchial pouch to the parathyroid and thymus where even secretion occurs. WOLLMAN and NÈVE [22] often observed UB follicles on the surface of the thyroid. The UB follicles show a gradual increase with age, occasionally attaining extreme dimensions. The largest follicles were noted in the thyroid glands of animals 18 month of age. The follicles were variable in shape, sometimes appearing as cysts or narrow tube-like formations. In some cases a central large cyst was present with small, bud-like cysts detached from its wall (Fig. 9). WOLLMAN and NÈVE also described follicles containing rod-like structures and epithelial pearls. Our findings were similar to, or identical with, those described by the authors quoted.

From 50 days of age, the cells of the follicular wall are separated by a slit from the mass filling the lumen. This is visible up to 18 month of age. The fact that it may be an artefact, cannot be excluded.

NÈVE and WOLLMAN [10] noted mixed follicles containing both thyroid follicular epithelium and U-cells in the thyroid gland of rats of all ages. In our material we have not observed mixed follicles, but we used Wistar rats, whereas NÈVE and WOLLMAN worked with Fischer rats. We have never noted argyrophilia and metachromasia in the UB follicles which shows that the follicular cells have definitely differentiated from the C-cells likewise of ultimobranchial origin (the latter cells always exhibited marked argyrophilic and metachromatic reactions). Thus, the ultimobranchial bodies migrating to the anlage of the thyroid carry two types of cells: C- and U-cells play an important part in calcitonin production, while the cells composing the UB follicles gradually degenerate and necrotize. The function of these cells is unknown.

It appears that glycoproteins are responsible for the PAS positive reaction yielded by the degenerating and necrotizing cells and cell fragments in the lumen of the UB follicles. Elucidation of this question needs further investigations.

REFERENCES

1. BUSSOLATI, G., PEARSE, A. G. E.: (1967) Immunfluorescent localization of calcitonin in the "C" cells of pig and dog thyroid. J. Endocr. **37,** 205—209. — 2. CALVERT, R.: (1972) Transitional cells in the postnatal thyroid gland of the rat. Anat. Rec. **174,** 341—360. — 3. CALVERT, R., ISLER, H.: (1970) Fine structure of a third epithelial component of the thyroid gland of the rat. Anat. Rec. **168,** 23—42. — 4. FOSTER, G. V., MACINTYRE, I., PEARSE, A. G. E.: (1964) Calcitonin production and the mitochondrion-rich cells of the dog thyroid. Nature (Lond.) **203,** 1029—1030. — 5. GOLDSTEIN, A. L., WHITE, A.: (1970) In: "Biochemical Actions of Hormones". G. Litwach, ed. Academic Press, New York. Pp. 465. — 6. DE GRANDI, P.: (1970) The routine demonstration of C cells in human and animal thyroid glands. Value of a simple silver stain. — Virchows Arch. ges. Path. Abt. B. Zellpath. **6,** 137—150. — 7. HIRSCH, P. F., GAUTHIER, G. F., MUNSON, P. L.: (1963) Thyroid hypocalcemic principle and recurrent laryngeal nerve injury as factors affecting the response to parathyroidectomy in rats. Endocrinology **73,** 244—252. — 8. KIRKEBY, S., ROMERT, P., GAUGUIN, J.: (1973) Origin of cholinesterase-containing follicle cells and parafollicular cells of the developing thyroid gland in the rat. Histochemie **37,** 243—250. — 9. KRACHT, J., HACHMEISTER, U., BREUSTEDT, H.-J.: (1968) Immunohistological studies on thyrocalcitonin in C-cells. Endokrinologie **52,** 395—401. — 10. NÈVE, P., WOLLMAN, S. H.: (1971) Fine structure of ultimobranchial follicles in the thyroid gland of the rat. Anat. Rec. **171,** 259—272. — 11. NONIDEZ, J. F.: (1931—32) The origin of the "parafollicular" cell, a second epithelial component of the thyroid gland of the dog. Amer. Anat. **49,** 479—505. — 12. PEARSE, A. G. E.: (1966) The cytochemistry of the thyroid C cells and their relationship to calcitonin. Proc. roy. Soc. B. **164,** 478—487. — 13. PEARSE, A. G. E., CARVALHEIRA, A. F.: (1967) Cytochemical evidence for an ultimobranchial origin of rodent thyroid C cells. Nature (Lond.) **214,** 929—930. — 14. PETKÓ, M.: (1974) The use of 1 : 9-dimethyl-methylene blue for the demonstration of thyroid C-cells. Stain Technol. **49,** 65—67. — 15. ROGERS, W. M.: (1927) The fate of the ultimobranchial body in the white rat (Mus norvegicus albinus). Amer. J. Anat. **38,** 349—376. — 16. SOLCIA, E., SAMPIETRO, R.: (1968) New methods for staining secretory granules and 5-hydroxy-tryptamine in the thyroid C cells. In: Calcitonin. Proceedings of the Symposium on Thyrocalcitonin and the C cells. (London 1967) Ed. S. Taylor, W. Heinemann Medical Books Ltd. London 1968. Pp. 127—132. 17. STOECKEL, M. E., PORTE, A.: (1970) Origine embryonnaire et différentiation sécrétoire des cellules à calcitonine (cellules C) dans la thyroïde fœtale du rat. Étude au microscope électronique. Z. Zellforsch. **106,** 251—268. — 18. SUGIYAMA, S.: (1940) The fate of the ultimobranchial body of the albino rat with special reference to the formation of the thyroid gland. Okajima Fol. anat. Jap. **19,** 333—341. — 19. VAN DYKE, J. H.: (1944) Behavior of ultimobranchial tissue in the postnatal thyroid gland: the origin of thyroid cystadenomata in the rat. Anat. Rec. **88,** 369—391. — 20. VAN DYKE, J. H.: (1955) Experimental thyroid metaplasia in the rat. Arch. Path. **59,** 73—81. — 21. WELSCH, U.: (1972) Die Entwicklung der C-Zellen und

des Follikelepithels der Säugerschilddrüse. Springer-Verlag, Berlin, Heidelberg, New York 1972. Pp. 12. — 22. WOLLMAN, S. H., NÈVE, P.: (1971) Ultimobranchial follicles in the thyroid glands of rats and mice. Rec. Progr. Hormone Res. **27**, 213—234. — 23. ZUCKERKANDL, E.: (1903) Die Entwicklung der Schilddrüse und der Thymus bei der Ratte. Anat. Hefte **21**, 1—28.

DIE MORPHOLOGISCHEN UND HISTOCHEMISCHEN VERÄNDERUNGEN DES POSTNATALEN ULTIMOBRANCHIALEN FOLLIKULUS IN DER RATTENSCHILDDRÜSE WÄHREND DES POSTNATALEN LEBENS

M. PETKÓ

Es wurden die morphologischen und histochemischen Veränderungen der ultimobranchialen Follikuli in Rattenschilddrüsen während des postnatalen Lebens, von der Geburt bis zum 18. Monat, untersucht.

In den Schilddrüsen von 10 Tage alten Tieren gelang es zum ersten Mal gut umschriebene ultimobranchiale Zellengruppen zu finden, die den Lumen noch nicht umfassten. Im Alter von 30 Tagen entstanden bereits die ultimobranchialen Follikuli mit einem Lumen. Ihr Volumen nahm mit dem Alter der Tiere zu, und bei alten Tieren kamen riesige Follikuli zustande. Die Follikuliformen zeigten sich, angefangen von einfachen Zysten bis zu den bizarren Formen, als ausserordentlich variabel; die Wandzellen und die Zelltrümmer im Lumen trennten sich von 50tägigen Alter an. Die Lumenzelltrümmer ergaben eine PAS-Reaktion. Die Zellen der ultimobranchialen Follikuli ergaben nie eine argyrophile und metachromatische Reaktion, was darauf hinweist, dass sie sich von den C-Zellen von ebenfalls ultimobranchialem Ursprung, die gute solche Reaktionen ergaben, unterscheiden.

МОРФОЛОГИЧЕСКИЕ И ГИСТОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ ПОСЛЕРОДОВОГО УЛТИМОЖАБЕРНОГО ФОЛЛИКУЛА ЩИТОВИДНОЙ ЖЕЛЕЗЫ У КРЫС В ТЕЧЕНИЕ ПОСЛЕРОДОВОЙ ЖИЗНИ

М. ПЕТКО

Морфологические и гистологические изменения улгиможаберного фолликула щитовидной железы было исследовано в течение послеродовой жизни с новорожденного до восемнадцатимесяцного возраста.

Обнаруживались хорошо описанные улгиможаберные группы клеток сперва в щитовидной железе десятидневного животного не образующие просвет. В тридцати дневнем возрасте уже видны улгиможаберные фолликулы имеющие просветом. Постепенно с возрастом увеличивалось их объем, в пожилом возрасте огромные фолликулы образовались. Обнаруживались разнообразные формы фолликула с простой кистой по странным видам. С 50-и дневного возраста пристеночные клетки и детрит клеток в просвете отделяются.

В просвете клеточный детрит дает реакцию ПАШ-а. Клетки улгиможаберного фолликула некогда не дают реакции аргирофилии и метакромазии, изображая различие от других улгиможаберных клеток »Ц« хорошо дающих эти реакции.

Dr. Mihály PETKÓ, DOTE Anatómiai, Szövet- és Fejlődéstani Intézet, H-4012 Debrecen, Pf. 14. Hungary

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First Department of Anatomy, Histology and Embriology,
Semmelweis University Medical School, Budapest

THE ARTERIAL AND VENOUS BLOOD SUPPLY OF THE SEPTUM PELLUCIDUM IN THE RAT

G. AMBACH, S. HORVÁTH and M. PALKOVITS

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The arterial and venous systems of the rat's septum pellucidum has been studied by means of the perfusion technique. The arteries may be classified into three groups. The branches of each of the three groups originate from the a. hemispherica and enter the septum from the frontal direction. The veins may be divided into an anterior and a posterior group. The anterior veins flow through the v. subcallosa into the sinus cavernosus, the posterior veins into v. cerebri magna. The arterial and venous blood supply of the individual nuclei of the septum are compar independent.

The septum pellucidum is a well developed organ in the rat containing numerous groups of nuclei [PALKOVITS, HORVÁTH in preparation]. Forming part of the limbic system, the septum is anatomically as well as functionally connected with both the hypothalamus and the other parts of the limbic system and specially with the hippocampus.

In recent years, an increasing number of experimental interventions (lesion, inplantation, fiber transection) has been performed in this region, but the details of the angioarchitecture of the septum have not been detected. Utilizing the recently elaborated double Indian ink perfusion technique [1], the arteries and veins of the septum in the adult rat were mapped.

Materials and methods

In 50 rats of 150 to 200 g body weight such Indian ink perfusion blood vessel preparations were produced, where the arteries were filled with red, the veins with blue [1]. Into the aorta of the rats anaesthetized with pentobarbital, blue Rotring Indian ink was injected until the dye had appeared in the right atrium; subsequently the arteries were filled with red Rotring Indian ink containing 5 to 7% gelatin. The brains were fixed in 8% formaldehyde for 10 days, then they were worked up partly for macroscopic preparations, partly for 500 μ thick frontal or sagittal serial sections. After alcoholic dehydration the sections were cleared in xylene. Exclusively arterial fillings with gelatinous red Indian ink were also performed.

Results

Arteries

On the basal surface of the brain, 1600—2000 μ laterally from the midline, the a. carotis interna runs forward and divides 1000—1200 μ behind the bregma into the a. cerebri media and the a. cerebri anterior (Fig. 1A) [1, 3].

Initially, the a. cerebri anterior runs along the external lower margin of the optic chiasma, then at 500—700 μ before the bregma plane it ascends gradually over the optic nerve and continues its course in the fissure longitudinalis cerebri (Fig. 1B). The a. olphactoria takes from it its origin at 700—900 μ before the bregma line and runs in ventral direction into the nasal cavity. Then the bilaterally running aa. cerebri anteriores are united at an acute angle in the midline, forming a single vessel, the *a. hemispherica.* (This is also called as anterior cerebral trunk [4] or common anterior trunk [6].)The a. hemispherica then forms a steep arch upward to the genu corporis callosi (Figs 1B, 2A) to run from there on the external surface of the corpus callosum in the median-sagittal plane backward to the rear end of the splenium corporis callosi, dividing again into two branches and supplying the median walls of the hemispheres, the convex surface of the brain it anastomoses with the branches of the a. cerebri media (Fig. 1B). The terminal branches form anastomoses with the a. cerebri posterior as well as with the anterior branches of the choroid plexus of the third and lateral ventricles.

From the section of the a. hemispherica below the corpus callosum originate the arteries supplying septum pellucidum they enter it substance from the anterior and inferior directions (Figs 1B, 1C, 2A).

The septal arteries may be classified into three groups (Table I).

1. The first group includes the *arteria infracallosa* and its branches. The a. infracallosa takes its origin from the a. hemispherica directly before the genu corporis callosi, 1800—2000 μ orally from the bregma plane. Then it makes a turn around the genu corporis callosi, dividing below it into two branches and takes its course backwards near the midline. It supplies the anterior two-thirds of the median upper region of the septum. Frequently, about 1000 μ before the bregma the a. infracallosa gives a branch running laterally, that participates in the blood supply of the upper lateral third of the septum (Fig. 2A).

2. The second group is formed by the branches of string trunk, the *a. subcallosa.* The most a. subcallosa originates from the a. hemispherica at 2000—2200 μ before the bregma plane (Fig. 2A), then it runs upward and backward to the region below the corpus callosum. In its slightly winding course it gives 4 to 7 branches from the front to the septum. Its uppermost branch, the *a. septalis superior* originates below the genu corporis callosi, 1400—1500 μ before the bregma. It runs caudally near the median-sagittal plane below the a. infracallosa parallel to the latter (Fig. 2A). The short branches of this section, the *rami minores anteriores* divide in the median upper part of the septum. At 900 to 1000 μ before the bregma line the a. septalis superior turns gradually upward and its 2 to 3 terminal branches, the *rr. minores posteriores* supply behind the dividing area of the a. infracallosa, the median upper septal region directly rostral to about 900 μ before the bregma a strong branch, the

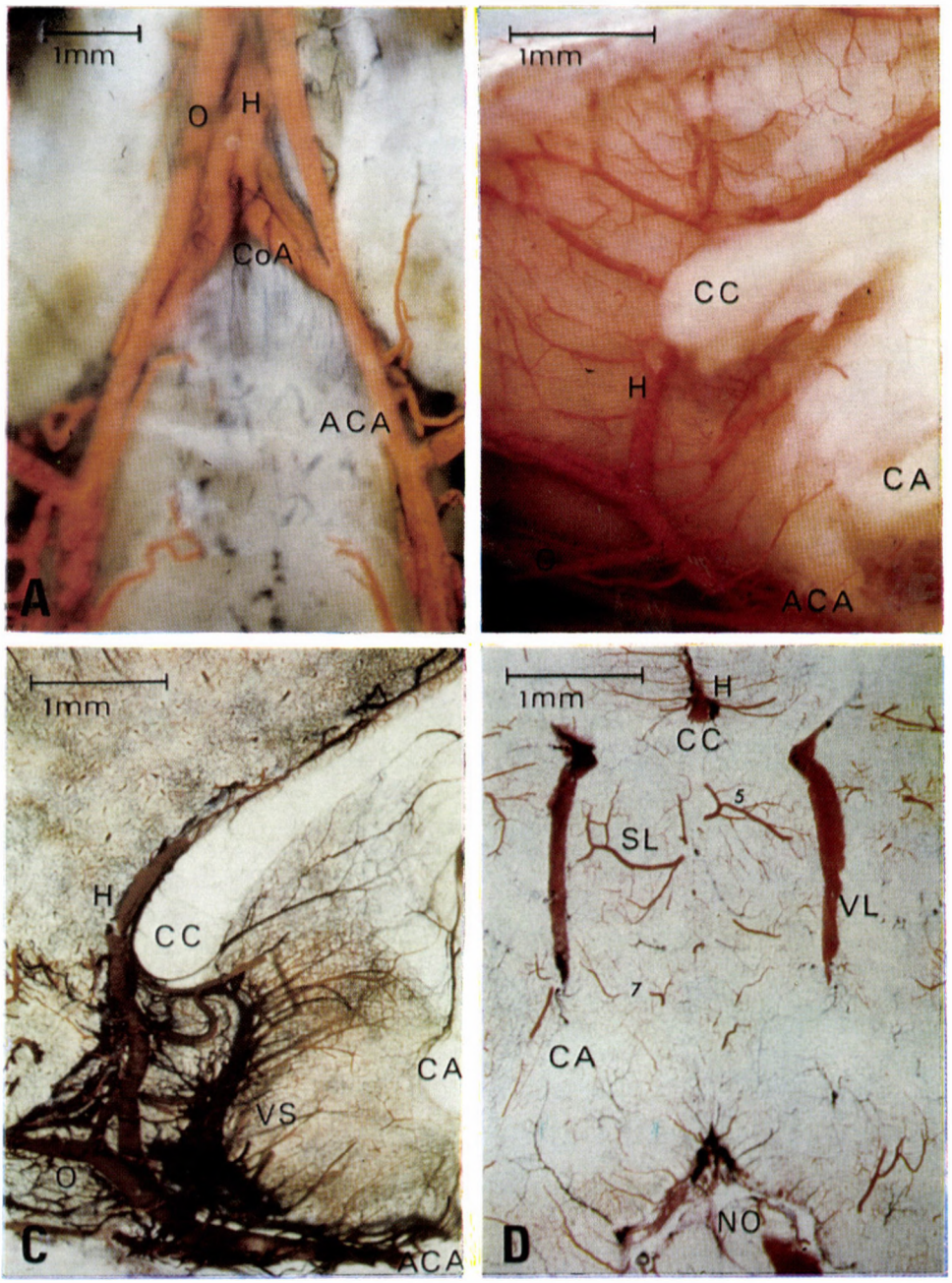
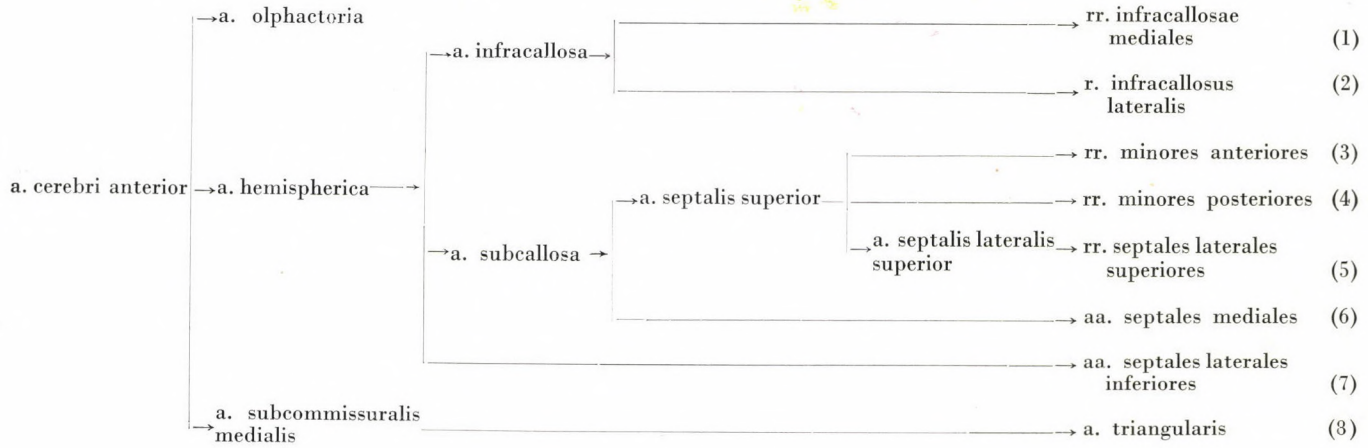


Fig. 1. A. Development of the a. hemispherica on the base of the brain; B. The a. hemispherica and its septal branches. Sagittal preparation; C. The a. hemispherica and its septal branches. 500 μ thick sagittal section; D. A. septalis lateralis superior. 500 μ thick frontal section; Numbering of vessels: See Table I. Abbreviations: See text

Table I

Arteries of the septum pellucidum in the rat



a. septalis lateralis superior makes a lateral turn, supplying with 2 to 3 branches the lateral superior region of the posterior two-thirds of the septum. This artery is well visible on sections in the frontal plane (Fig. 1D). From the a. subcallosa, below the a. septalis superior, 3 to 5 parallel thin arteries take their origin; these are the *aa. septales mediales* which enter the septum from the frontal direction and supply the central and lower thirds of the anterior medial part of the septum (Fig. 2A).

3. The third group is formed by 3 to 6 arteries diverging fan-like *(aa. septales laterales inferiores)*; they originate from the a. cerebri anterior or from the initial section of the a. hemispherica, 800—1000 μ before the bregma (Fig. 2A). These vessels enter from below into the substance of the septum and branch off laterally throughout the entire length of the septum. One of these arteries, the *a. subcommissuralis medialis* runs backward and upward in the median-sagittal plane, encircles the central part of the commissura anterior and supplies the median inferior septal region over the commissura anterior (Fig. 2A).

Veins

In contrast to the arterial supply the venous drainage of the septum is two-directional: the anterior group flows into the sinus cavernosus and the posterior group drains the blood into the v. Galeni (Fig. 3A; Table II).

The veins of the septal region before the commissura anterior are collected into an important venous trunk, the v. subcallosa (Figs. 3A, 3B, 4A). This forms numerous anastomoses with the veins running downward from the cortex. In this way below the corpus callosum, along the a. hemispherica, a venous plexus infracallosus is formed (Fig. 3B). The v. subcallosa is connected backward over the n. opticus with the v. perioptica and by a stronger branch running in frontal direction with sinus cavernosus.

1. The upper vein of the anterior group, the *v. infracallosa* originates with small branches below the corpus callosum. It runs forward near the midline, then flows into the v. subcallosa or the upper of the plexus infracallosus.

It drains the venous blood of the median upper third of the septum. The collecting area of the vein corresponds mostly to the supplying region of the a. infracallosa (Figs 3B, 4A). The veins originating at 300—400 μ before the bregma plane from the dorsal and median thirds of the septum (vv. septales anteriores superiores) run forward for about 1000—1200 μ, then flow into the v. subcallosa. These veins collect the blood from the anterior two-thirds of the supplying region of the a. septalis superior (Fig. 4A).

From the lateral and inferior part of the frontal two-thirds of the septum, 5 to 8 veins drain the blood into the v. subcallosa; these are called the *vv. septales mediales* and *laterales*. Usually, 3—4 median and 2—4 lateral veins may be found. The median veins originate above the commissura anterior and

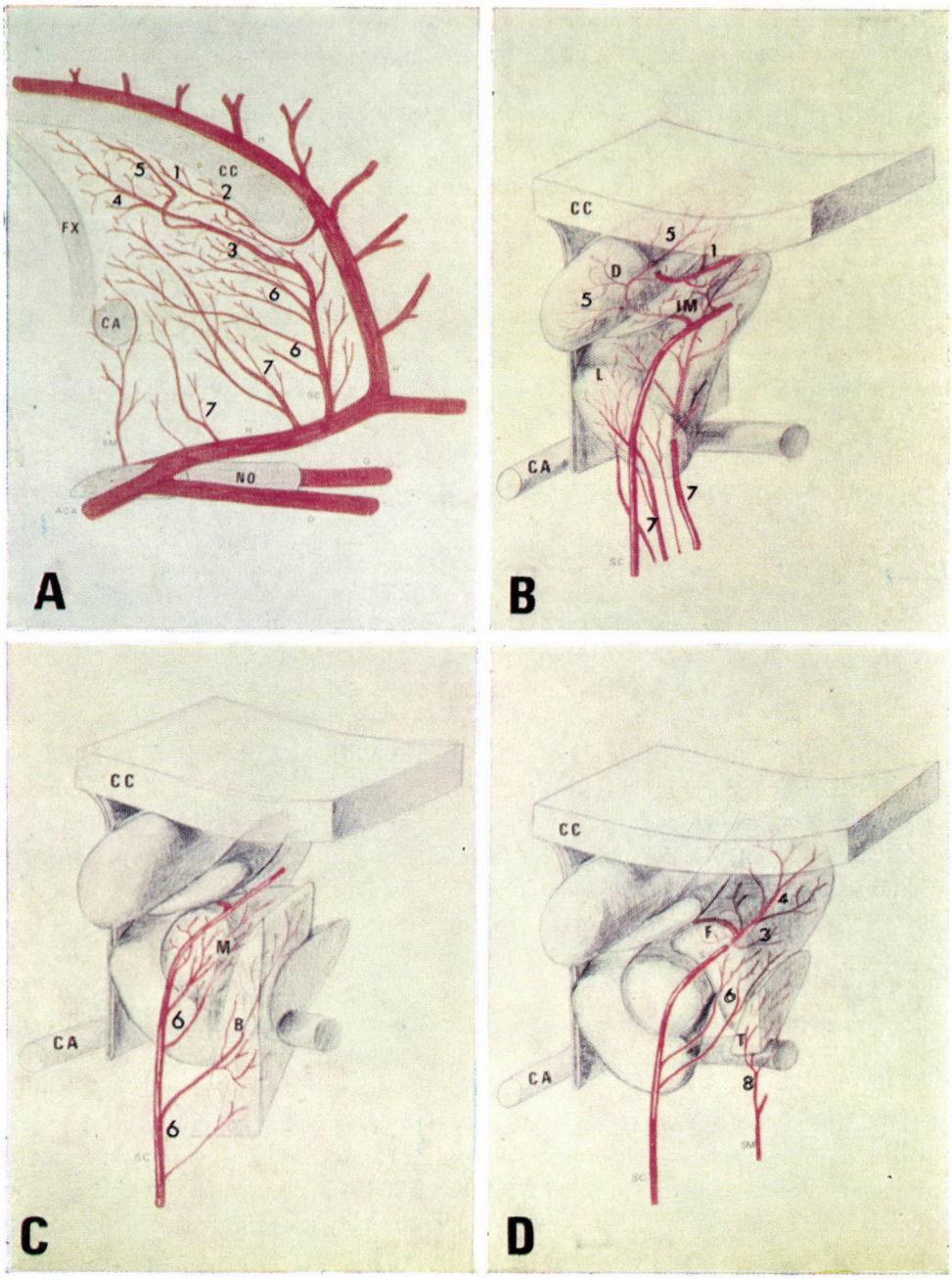


Fig. 2. Schematic drawing of the septal arteries. A. Septal arteries in the sagittal plane; B. Arteries of the nucleus septalis dorsalis, pars intermedia and nucleus septalis lateralis; C. Arteries of the nucleus septalis medialis and the nucleus tractus diagonalis pars septalis; D. Arteries of the nucleus septalis fimbrialis and nucleus septalis triangularis. Numbering of vessels: See Table I. Abbreviations: See text

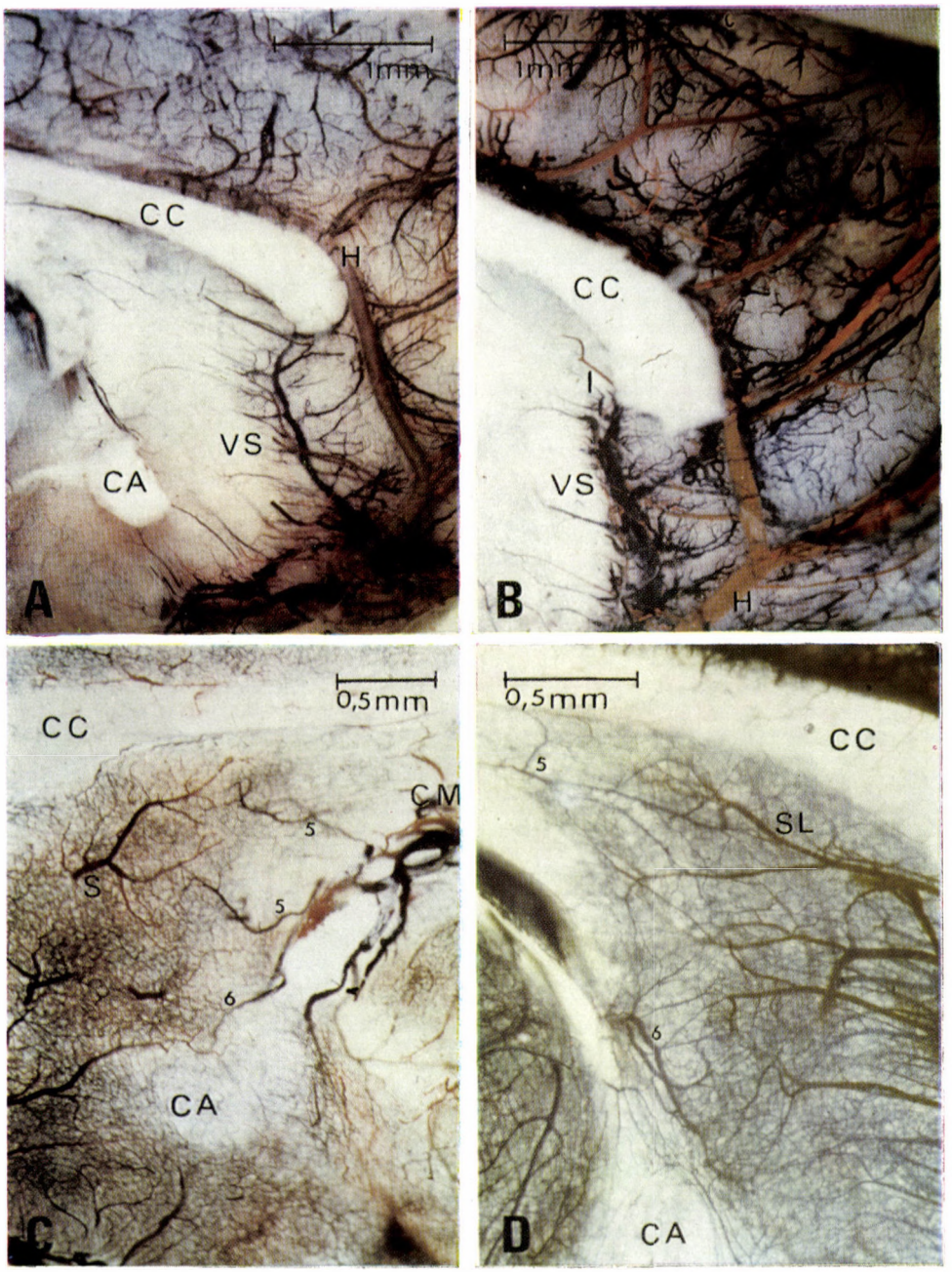


Fig. 3. Macroscopic and microscopic preparations of the veins draining the septal blood; A. V. subcallosa and its septal branches. Sagittal preparation; B. Anterior veins of the septum. Sagittal preparation; C. Posterior-superior veins of the septum. 500 μ thick sagittal section; D. Posterior-inferior veins of the septum. 500 μ thick sagittal section; Numbering of vessels: See Table II. Abbreviations: See text

Table II

Veins of the septum pellucidum in the rat

Anterior veins

(1) rr. infracallosae → v. infracallosa →

(2) vv. septales anteriores superiores →

(3) vv. septales mediales →

(4) vv. septales laterales →

→ v. subcallosa → sinus cavernosus

Posterior veins

(5) vv. septales posteriores superiores →

(6) vv. septales posteriores inferiores ←

→ v. cerebri magna (Galeni)

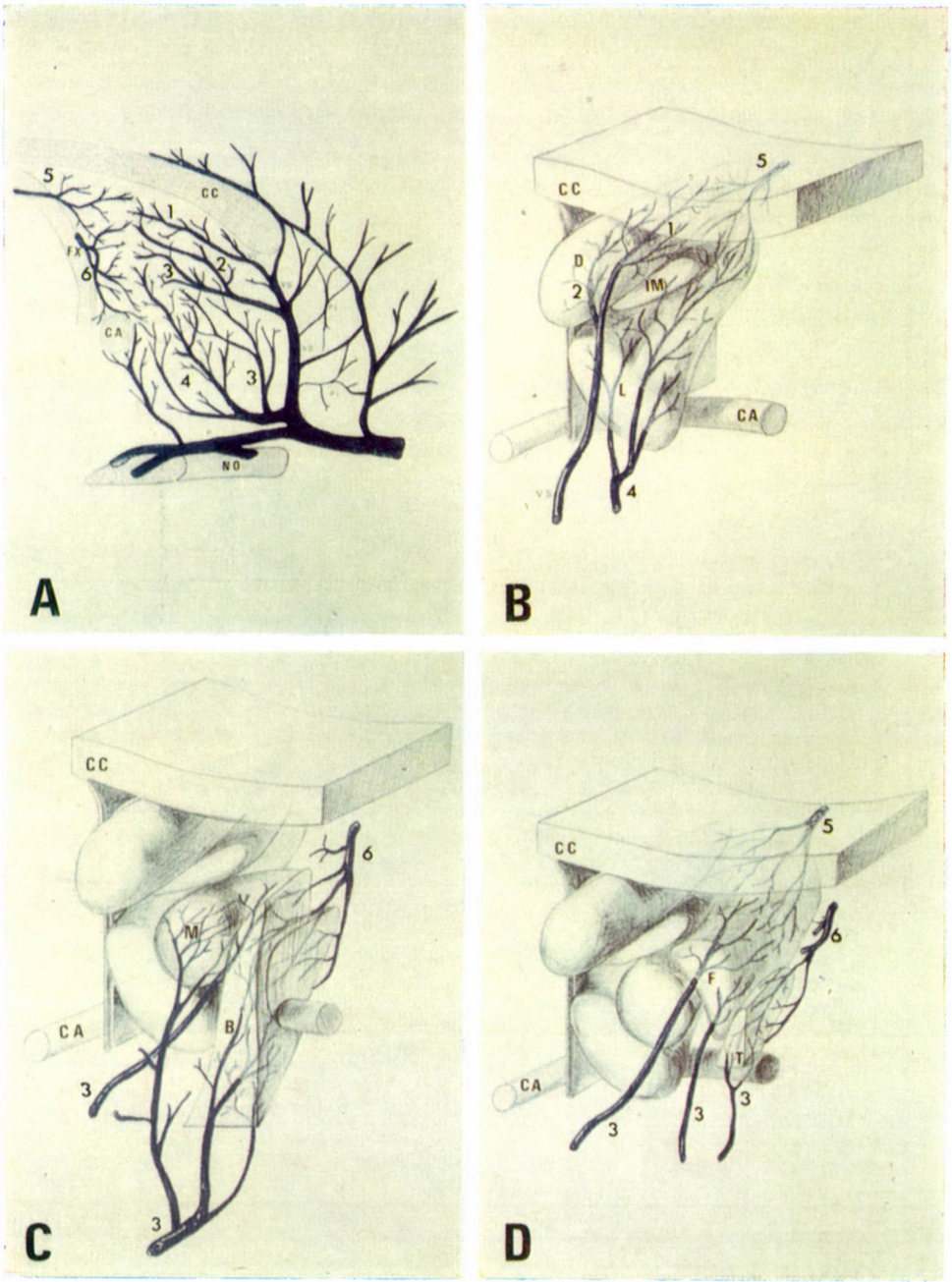


Fig. 4. Schematic representation of the septal veins. A. Principal septal veins: diagram in the sagittal plane; B. Veins of the nucleus septalis dorsalis, pars intermedia and nucleus septalis lateralis; C. Veins of the nucleus septalis medialis and nucleus tractus diagonalis; D. Veins of the nucleus septalis fimbrialis and nucleus septalis triangularis. Numbering of vessels: See Table II. Abbreviations: See text

transport the blood from the median and inferior part of the frontal two-thirds of the septum; the lateral veins take their origin from the lower part of the identical region and run towards the v. subcallosa. They are frequently collected into a common trunk and flow into the v. subcallosa (Fig. 4A). These veins are frequently overlapping with the dorsal-inferior veins.

2. From the dorsal one-third of the septum the veins are connected with the anterior veins of the hippocampus and are draining backward through the v. Galeni. The superior veins *(vv. septales posteriores superiores)* originate below the corpus callosum with several small branches from the posterior-superior one-third of the septum. The originating branches may be found at 0—300 μ before the bregma plane in the full width of the septum (Figs 3C, 4A).

The inferior veins *(vv. septales posteriores inferiores)* collect the blood from the middle and lower part of the posterior one-third of the septum as well as from the region before the commissura anterior and in connection with the superior veins, together with the anterior veins of the hippocampus flow into the v. Galeni (Fig. 3D).

Frequently, the veins of the anterior and posterior groups are overlapping, particularly in the median and inferior lateral parts of the septum.

Variations

Arteries. In 25% of the cases, the upper one-third of the a. subcallosa is connected by a communicating branch with the a. hemispherica. Sometimes, instead of the a. subcallosa the arteries classified into the second group originate from the a. hemispherica. Infrequently, the branches of the third group originate with one single common trunk at the initial section of the a. hemispherica. The arterial supply of the two halves of the septum is frequently asymmetrical.

Veins. In the venous system of the septum, on the collecting region of the veins draining in anterior or posterior direction considerable overlapping and numerous variations are frequent. Asymmetry of the venous drainage of the septal halves is common. In the venous drainage of the nuclei, groups of vessels are more dominant than single vessels; thus the variations might be even numerous.

Discussion

In the rat the arteries of the septum pellucidum take their origin exclusively from the system of the a. cerebri anterior, in contrast with the rabbit where the branches of the a. cerebri media too participate in the blood supply of the septum [5].

The septum similarly to the hypothalamus [1—3] possesses an abundant network of blood vessels. Even where larger branches are dissected they are favourable possibilities for the relatively undisturbed blood supply of the septal nuclei, thus from this aspect the chances of surgical intervention are promising. At the same time, when the divisions of the vessels are projected together with the cytoarchitecture of the septum (Palkovits, Horváth; in preparation) it is seen that the individual septal nuclei have a comparatively independent blood supply.

The nucleus septalis dorsalis is supplied by the branches of the a. septalis lateralis superior (Fig. 2B); sometimes with the participation of the rr. infracallosae. Contrarily, the pars intermedia of the dorsal nucleus is supplied in its great majority by these latter rami, whereas the a. septalis lateralis superior gives branches only to the anterior part of this subdivision (Fig. 2B). In the region of the nucleus septalis lateralis the aa. septales laterales inferiores, which belong to the third group, are dividing (Fig. 2B). The arteries originating medially from the a. subcallosa, the aa. septales mediales supply the nucleus septalis medialis and the pars septalis of the nucleus tractus diagonalis (Fig. 2C). In the major part of the region of the nucleus septalis fimbrialis the a. septalis superior divides, while in the anterior-inferior part of the nucleus some of the branches of the aa. septales mediales terminate (Fig. 2D). The nucleus septalis triangularis possesses an independent artery, the a. septalis triangularis, which is the median terminal branch of the a. subcommissuralis medialis (Table I).

The septal veins flow in two directions: the anterior group flows into the sinus cavernosus, the posterior group into the v. Galeni. The latter veins communicate with those of the hippocampus.

When the venous system of the individual septal nuclei is studied, it is seen that compared with the arteries the overlapping in the dividing region of the veins is much more considerable. The two directional drainage is valid in the case of all nuclei; from each nucleus venous branches are collected in both the anterior and posterior direction.

From the nucleus septalis dorsalis (together with the pars intermedia) frontally the vv. septales anteriores, dorsally the vv. septales posteriores superiores are collected. (Fig. 4B). From the nucleus septalis lateralis the vv. septales laterales (Fig. 4B), backwards the vv. septales posteriores superiores (sometimes also the inferiores) drain the blood. The nucleus septalis medialis and the nucleus tractus diagonalis pars septalis have common veins: forward the vv. septales mediales, backward the vv. septales posteriores inferiores are collected (Fig. 4C). The vv. septales mediales drain forward the blood of the nucleus septalis fimbrialis and the nucleus septalis triangularis (Fig. 4D). Backward from the former nucleus the vv. septales posteriores superiores and inferiores, while from the latter only lower branches, originate (Fig. 4D).

Abbreviations

ACA = A. cerebri anterior
B = Nucleus tractus diagonalis (Broca)
CA = Commissura anterior
CC = Corpus callosum
CM = V. cerebri magna (Galeni)
CoA = A. communicans anterior
D = Nucleus septalis dorsalis
F = Nucleus septalis fimbrialis

FX = Fornix
H = A. hemispherica
I = A. infracallosa
IM = Nucleus septalis dorsalis pars intermedia
L = Nucleus septalis lateralis
M = Nucleus septalis medialis
NO = N. opticus
O = A. olfactoria
P = V. perioptica
S = A. septalis superior
SC = A. subcallosa
SL = A. septalis lateralis superior
SM = A. subcommissuralis medialis
T = Nucleus septalis triangularis
VI = V. infracallosa
VL = Ventriculus lateralis
VS = V. subcallosa

REFERENCES

1. Ambach, G., Palkovits, M.: (1974) Blood supply of the rat hypothalamus. I. Nucleus supraopticus. Acta morph. Acad. Sci. hung. **22,** 291—310. — 2. Ambach, G., Palkovits, M: 1974 Blood supply of the hypothalamus. II. Nucleus paraventricularis. Acta morph. Acad. Sci. hung. **22,** 311—320. — 3. Ambach, G., Palkovits, M.: (1975) Blood supply of the rat hypothalamus. III. Regio anterior hypothalami (Nucleus suprachiasmatis, nucleus hypothalamicus anterior, nucleus periventricularis). Acta morph. Acad. Sci. hung. **23,** 21—43. — 4. Moffat, D. B.: (1961) The development of the anterior cerebral artery and its related vessels in the rat. Amer. J. Anat. **108,** 17—30. — 5. Prolo, D. J., Stilwell, D.: (1962) Arterial supply to the diencephalon and some associated areas of the rabbit brain. J. comp. Neur. **109,** 229—254. — 6. Scremin, O. U.: (1970) The vascular anatomy of the rat's hypothalamus in stereotaxic coordinates. J. comp. Neur. **139,** 31—52.

DIE ARTERIELLE UND VENÖSE BLUTVERSORGUNG DES SEPTUM PELLUCIDUM BEI RATTEN

G. AMBACH, S. HORVÁTH und M. PALKOVITS

Die Autoren untersuchten das arterielle und venöse System des Septum Pellucidum mit kombinierten doppelten Perfusionstechnik bei Ratten.

Die Arterien sind in drei Gruppen zu teilen. Die Äste aller Gruppen entspringen der a. hemispherica und gelangen von vorne in den Septum Pellucidum. Die Venen sind in vordere und hintere Gruppen zu teilen. Die vordere münden sich durch der v. subcallosa in den sinus cavernosus, die hintere in die v. cerebri magna. Die arterielle und venöse Blutversorgung der einzelnen Kernen des Septum Pellucidum ist relativ selbständig.

АРТЕРИАЛЬНОЕ И ВЕНОЗНОЕ КРОВОСНАБЖЕНИЕ ПЕРЕГОРОДКИ КРЫСЫ

Г., АМБАХ, Ш. ХОРВАТ и М. ПАЛКОВИТШ

Исследовалась артериальная и венозная система перегородки крысы методом двойной перфузии туши.

Артерии разделяются на три группы. Ветри из каждой группы начинаются от а. хемисферика и проходят к перегородке спереди. Вены разделяются на переднюю и заднюю группы.

Передние вливаются через в. субкаллоза в синус кавернозус а задние в в. церебри магна.

Артериальное и венозное кровоснабжения каждого ядра перегородки считаются самостоятельными.

Dr. György Ambach
Dr. Sándor Horváth
Dr. Miklós Palkovits
Semmelweis Orvostudományi Egyetem,
I. Anatómiai Intézete
1450 Budapest, Pf. 95., Hungary

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Department of Pathology, Péterfy Hospital; Department of Pathology, Postgraduate Medical School, Department of Urology, Semmelweis University Medical School, Budapest

EFFECT OF REHYDRATION OF RAT LIVER TISSUE AFTER WATER DEPRIVATION

I. Bartók, Sz. Virágh and J. Menyhárt

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Male albino rats were deprived of water for 6 days, then they were allowed to drink tap water ad libitum. The structure of the liver was examined by light and electron microscopy, and the protein and dry matter contents, oxygen consumption and glucose-6-phosphatase activity of the liver were determined after rehydration. At 10 minutes, the mitochondria showed signs of division and a peculiar transformation of the cristae. At 60 minutes, the membranes of the rough endoplasmic reticulum were found to have proliferated. At 12 hours, the smooth-surfaced membranes showed hypertrophy and the bile canaliculi were distended. At 24 hours all rehydration induced organelle alterations were declining. The biochemical findings agreed well with the fine structural changes and both were indicative of an enchanced functional capacity of the liver cells during rehydration.

The fine structural changes induced in the rat liver by dehydration due to water deprivation were described previously [1]. Six-day thirsting was followed by swelling of the mitochondria, quantitative decrease of rough and smooth surfaced endoplasmic reticulum (ER), fragmentation and dilatation of rough-surfaced ER (RER) cisternae and a reduction in size of the Golgi complexes. There was also a marked constriction of the lumen of bile canaliculi, and hepatic oxygen consumption and glucose-6-phosphatase activity were decreasing. These alterations indicated a general reduction of the functional capacity of the liver.

The present paper reports on further studies on the behaviour of dehydration-injured cell organelles after rehydration, with regard to the temporal course of organelle alterations.

Materials and methods

Male albino rats weighing 330—370 g, and maintained on a semisynthetic diet were deprived of water for 6 days and were subsequently allowed to drink tap water *ad libitum*. The haematocrit was regularly recorded. Five rats were killed on the sixth day of water deprivation and five each at 10, 20 and 60 minutes, and 12 and 24 hours after the beginning of rehydration. Average water consumption of the rehydrated animals was 10 ml during the first 10 minutes, and 15, 20, 60 and 70 ml subsequently. Rats killed after 10, 20 and 60 minutes did not receive food, whereas those killed later were fed normally. The livers of the water deprived and rehydrated animals as well as of the five rats normally provided with food and water were removed immediately after sacrifice and examined by the following methods.

1. Dry matter content was determined from a specimen weighing about 150 mg, devoid of capsule, after overnight drying at 90°C.

2. Protein content was determined by the micro-Kjeldahl technique.

3. Oxygen consumption was measured by the Warburg technique, in the presence of 0.01 M Glucose substrate.

4. G-6-Pase activity was estimated by the method of BERGMEYER [2], using G-6-P as substrate.

5. Blocks for light microscopy were fixed in 10% neutral formalin, embedded in paraffin, and the sections were stained with haematoxylin and eosin.

6. Small tissue blocks for electron microscopy were fixed in 4% glutaraldehyde and 1% $HOsO_4$ buffered according to Millonig, then dehydrated in alcohol and embedded in Durcupan ACM. Thick sections were stained with toluidine blue, thin sections with uranyl acetate and lead citrate. Electron micrographs were made on a SEM-1 electron microscope.

Results

Haematocrit, hepatic dry matter and protein contents, oxygen consumption and G-6-Pase activity of the liver of normal, dehydrated and rehydrated rats are shown in Table I. Hepatic dry matter and protein content had approximated the normal value after rehydration for 1 hour and reached it after 12 hours. Oxygen consumption had normalized one hour after rehydration and persisted at that level throughout. The G-6-Pase activity increased at a slower rate, but after 12-hour rehydration it markedly exceeded the normal hepatic activity and rose further until 12 hours.

Table I

Haematocrit, hepatic dry matter and protein contents, oxygen consumption and glucose-6-phosphatase activity in normal, dehydrated and rehydrated rats

	Haematocrit, per cent	Hepatic dry matter content, per cent	Hepatic protein content, per cent	QU_2 ±SE	Glucose-6-phosphatase activity*
Normal	43 (41—48)	31.4 (28—33)	22.1 (19—23)	9.7 ±1.6	23.5 ±3.0
Dehydrated	53 (51—56)	39.2 (37—42)	30.8 (27—32)	6.1 ±0.9	16.4 ±2.2
10-minute-rehydrated	—	37.2 (35—39)	28.3 (25—30)	6.7 ±1.1	17.1 ±2.3
20-minute-rehydrated	—	—	27.4 (26—30)	7.6 ±1.0	19.6 ±2.9
1-hour rehydrated	49 (48—51)	34.7 (32—37)	25.3 (22—27)	10.1 ±1.8	18.9 ±2.7
12-hour rehydrated	—	32.5 (30—35)	23.1 (22—26)	10.9 ±1.7	30.6 ±4.4
24-hour rehydrated	46 (43—49)	32.0 (29—36)	22.9 (19—24)	10.1 ±2.0	37.4 ±4.8

* = μ mole PO_3 gm liver/min

In H. E. — stained sections from dehydrated rats the liver cells appeared shrunken and the average number of nuclei found in 20 high-power fields (×700) was 23 in contrast to the normal average of 12. Shrinking of the cells disappeared gradually on rehydration and after 1 hour the average number of nuclei in one field did not differ from the normal.

Electron microscopy

In liver cells of rats rehydrated for 10 minutes mitochondrial swelling disappeared. Many mitochondria — more in the periportal than in the centrolobular hepatocytes — showed phenomena indicative of autoreproduction: parallel membranes, continuous with the inner limiting membrane, extended transversally into the matrix space, dividing it into 2 or 3 parts (Fig. 1); other mitochondria became elongated and either assumed a dumbbell-shape owing to constriction of the central part (Fig. 2) or exhibited ramifications. In many other mitochondria, unusually long cristae, ordered parallel to the outer membrane, were seen. The intracristal space contained a peculiar electron dense substance (Fig. 4) which consisted of alternating dense and light lines. Mitochondria exhibiting this peculiar structure were found in material fixed in both OsO_4 and glutaraldehyde; they occurred in all parts of lobules. These mitochondrial changes have been described in greater detail in a previous paper [1].

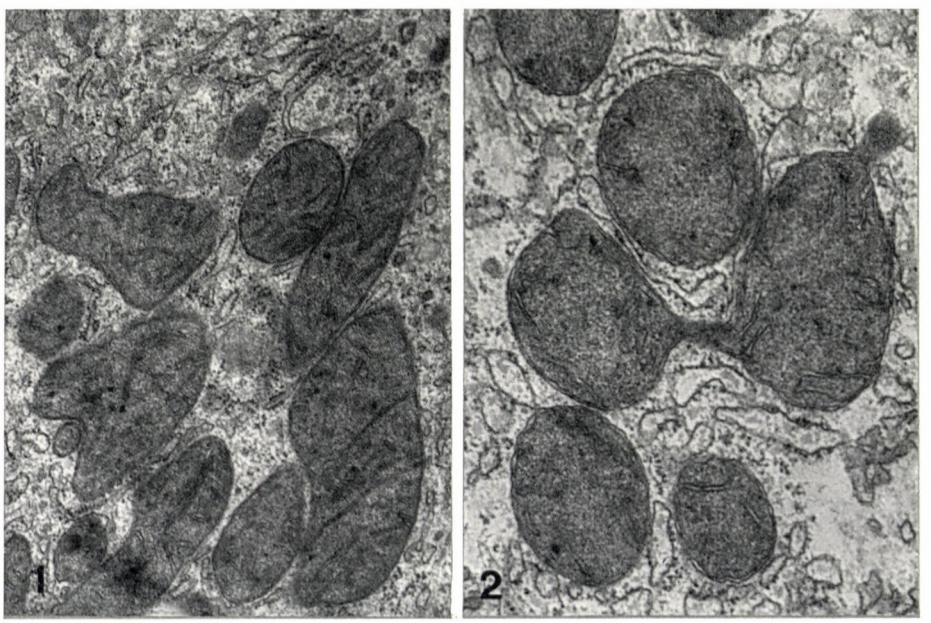


Fig. 1. 10-minute rehydration. Dividing mitochondrium: transversal double membranes separate the matrix into three parts. ×24 000

Fig. 2. 10-minute rehydration. Dividing dumbbell-shaped mitochondrium. ×33 000

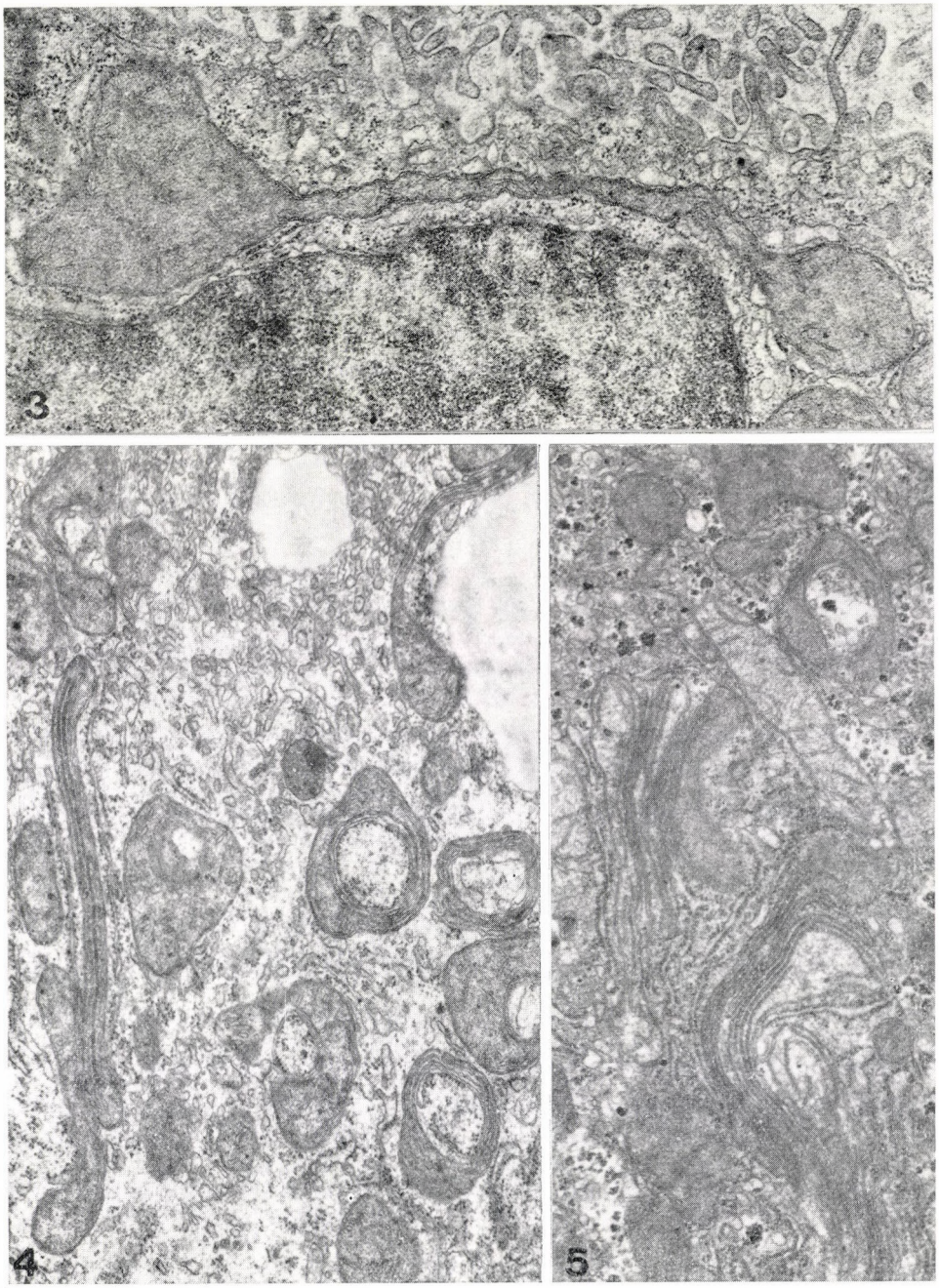


Fig. 3. 20-minute rehydration. Dividing dumbbell-shaped mitochondrium. ×25 000
Fig. 4. 10-minute rehydration. Mitochondria of different shapes with elongated cristae, extending parallel to the inner limiting membrane. The intracristal space of the elongated mitochondrium contains electrondense material (OsO_4 fixation). ×23 000
Fig. 5. 20-minute rehydration. Many longitudinal parallel inner membranes inside the elongated mitochondria (glutaraldehyde fixation). X 23 000

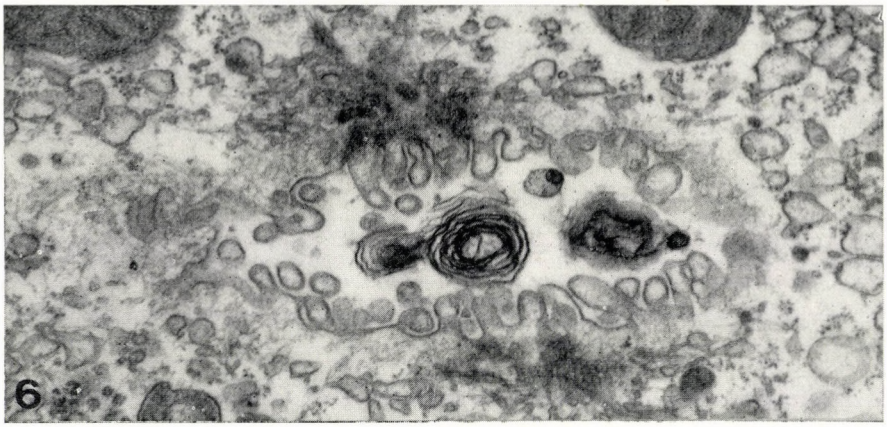
After 10-minute rehydration a certain dilatation and fragmentation of the RER persisted, but the change was much less expressed than during dehydration. Single autophagic vacuoles were seen in several cells. The lumen of some bile canaliculi contained a myelin figure-like, concentrically ordered lamellar substance (Fig. 6).

After rehydration for 20 minutes, the structural changes induced by dehydration disappeared completely. Dividing mitochondria and mitochondria with longitudinal cristae were still present in fair numbers, although less abundantly than previously (Figs 3 and 5). Elongated parallel DER profiles were seen. The smooth ER(SER) formed a network of membranes containing glycogen granules. The cisternae and vacuoles of the Golgi complex increased in number and either a homogeneous or a granular substance was abundantly present inside them.

After rehydration for 60 minutes, dividing mitochondria and longitudinal cristae were rarely seen. The RER membranes increased in number so that 30 or more parallel cistern profiles were frequent (Fig. 7). The SER formed a circumscribed compact network in several cytoplasmic areas. One or two autophagic vacuoles were still present in the cells. Several bile canaliculi were distended and some had either an irregular amorphous, or a concentrically ordered lamellar substance in the lumen.

After 12 hour rehydration, no dividing mitochondria were found, but in the periportal and centrolobular hepatocytes some mitochondria contained longitudinal cristae. Many liver cells in the centrolobular and periportal areas showed a marked increase of SER. The SER tubules formed a continous, contorted and arborizing network that traversed the entire cytoplasm (Fig. 8). Glycogen was not present in such hepatocytes whereas large fields of glycogen were seen in others. Lipid droplets, part of which were surrounded by SER tubules closely attached to the margin of the drop, were frequent in the glycogen fields (Fig. 10). While at shorter periods of rehydration single, if any, autophagic vacuoles were seen, 4 to 5 of them were found to have appeared simultaneously in some hepatocytes (Fig. 12). Marked distension of bile canaliculi was frequently seen in all lobular zones. The circumscribed dilated part of the lumen was occasionally bulging into the cytoplasm of liver cells in a sac-like manner (Fig. 11). An amorphous or lamellar substance was often present in the canalicular lumen (Fig. 9).

After rehydration for 24 hours, mitochondria with longitudinal cristae were rare and were found only in centrolobular hepatocytes. Several bile canaliculi were still distended, their lumen comprising the same substance as before. The SER was still hypertrophic in part of the hepatocytes, mainly in the centrolobular zone, yet less abundant than earlier.



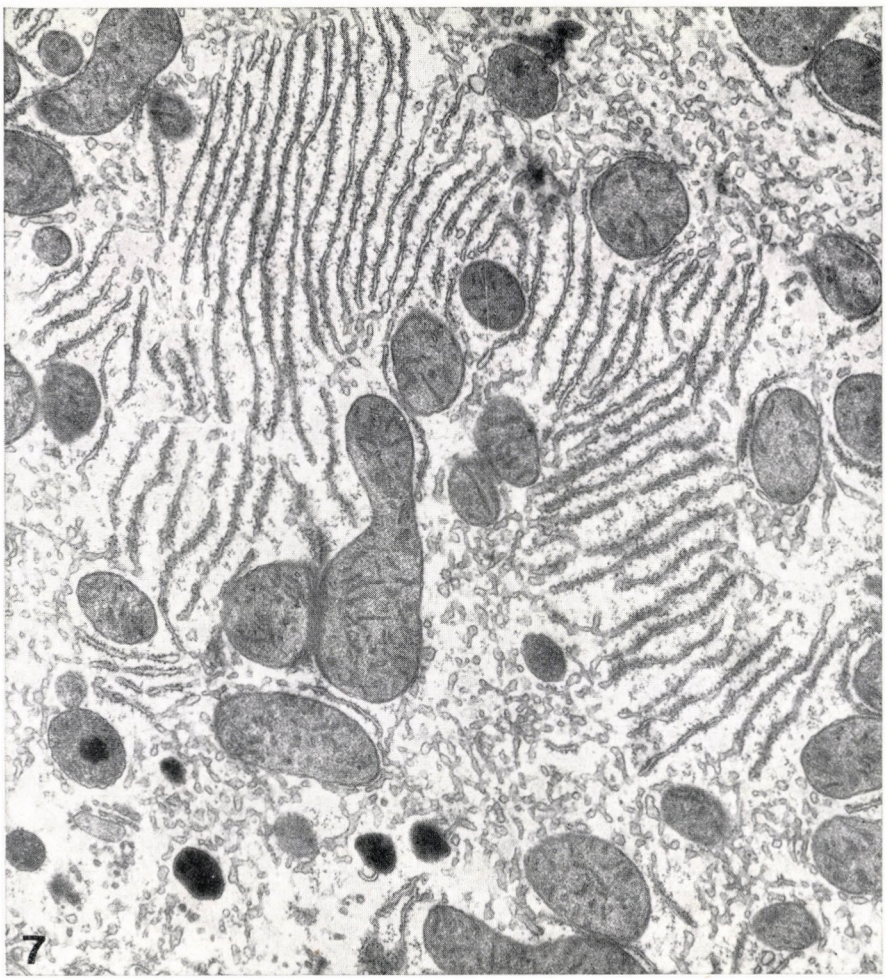


Fig. 6. 10-minute rehydration. A concentrically ordered lamellar substance inside the bile canaliculus. ×24 000

Fig. 7. 1-hour rehydration. Proliferation of rough-surfaced endoplasmic reticulum. ×13 800

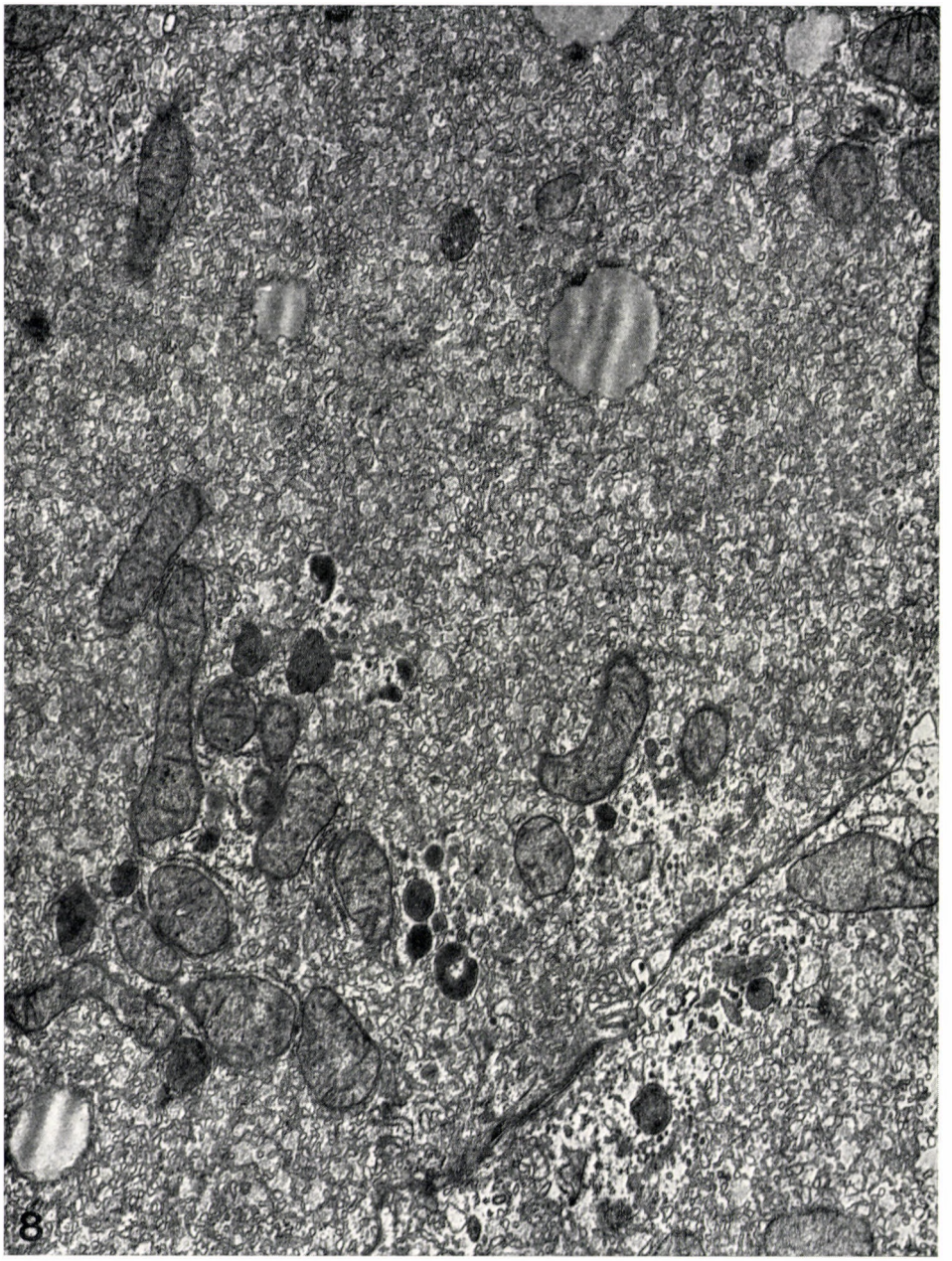


Fig. 8. 12-hour rehydration. Hypertrophy of smooth-surfaced endoplasmic reticulum. ×15 000

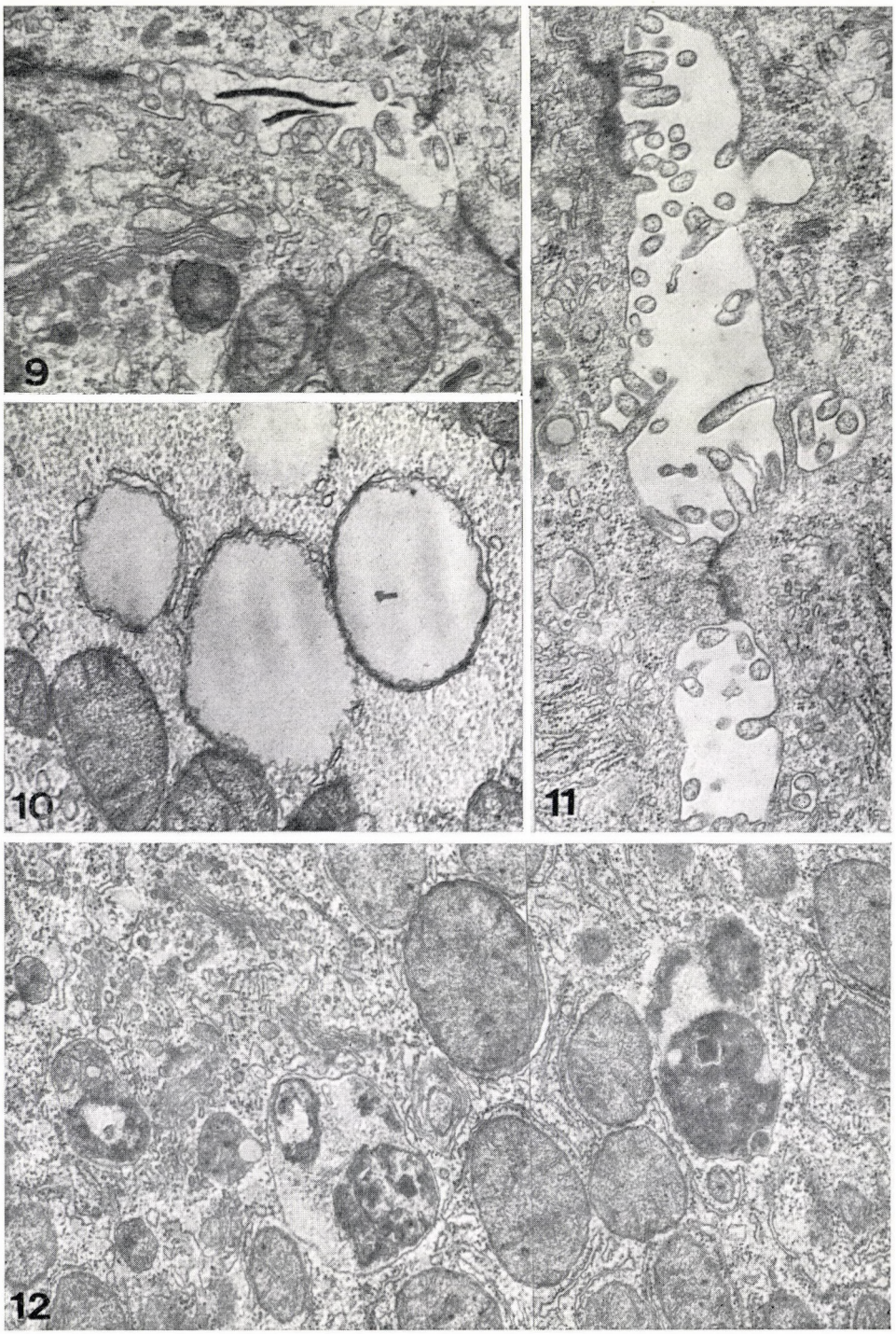


Fig. 9. 12-hour rehydration. Lamellar substance inside the bile canaliculus. ×24 000
Fig. 10. 12-hour rehydration. Lipid droplets surrounded by profiles of smooth-surfaced endoplasmic reticulum. Note increase of glycogen. ×22 000
Fig. 11. 12-hour rehydration. Circumscribed sac-like dilatation of a bile canaliculus. × 22 00
Fig. 12. 12-hour rehydration. Hepatocyte containing three autophagic vacuoles. ×22 0000

Discussion

The present results indicate that in the liver cells of albino rats morphological changes induced by prolonged dehydration normalize rapidly. Apart from the disappearance of dehydration-induced changes, certain structural alterations are taking place, which suggest an increase of the functional capacity of hepatocytes during rehydration.

Ten minutes after the beginning of rehydration many mitochondria showed phenomena that are known to signify mitochondrial division in both plant [5] and animal cells (3, 12, 13]. The beginning of mitochondrial division within a few minutes after drinking indicates an extraordinarily rapid accommodation of these organelles to the functional requirements of the hepatocytes. Oxygen consumption of the liver rose parallel to the progress of mitochondrial division and reached the normal level after its termination.

Simultaneously with mitochondrial autoreproduction, the cristae of many mitochondria became elongated, arranged longitudinally, and a substance showing a periodic pattern made appearance in the intracristal space in every zone of the lobules. A similar transformation of the inner mitochondrial membrane has been observed by several investigators under different conditions in hepatocytes and other cells (see in [1]), but its exact role is still unclear. According to our own findings and to others [15], the change is not due to fixation. Elongation of the cristae enlarges the surface of the inner membrane 3 to 4 times and the elongated membranes show a cytochrome oxidase activity [8]. In the present experiment, the longitudinal transformation of the mitochondrial cristae, the autoreproduction of mitochondria and the increase in hepatic oxygen consumption took place simultaneously. There is, accordingly, reason to suppose that the formation of longitudinal cristae might be related to the enhanced functional state of the mitochondria.

After rehydration for 1 hour, changes of the ER became predominant. Proliferation of the RER was observed after 60 minutes and in many liver cells the SER showed a marked hypertrophy after 12 hours. SER is known to be the principal site of oxidative detoxification of lipid-soluble exogenous substances [4, 6, 10] but it also plays a major role in the metabolism of endogenous substances such as steroid hormones, cholesterol [4, 6, 7] fatty acids [4], bile salts [11] and bilirubin [9]. The circulatory disorder arising during dehydration gradually normalizes on rehydration, to judge from the respective haematocrit readings. Thus, presumably, a considerable amount of endogenous substances enters the liver cells and undergoes metabolic transformation in the SER. The hypertrophy of the smooth membranes was accompanied by an elevation of the G-6-Pase activity, indicating a simultaneous increase of the system's functional activity. Hypertrophy of the SER was preceded by multiplication of the rough-surfaced membranes. This agreed well with the impli-

cation that protein synthesis involved in the neogenesis of smooth membranes takes place — at least partly — in the RER [4, 6, 7, 14]. As the ER plays a role in fatty acid metabolism too [4], there is reason to suppose that the close topographic association between lipid droplets and SER tubules implies a functional relation between these components.

Twelve hours after rehydration the autophagic vacuoles temporarily increased in number. The amorphous or lamellar substance found in the bile canaliculi had probably been released from the autophagic vacuoles. The distension and intracellular sacculation of the bile canaliculi might be related with the increased secretion of bile[16].

It is concluded from the present findings that rehydration following upon prolonged water deprivation induces in the hepatocytes a rapid and intensive neogenesis of mitochondria and ER. It was shown previously [1] that blood circulation and functional capacity of the rat liver diminished during dehydration. On rehydration the circulation improved gradually, as judged from the rise of the haematrocit. The fluid content of the liver cells increased as shown by the reduction of dry matter content and protein concentration, and, in all probability, the amount of substances transported to the liver also increased. It appears, therefore, that the multiplication of cell organelles is related to the increase in the functional capacity of hepatocytes. The surprisingly rapid autoreproduction of mitochondria can be interpreted as the earliest manifestation of accommodation to the increased metabolic and energy requirements. This is followed by proliferation of the RER, which indicates an increased protein synthesis and membrane neogenesis. Finally the SER hypertrophies, apparently to enlarge its capacity for the metabolic transformation of the increased amount of endogenous substances entering into the hepatocytes during rehydration.

REFERENCES

1. Bartók, I., Virágh, Sz., Menyhárt, J.: (1973) Prompt divisions and peculiar transformation of cristae in liver mitochondria of rats rehydrated after prolonged water deprivation. J. Ultrastruct. Res. **44**, 49. — 2. Bergmeyer, H. M.: (1962) Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim 1962, P. 788. — 3. David, H.: (1969) Struktur der Mitochondrien. In: Bielka H. (Ed) Molekulare Biologie der Zelle. Fischer, Jena 1969, P. 466. — 4. Ernster, L., Orrenius, S.: (1973) Dynamic organisation of endoplasmic reticulum membranes. Drug Metab. Dispos. **1**, 66. — 5. Hanzely, L., Schjeide, O. A.: (1971) Fine structural observations of mitochondria undergoing division in Allium sativum root tip cells. Cytobiologie **4**, 207. — 6. Jones, A. L., Fawcett, D. W.: (1966) Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by phenobarbital (with a review on the function of this organelle in liver). J. Histochem. Cytochem. **14**, 215. — 7. Orrenius, S., et al: (1968) The hydroxylating enzyme system of liver endoplasmic reticulum. In: J. C. Gran (Ed): Structure and function of the endoplasmic reticulum in animal cell. Universitetsforlaget, Oslo, 1968, p. 81. — 8. Reith, A., Riede, U. N., Rohr, H. P.: (1972) Atypische Lebermitochondrien, adaptive Sonderformen? II. Cytochemisch-morphometrische Untersuchung. Cytobiologie **6**, 163. — 9. Remmer, H.: (1970) The role of the liver in drug metabolism. Amer. J. Med. **49**, 617. — 10. Remmer, H., Merker, H. J.: (1963) Drug induced changes in the liver endoplasmic

reticulum. Association with drug metabolizing enzymes. Science **142,** 1657. — 11. SCHAFFNER, F., POPPER, H.: (1969) Cholestasis is the result of hypoactive hypertrophic smooth endoplasmic reticulum in the hepatocyte. Lancet **2,** 355. — 12. TANDLER, B., et al.: (1969) Riboflavin and mouse hepatic cell structure and function. II. Division of mitochondria during recovery from simple deficiency. J. Cell Biol. **41,** 477. — 13. TANDLER, B., HOPPEL, C. L.: (1973) Division of giant mitochondria during recovery from cuprizone intoxication. J. Cell Biol. **56,** 266. — 14. TAYLOR, J. M., et al.: (1967) The synthesis and degradation of membrane proteins. Drug Metabol. Dispos. **1,** 84. — 15. VALDIVIA, E., BERGER, J. E.: (1973) Atypical electron microscope structures in liver mitochondria. Path. Microbiol. **39,** 112. — 16. VIRÁGH, SZ., BARTÓK, I.: (1967) An electron microscopic study of the regeneration of the liver following partial hepatectomy. Amer. J. Path. **49,** 825.

REHYDRATATIONSEFFEKT IN DER LEBERSTRUKTUR VON RATTEN NACH DÜRSTEN

I. BARTÓK, SZ. VIRÁGH und J. MENYHÁRT

Die Verfasser liessen männliche weisse Ratten 6 Tage lang dürsten und ernährten diese nur mit Trockenfutter. Sodann erhielten die Tiere *ad libitum* Leitungswasser. Während der Rehydratation prüften sie mit Hilfe von Licht- und Elektronenmikroskop die Leberstruktur sowie den Trockensubstanz- und Eiweissgehalt, den Sauerstoffverbrauch und die Glukose-6-Phosphatase-Aktivität der Leber. 10 Minuten nach Beginn der Rehydratation zeigten sich in zahlreichen Mitochondrien Anzeichen einer Teilung, und die Cristae wiesen eine eigentümliche Umwandlung auf. Nach 60 Minuten nahm die Zahl der Membranen des rauhen endoplasmatischen Retikulums zu. 12 Stunden später entstand eine Hypertrophie im Reticulum des glatten endoplasmatischen Retikulums, autophagische Vacuola reicherten sich an, und die Gallencanaliculi dehnten sich aus. Nach 24stündiger Flüssigkeitsaufnahme zeigte die Leberzellenstruktur eine nahezu normales Bild. Die Ergebnisse der biochemischen Prüfungen standen in Einklang mit den Veränderungen in der Feinstruktur, und diese wiesen insgesamt auf eine Erhöhung der funktionellen Kapazität der Leberzellen während der Rehydratation hin.

ВЛИЯНИЕ РЕГИДРАЦИИ НА ТКАНЬ ПЕЧЕНИ ПОСЛЕ ДИЕТЫ ЛИШЕННОЙ ЖИДКОСТИ У КРЫС

И. БАРТОК, С. ВИРАГ и Й. МЕНЪХАРТ

Белые крысы мухского рода были содержены на безводной диете в течение 6 дней, кормили их только сухим пищевым веществом. Далее животные получили простую воду *ad libitum*. Во время регидрации оптически и электронномикроскопически исследовалось структура печени, состав сухого вещества и белки печени, расход кислорода и активность глукоз-6-фосфатазы. Обнаруживалось за 10 минут после начала пойки воды признаки деления во многих митохондриях, появилось переобразование своеобразных митохондриальных гребешок.

За 60 минут обнаруживалось нарастание мембранов зернистой эндоплазматической сети, за 12 ч. гипертрофия незернистой эндоплазматической сети, и нарастание автофаготических вакуол и расширение желчных каналцев. В течение 24 часов после введения жидкости структура клеток печени оказывалась почти нормальной.

Результаты биохимических исследований совпадаются ультраструктурными изменениями и эти вместе касаются функциональной мощности клетки печени во время регидрации.

Dr. István BARTÓK, Fővárosi Péterfy Sándor utcai Kórház 1441 Budapest, Pf. 76., Hungary

Dr. Szabolcs VIRÁGH, Orvostovábbképző Intézet, 1389 Budapest, Pf. 112., Hungary

Dr. Jenő MENYHÁRT, Semmelweis Orvostudományi Egyetem, Urológiai Klinika, MTA Kutató Lab., 1450 Budapest, Üllői út 78., Hungary

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Department of Histology, Medical Institute, Vladivostok, U.S.S.R.

A COMPARATIVE CHARACTERISTIC OF EFFECTOR INNERVATION OF CEREBRAL ARTERIES IN MAMMALS AND HUMANS

P. A. Motavkin, G. S. Vlasov and L. D. Palashchenko

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The neural fibres of cerebral arteries in humans, rats, guinea-pigs, rabbits, cats, dogs, swine, cows and horses have been studied using the methods of Flack-Hillarp and Koelle. The large arteries of the carotid and vertebral systems bear dense cholinergic and adrenergic plexuses formed by transversal and longitudinal neural fibres, which are located in the superficial and deep adventitial layers. The highest density of cholinergic and adrenergic fibres was observed on the arteries of humans and large mammals. Depending on the density of cholinergic and adrenergic fibres in the said arteries, the animals may be listed in the following order: rats, guinea-pigs, rabbits, cats, dogs, swine, cows and horses. Basic differences in the structure of neural plexuses of humans and animals were not observed.

Different methods have been used for clarifying the functional significance of neural plexuses on cerebral vessels. Carlyle and Grayson [1], Sokoloff [18], Rosenblum [17], Politoff and Macri [16], Kobayashi et al. [11], Nielsen and Owman [13] and Harper et al. [8] noted that on sympathetic stimulation or introduction of catecholamines the diameter of the pial arteries decreases whereas excitation of the vagal nerve or introduction of acetylcholine the said arteries dilate.

Further advances in the study of vasomotor innervation are depending on the histological techniques used for revealing the meditors and enzymes in the neural fibres. The specific histochemical fluorescence method [5], allowed to describe the adrenergic neural plexuses on the cerebral blood vessels of mammals and humans [2, 3, 6, 9, 10, 12, 14, 15, 19, 20, 23, 24]. The cerebral vascular system possesses both adrenergic and cholinergic innervation [4, 21, 22]. Most authors used only 1—3 mammal species to study the innervation of cerebral vessels, [4] being the only ones to have presented evidence obtained in numerous animals e.g. mice, rats, rabbits, guinea-pigs, and cats.

The above-cited references convincingly indicate the presence of a morphological substrate for neural regulation of cerebral blood circulation. Most of the relevant papers, however, were based chiefly on the study of laboratory animals. Farm animals have not been used, neither have the cerebral blood vessels of humans been sufficiently studied. Yet, such evidence appears to be indispensable for a comparative characterisation of the cholinergic and adrenergic innervation of cerebral vessels.

Materials and methods

Ten each of sexually mature rats, guinea-pigs, rabbits, cats, dogs, swine, cows, horses and the cerebral arteries of 10 humans, who had died in accidents in wintertime, were used in the study. The brains were removed immediately. The following arteries were examined: internal sphenoid; middle, anterior and posterior cerebral; main, vertebral and cerebellar; and pia mater arteries.

Intracranial arteries (diameter up to 800 μ) of the carotid and vertebral systems were isolated with the pia mater. The larger vessels were cut longitudinally and mounted on slides. At the same time 25—40 μ thick transversal and tangential cyrostate sections were prepared. Adrenergic fibres were examined by means of the histochemical fluorescence method of Falck-Hillarp. All preparations were dried *in vacuo* ($5 \cdot 10^{-4}$mm Hg) at room temperature for 5—10 min. After drying, the samples were treated with gaseous formaldehyde at 80°C for 1 hr in closed glass vessels containing paraformalin at standardized humidity in a thermostat. Depending on the subjects examined, relative humidity varied from 65 to 75%. The preparations were studied in a luminescent microscope in blue-violet light of 410—480 mμ. The results were photographed on X-ray film. Samples not treated with gaseous formaldehyde were used as control to check the specificity of luminescence. Acetylcholinesterase (3.1.1.7) and cholinesterase (3.1.1.8) were estimated in cholinergic neural fibres by Koelle's method using iodide and bromide salts of acetylthiocholine and butyrylthiocholine. Control material was kept in 10^{-5} M eserine solution. The optimum exposition time in the incubation solution was determined experimentally. The preparations were maintained in the medium at 37°C, pH 6.2—6.8, for 0.5—4 hr. The concentration of longitudinal and transversal conductors per sq. mm. was counted to make a quantitative determination of cholinergic and adrenergic neural plexuses in the arteries.

Results and discussion

Cholinergic and adrenergic neural plexuses were observed in all the arteries of the carotid and spinal systems of every animal studied.

The cholinergic fibres were light-brown and brown in colour. Neural conductors with high acetylcholinesterase activity contrasted sharply with the vascular wall where enzymatic activity was lacking. Copper sulphide, a final product of the histochemical reaction, falls out non-uniformly along the conductor length to form segments of varicose intumescences with large-granular structure. Adrenergic fibres have bright-green fluorescence, and also contain varicose intumescences with more intensive fluorescence. Deep adventitia layers were not observed in the total preparations with thick vascular walls due to tissue autofluorescence and fluorescence of the superficial plexus. In such cases, the number of neural fibres was not counted.

In arteries and branches over 400 μ in diameter two networks were detected in a single neural plexus (Figs 1—4). A superficial large-loop network was formed chiefly by periadventitial thick neural bundles (10—90 μ) with longitudinal, transversal and spiral-like directions. From the outer plexus, fibre bundles branch to form, on the boundary with the middle pia mater, a dense deep network consisting of thin chiefly circular neural fibres. The smaller the vessel, the less the number of superficial neural conductors and the lower the density of neural fibres in the deep plexus. In arteries less than 400 μ in diameter only one plexus was seen (Figs 3, 4c).

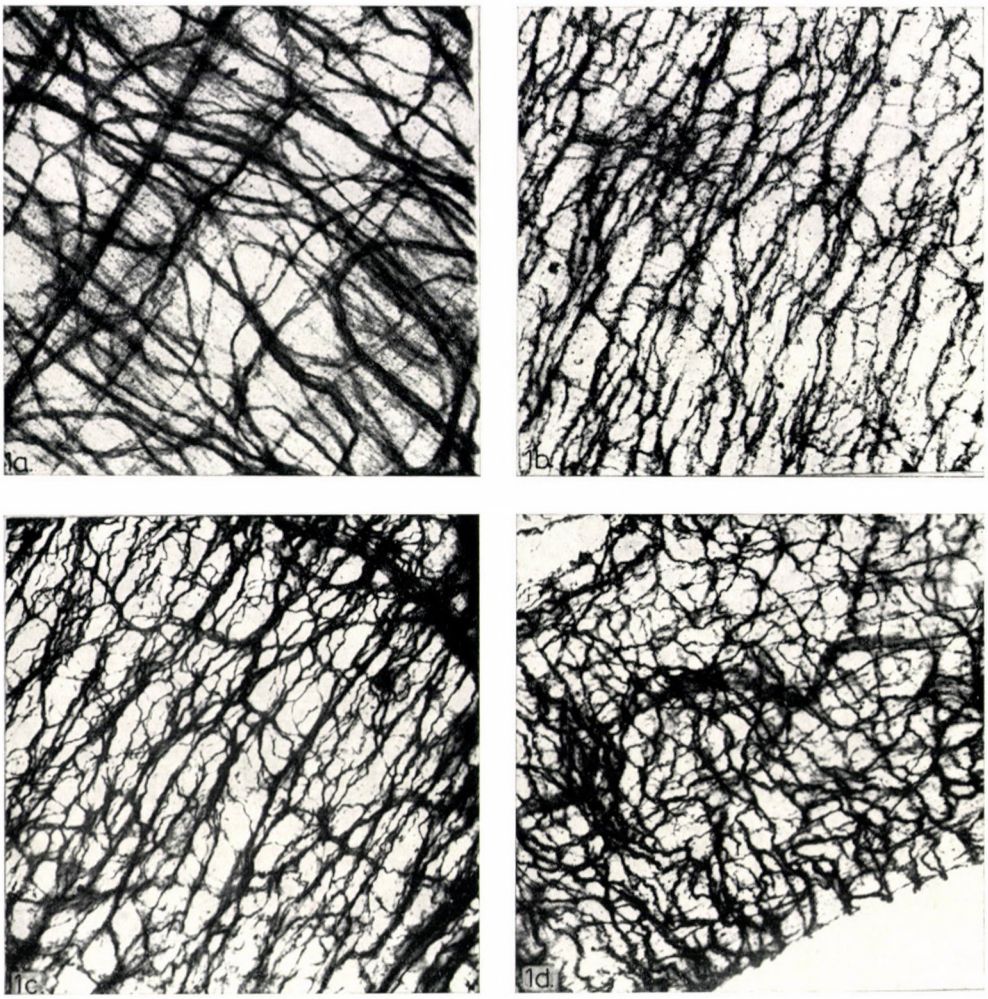

Fig. 1. Cholinergic neural plexuses. Method of Koelle. ×80: a. Middle cerebral artery of the human; b. Middle cerebral artery of the horse; c. Middle cerebral artery of the cow; d. Middle cerebral artery of the dog

In the large vessels of the cerebral base, the density of cholinergic conductors was higher than that of adrenergic fibres. Thus, the density of the two kinds fibre per sq. mm. of the middle artery in the brain of a dog was 74.18 ± 2.34 and 67.64 ± 3.28 respectively ($P < 0.001$) (Figs 1d, 4b). The small arteries in the pia mater of rats, guinea-pigs, rabbits, dogs, cats, swine, cows, horses and humans contained the same number of cholinergic fibres. Thus, for instance, the density of cholinergic and adrenergic conductors per sq. mm. in the pial arteries (diameter 300 μ) of rabbits and guinea-pigs was 54.61 ± 1.04 and 53.45 ± 1.82 and 52.34 ± 2.36 and 55.61 ± 3.24 ($P > 0.5$), respectively.

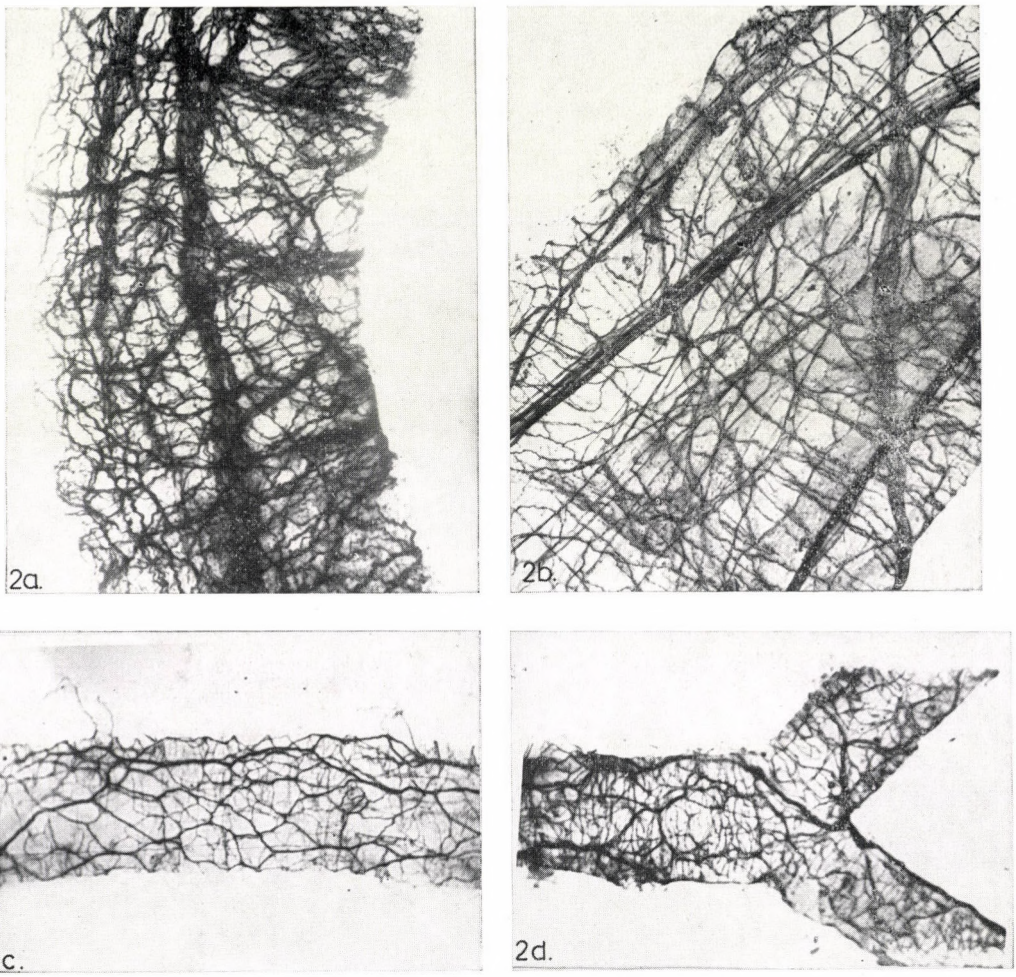

Fig. 2. Cholinergic neural plexuses. Method of Koelle. a, Branch of the middle cerebral artery of the swine. ×80; b. Middle cerebral artery of the cat. ×100; c. Middle cerebral artery of the guinea-pig. ×200; d. Anterior cerebral artery, middle cerebral artery and anterior branch of the rat's internal carotid artery. ×200

In arteries less than 200 μ in diameter transversal fibres predominated among the cholinergic and adrenergic plexuses. In thinner arteries, the density of transversal fibres was less, while in arteries with a lumen of 25—100 μ there were more longitudinal conductors.

The highest density of fibres was found in the intracranial part of the internal sphenoid artery and the first sections of the anterior and middle cerebral arteries, further in cats, in the intracranial branch of the internal maxillary artery.

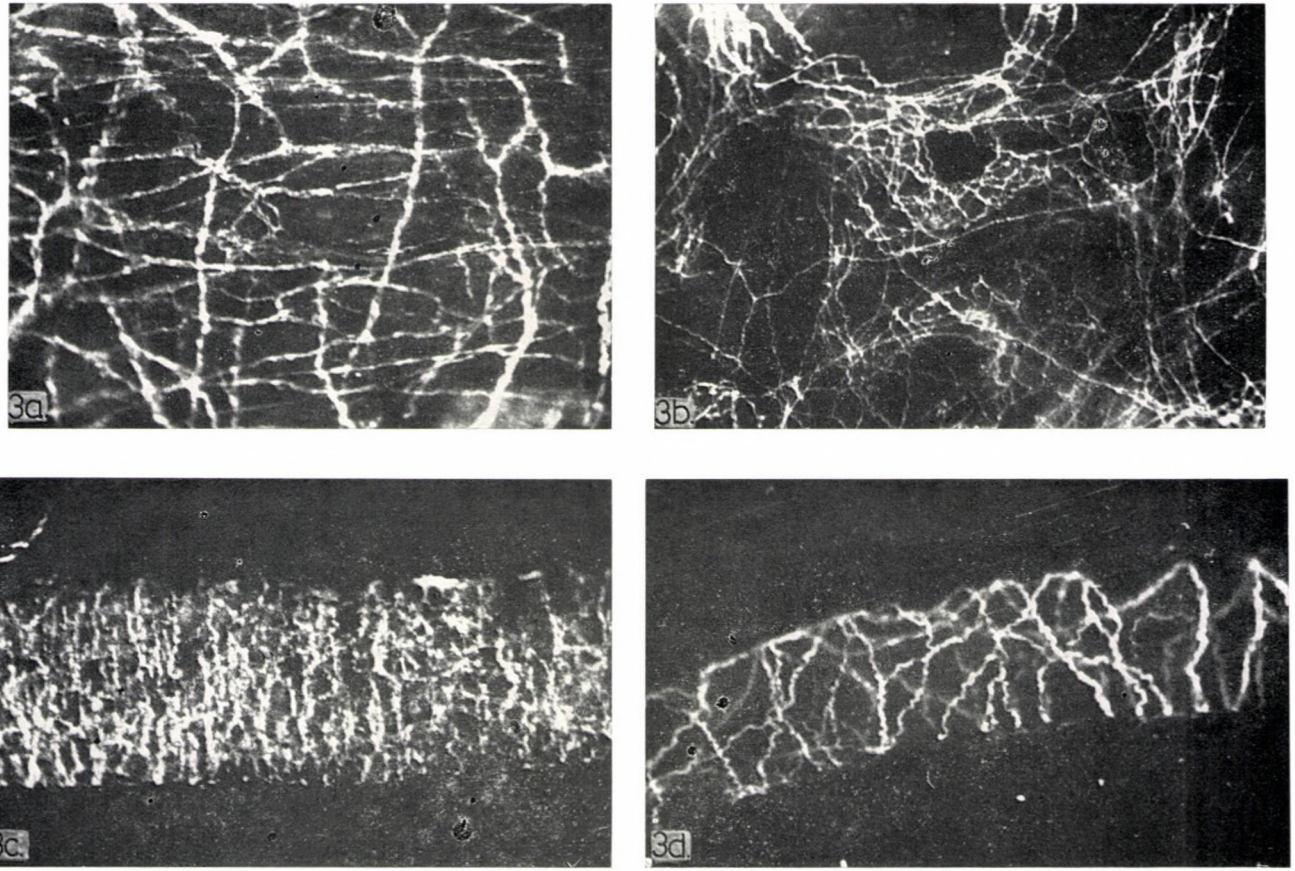

Fig. 3. Adrenergic neural plexuses. Method of Falck-Hillarp, ML-2, a. Middle cerebral artery of the cat. ×200; b. Small arteries in the pia mater of the cat. ×200; c. Middle cerebral artery of the rat. ×150; d. Branch of the guinea-pig's middle cerebral artery. ×200

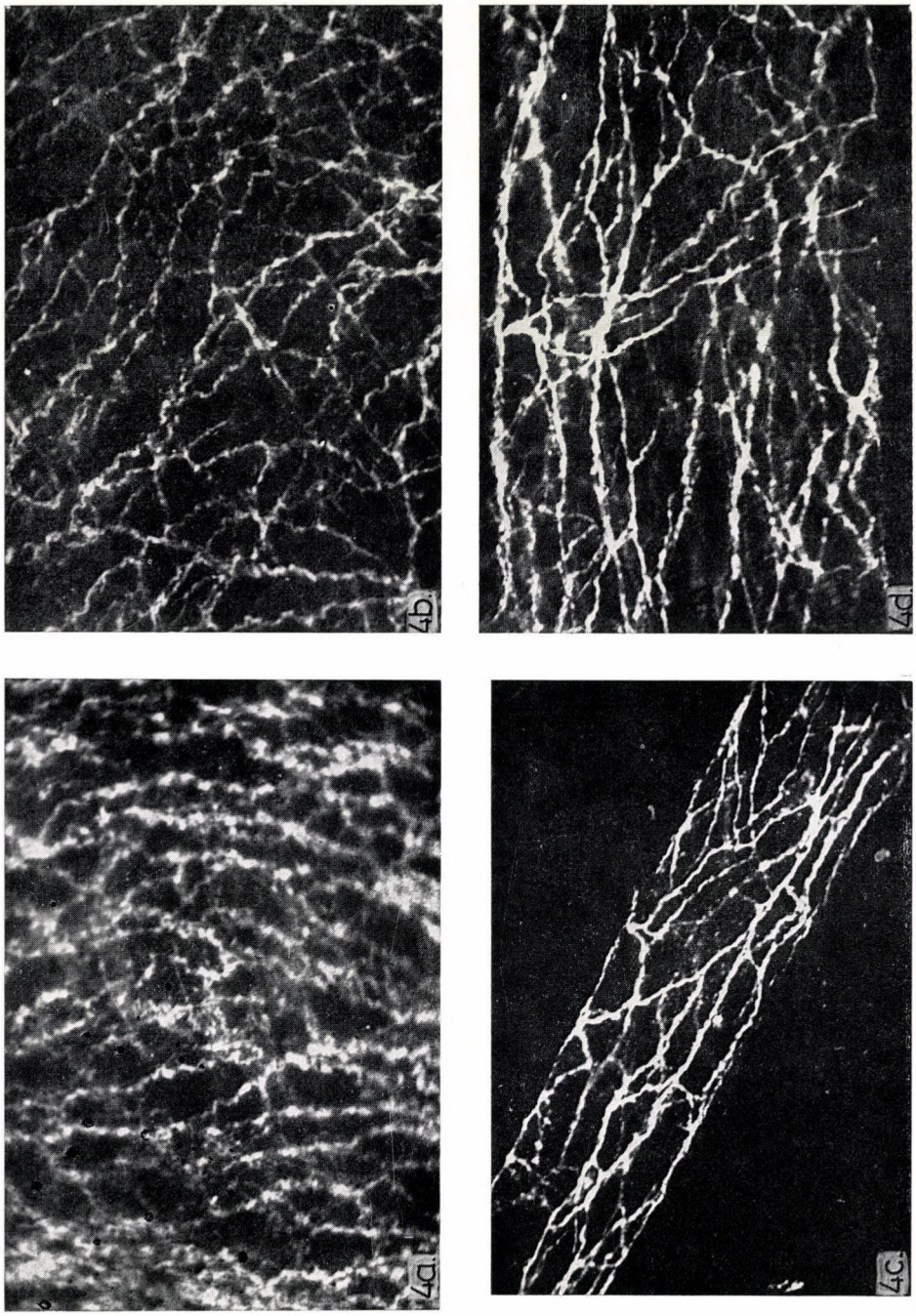

Fig. 4. Adrenergic neural plexuses. Method of Falck-Hillarp, ML-2 a. Middle cerebral artery of the cow. ×200; b. Middle cerebral artery of the dog. ×200; c. Branch of the dog's middle cerebral artery. ×200; d. Middle cerebral artery of the rabbit. ×100

The vessels of the vertebrate system, namely the main spinal, cerebellar and posterior cerebral arteries, displayed a lower density of neural fibres in all the animals. Thus, the densities of cholinergic neural fibres per sq. mm in the cat's middle cerebral artery and the caudal direction were 67.88 ± 1.48 and 52.49 ± 1.73 respectively ($P < 0.001$). A similar regularity was characteristic of the adrenergic fibres, too.

Thus no basic differences were observed in the structure and density of cholinergic and adrenergic plexuses. The present study on the concentrations of neural fibres in cerebral arteries showed especially dense cholinergic and adrenergic neural plexuses to be typical of humans and large mammals. According to the density of cholinergic and adrenergic fibres in the said arteries, the animals may be listed in the following order: rats, guinea-pigs, rabbits, cats, dogs, swine, cows and horses.

REFERENCES

1. Carlyle, A., Grayson, J.: (1956) J. Physiol. (Lond.) 133, 10—30. — 2. Csillik, B., Jancsó, G., Tóth, L., Kozma, M., Kálmán, G., Karcsu, S.: (1971) Acta anat. (Basel) 80, **1**, 142—151. — 3. Donath, T.: (1968) Acta morph. Acad. Sci. hung. **16**, 3, 285—293. — 4. Edvinsson, L., Nielsen, K., Owman, Ch., Sporrong, B.: (1972) Z. Zellforsch. 134, **3**, 311—325. — 5. Falck, B.: (1962) Acta physiol. scand. **56**, 197, 1—25. — 6. Falck, B., Mchedlishvili, G., Owman, Ch.: (1965) Acta pharmacol. (Kbh.) **23**, 133—142. — 7. Falck, B., Nielsen, K., Owman, Ch.: (1968) Scand. J. clin. Lab. Invest. **102**, 96—98. — 8. Harper, A. et al.: (1972) Arch. Neurol. **27**, 1—6. — 9. Kajikawa, H.: (1968) Arch. Jap. Chir. **37**, 473—482. — 10. Kajikawa, H.: (1969) Arch. Jap. Chir. **38**, 227—235. — 11. Kobayashi, S., Rhoton, A., Waltz, A.: (1969) Neurology (Minneap.) **19**, 307—308. — 12. Nielsen, K., Owman, Ch.: (1967) Brain Res. **6**, 773—776. — 13. Nielsen, K., Owman, Ch.: (1971) Brain Res. 27, 33—42. — 14. Ohgushi, N.: (1968) Arch. Jap. Chir. **37**, 294—302. — 15. Peerless, S., Yasargil, M.: (1971) J. Neurosurg. **35**, 148—154. — 16. Politoff, A., Macri, F.: (1966) Int. J. Neuropharmacol. **5**, 155—162. — 17. Rosenblum, W.: (1965) Angiology **16**, 485—507. — 18. Sokoloff, L.: (1959) Pharmacol. Rev. **11**, 1—85. — 19. Spoendlin, H., Lichtensteiger, W.: (1967) Arch. Ohr.-, Nas. - u. Kehlk.-Heilk. **189**, 346—359. — 20. Бородуля А. В., Плечкова Е.К.: (1973) Acta anat. (Basel) **86**, 410—425. — 21. Мотавкин П. А., Довбыш Т. В.: (1970) Бюл. экспер. биол. и мед. **70**, 7, 113—116.—22. Мотавкин П. А., Довбыш Т. В. (1971) Acta morph. Acad. Sci. hung. **19**, 2, 159—173. — 23. Мотавкин П. А., Довбыш Т. В.: (1972) Ж-л невропатологии и психиатрии. 72, **7**, 1007—1011. — 24. Мотавкин П. А., Осипова Л. П.: (1973) Z. mikrosk.-anat. Forsch. (Leipzig) **87**, 3, 365—378. — 25. Мотавкин П. А., Палащенко Л. Д.: (1973) Acta morph. Acad. Sci. hung. **21**, 3, 227—238.

VERGLEICHENDE UNTERSUCHUNG DER EFFEKTORINNERVATION DER ZEREBRALEN ARTERIEN BEI SÄUGETIEREN UND MENSCHEN

P.A. MOTAWKIN, G.S. WLASOW und L.D. PALASCHENKO

Mit Hilfe der Falck-Hillarpschen und Koelleschen Methode wurden die Neurofibrillen in den zerebralen Arterien von Menschen und Säugetieren (Ratten, Meerschweinchen, Kaninchen, Katzen, Hunden, Schweinen, Kühen und Pferden) untersucht. An der Karotis und den grossen Arterien des vertebralen Systems waren dichte cholinerge und adrenerge Plexi aus Quer- und Längsfibrillen in den oberflächlichen und tieferen adventitiellen Schichten zu beobachten. Die stärkste cholinerge und adrenerge Fibrillenkonzentration zeigte sich an den menschlichen sowie den Arterien der grossen Säugetiere. Aufgrund der steigenden Cholinerg- und Adrenerg-Fibrillenkonzentration in den gleichlautenden Arterien der Tiere konnte fol-

gende Reihenfolge aufgestellt werden: Ratten, Meerschweinchen, Kaninchen, Katzen, Hunde, Schweine, Kühe und Pferde. Es zeigte sich kein Unterschied von grundlegendem Charakter in der Struktur der Nervenplexi zwischen dem Menschen und den untersuchten Tieren.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ЭФФЕКТОРНОЙ ИННЕРВАЦИИ АРТЕРИЙ ГОЛОВНОГО МОЗГА МЛЕКОПИТАЮЩИХ И ЧЕЛОВЕКА

П. А., МОТАВКИН, Г. А., ВЛАСОВ и Л. П. ПАЛАЩЕНКО

Изучались нервные волокна артерий головного мозга человека и млекопитающих (крыс, морских свинок, кроликов, кошек, собак, свиней, коров и лощадей) методами Фалька—Хилларпа и Келле. На крупных артериях каротидной и вертебральной систем имеются густые холин- и адренергические сплетения, образованные поперечными и продольными нервными волокнами, расположенными в поверхностных и глубоких слоях адвентиции. Наиболее высокая концентрация холин- и адренергических волокон установлена на артериях человека и крупных млекопитающих. В соответствии с возрастающей концентрацией холин- и адренергических волокон в одноименных артериях животные могут быть расположены в следующий ряд: крысы, морские свинки, кролики, кошки, собаки, свиньи, коровы, лошади. Принципиальных различий в строении нервных сплетений у человека и изчуаемых животных не установлено.

Dr. P. A. Motavkin
Dr. G. S. Vlasov
Dr. L. D. Palashchenko
Medical Institute, 690747 Vladivostok, U.S.S.R.

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Institute of Forensic Medicine, Semmelweis University Medical School, Budapest

EFFECT ON THE HEART MUSCLE OF EXPERIMENTAL CARBON MONOXIDE POISONING

I. Balogh, P. Sótonyi and E. Somogyi

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The electron microscopic technique failed to reveal ultrastructural alterations specific for carbon monoxide poisoning. In this contact, the specificity of the lead salt detection method of calcium determination is discussed.

With the increase of motor vehicle traffic environmental air pollution has become grave [5, 8] and carbon monoxide occurs at highest concentration in the urban atmosphere [11, 19, 32].

The most frequent sources of carbon monoxide poisoning are in addition to the exhaust gases, the industrial and generator gases, the explosion gases developing in mines as well as carbon monoxide liberated during the incomplete combustion of coal and the lighting gas particularly in towns [14, 18, 26, 28, 35].

Of the effects of carbon monoxide, chiefly its action on central nervous system has been studied [14, 17]. Only few data [24, 37] deal with the changes induced in the heart especially at the ultrastructural level.

The present examinations were aimed at studying the effect of acute carbon monoxide poisoning on the heart muscle.

Materials and methods

The examinations were carried out on isolated rat hearts treated by perfusion according to Langendorff. After heparinization and decapitation the hearts were immediately removed and perfused through the coronaries with Krebs-Henseleit bicarbonate buffer solution containing 10 mM glucose. The temperature of the solution was adjusted to 31°C by a thermostat and bubbled through with a mixture of O_2 and CO_2, or CO, N_2 and CO_2. The perfusion solution contained 95% O_2 and 5% CO_2, the CO solution contained 45.10 vol% CO, 4.77 vol% CO_2 and 50.13 vol% N_2. Calcium was added at concentrations of 0; 0.075; 0.1506; 0.325; 0.625; 1.3 and CO perfusions was done for 1, 2, 5, 10, 15, 20 or 30 min.

The heart was fixed in 3% glutaraldehyde solution of 31°C, and specimens were obtained from the middle layer of the left ventricle surrounding the left descending coronary. The material was fixed in 1% OsO_4, dehydrated and embedded in Araldit (Durcupan ACM, Fluka). In addition to the routine electron microscopic technique, for the localization of calcium the ammonium oxalate [9, 25], potassium oxalate and lead acetate methods were applied.

Succinodehydrogenase was estimated according to KERPEL-FRONIUS and HAJÓS [23] unfixed specimens which after dehydration were embedded in Araldit.

Ultrathin sections were prepared with the LKB ultrotom, then examined with a JEM 100B electron microscope at 60 kV acceleration voltage.

Results

The earliest change was a dilatation of the sacrotubular, and T-system. After 20 to 30 min perfusion with CO or with a solution containing calcium below the physiological concentration, the walls of the sacrotubular system were also demaged. The capillaries were dilated, the pericapillary spaces were also extended. The enlarged pinocytotic vesicles referred to increased transport of materials. The pericapillary spaces and surrounding fibres contained oedema fluid. The glycogen content was maintained. In the mitochondria, after 1—2 min perfusion, as one of the earliest changes, one of the cristae became elongated, then stretched across to the opposite side, where a stricture appeared on the mitochondrium and thus practically two daughter-mitochondria developed. At some sites the changes involved only parts, especially the peripheral parts, of the mitochondrium (Plate II; Fig. 3). Subsequently a circular arrangement of the mitochondrial cristate, later their fragmentation, then complete lysis of the inner structure occurred. In the first 5 to 10 min the mitochondrial damage was restricted to the immediate vicinity of the capillaries, then after 30 min also in more remote areas. In the muscle fibres the sarcomeres were contracted and reduced in length after 2 min perfusion, when a disintegration, stretching and breaking up of the Z-disk, also appeared (Plate I; Figs 1, 2). Beside the hypercontracted areas hyperrelaxed parts could also be observed (Plate II; Figs 1, 3, 4). In the intercalated disks the intercellular space was widened. At hypercontraction on both sides of the Z-disks strongly electron scattering material could be observed (Plate II; Fig. 2).

Among the applied calcium detection procedures the lead-acetate assay was applied as a routine method. It gave a positive reaction in the mitochondria and in the dilated sacrotubular systems, some of them with damaged walls (Plate III; Figs 1, 3). At some sites a positive reaction was seen along the Z-disk as well as in the area of hypercontraction (Plate III; Fig. 2). In the heart muscle injured by carbon monoxide and perfused with a physiological amount of calcium the changes develop later than if the perfusing solution contains a low concentration of calcium. If the perfusion is carried out without calcium and CO is subsequently added, the changes appeared as soon as 1—2 min (Plate III; Fig. 4) Otherwise they appeared only after 20 to 30 min. The succinodehydrogenase reaction failed to show any significant change in any of the experimental groups.

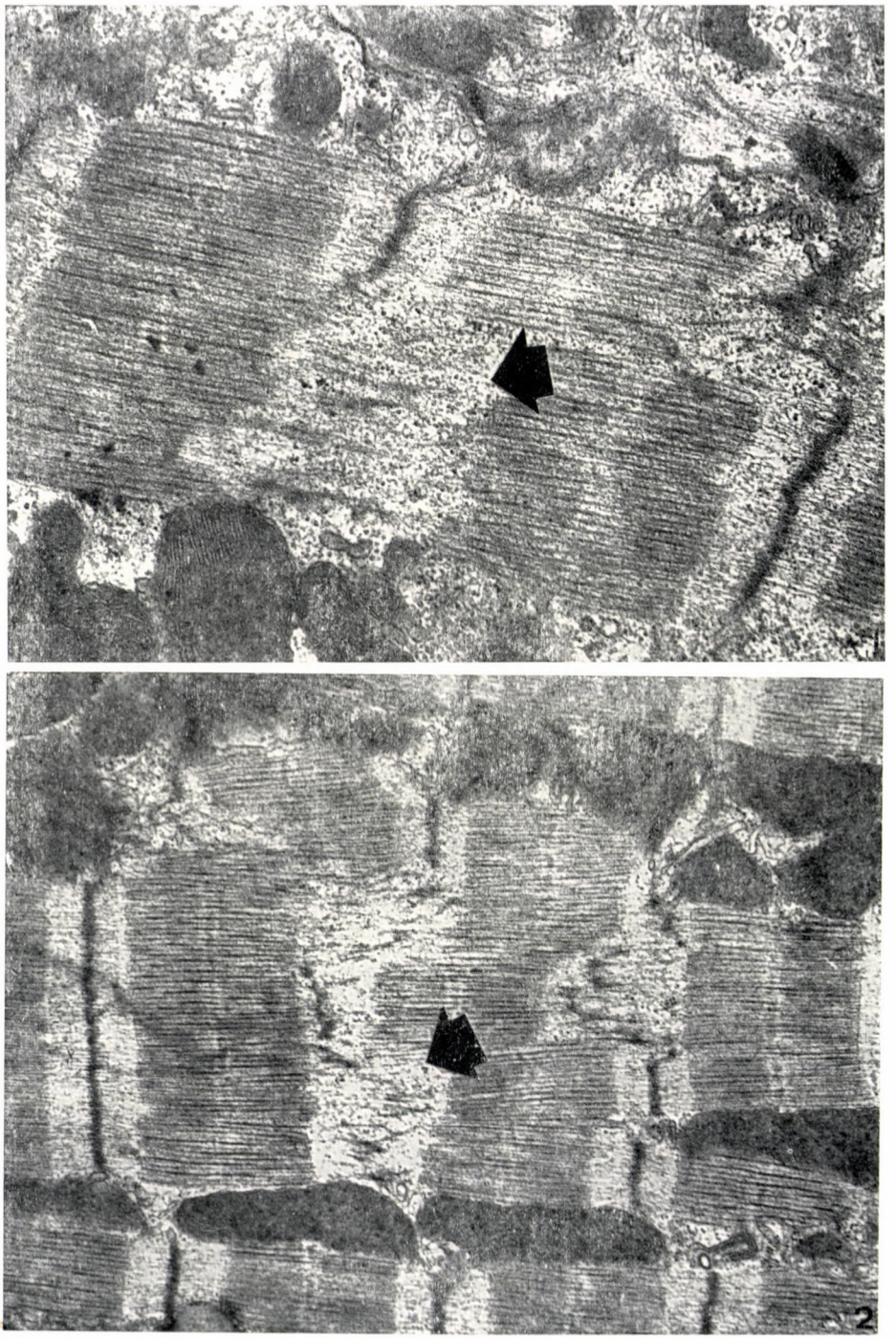

Plate I, Figs 1, 2
Fragmentation and disintegration of Z-membranes (arrows) in CO-poisoned heart muscle. 1—2 = ×36 000

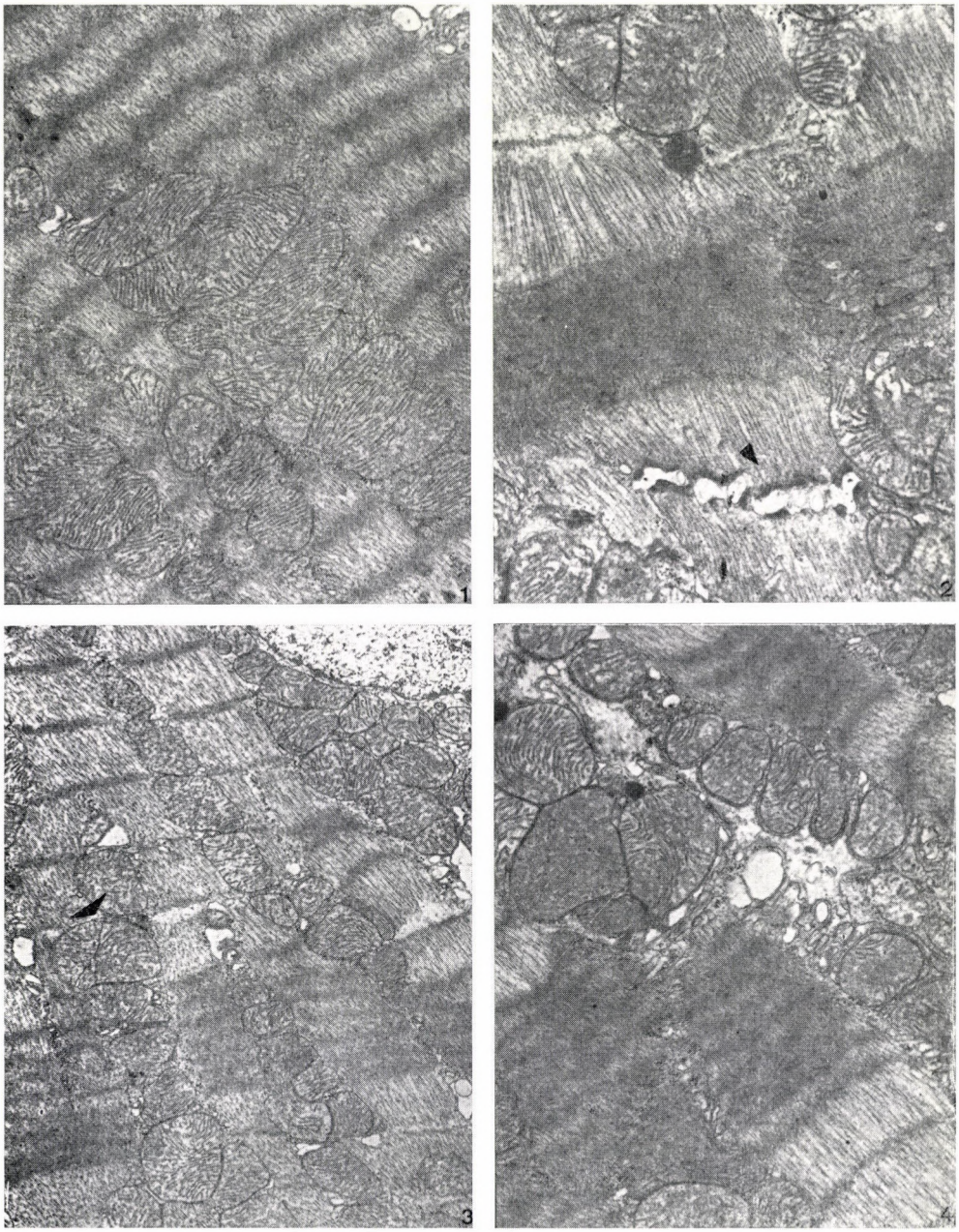

Plate II,

Fig. 1. The sarcomere is shortened. Hypercontracted areas in the heart muscle. ×18 000

Fig. 2. On both sides of the hypercontracted fibres hyperrelaxation and torn fibres may be observed. The intercalated disk is drawn out (arrow). ×12 000

Fig. 3. Hypercontracted fibres and characteristic bridges (arrow) in the mitochondria: ×18 000

Fig. 4. Hypercontracted fibres, incipient tearing of the fibrils. ×18 000

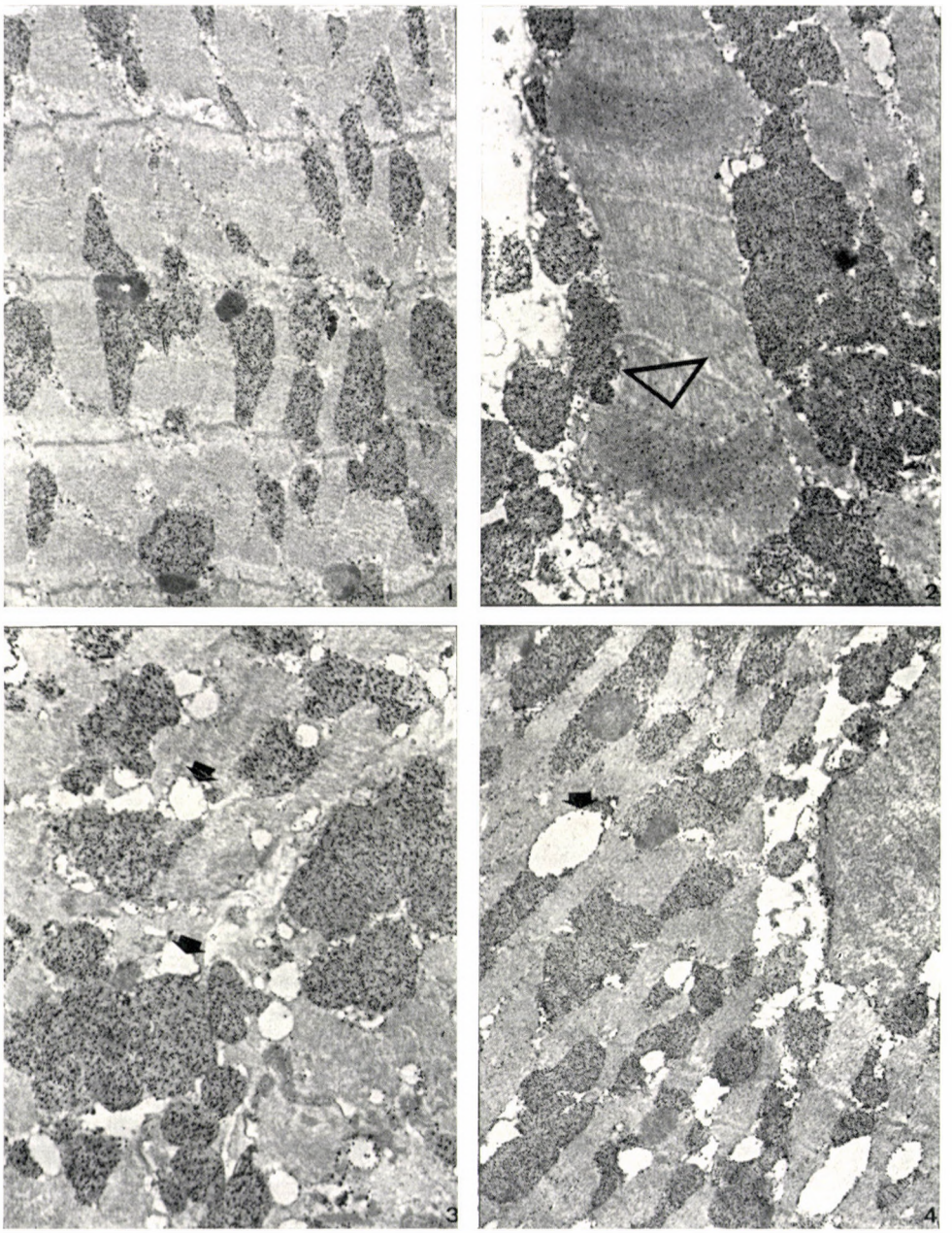

Plate III.

Fig. 1. Electron microscopic histochemical calcium detection in control heart muscle. ×14 400

Fig. 2. Lead precipitate (arrow) in hypercontracted sarcomeres. ×13 440

Fig. 3. CO-poisoned heart muscle. Positive reaction in the walls of the dilated sacrotubular system (arrows) and the mitochondria. ×14 000

Fig. 4. Perfusion in the absence and in the presence of CO. The sacrotubular system is markedly dilated (arrow), its wall is fragmented at numerous sites, the heart muscle is disintegrated in some parts. ×14 000

Discussion

Carbon monoxide responsible for one of the most frequent [28]; it is absorbed by the erythrocytes and bound to haemoglobin. The affinity of haemoglobin to carbon monoxide is 200 fold of its affinity to oxygen. In muscle, carbon monoxide is bound to myoglobin, but the distribution of CO between haemoglobin has not been clarified [1, 10].

Carboxyhaemoglobin shifts the normal oxygen dissociation curve to the left and induces tissular hypoxia. In addition to the concentration of the inhaled gas, its injurious effect is connected with the actual state of tissue metabolism, with respiratory function as well as with the action of the heart [2, 3].

In subjects with anaemia, atherosclerosis or coronary sclerosis, a low carbon monoxide concentration may already induce severe alterations, even in detail [34]. Carbon monoxide inhibits the activity of glucose-6-phosphate dehydrogenase and lactic acid-dehydrogenase; it has no effect on malonic acid dehydrogenase and it reduces the number of lysosomes [7, 39]. For its effect on the heart muscle cells, the tissue hypoxia and the inhibition of ferroprotein (cytochrome, myoglobin) due to carboxyhaemoglobin formation are responsible. The mechanism of cellular ferroprotein inhibition is not quite clear, partly because the cellular effects of carbon monoxide cannot be separated from the effects produced by carboxyhaemoglobin *in vivo* [3, 6, 22].

According to clinical data, after acute CO poisoning changes pointing to heart muscle cell injury may be absent, or appear immediately or several days later [20, 21]. Petechial or diffuse bleedings may develop in the pericardium, endocardium and the papillary muscles. In the myocardium, fatty degeneration or necrosis may develop [16, 21].

The phenomena observed by us, namely the elongation of the crista and the division the mitochondria occur also in other materials, thus in the liver mitochondria of dehydrated rats after rehydration [4] and under the effect of triiodo-thyronine and riboflavin deficiency in heart and liver mitochondria [30, 31]. In the heart muscle intracellular oedema, dilatation of the sarcoplasmic reticulum and mitochondrial changes were observed following the inhalation of 1 vol% CO [37].

Formation of a hypercontracted stripe was observed in potassium deficiency [33], following electric shock [36] and after open heart surgery [38].

For the electronmicroscopic histochemical detection of calcium, several methods have been elaborated [9, 12, 13, 25, 27, 29]. The examinations demonstrated that in the mammalian heart muscle beside the sacroplasmatic reticulum other cellular organelles have also a role [13].

The changes found in the mitochondria developed early; the functionally and partly structurally lost parts seem to be demarcated by a membrane

from the intact regions. In the immediate surroundings of the capillaries, severe mitochondrial changes are developing rapidly. The fragmentation and disintegration of the Z-disk may also be considered as an early effect of hypoxia, but this change appears later than the dilatation of the sacrotubular system.

We have obtained correctly evaluable and experimentally well-reproducible reactions with the lead-acetate method. The lead precipitate pointing to the highest amount of calcium was found corresponding to the mitochondria, and frequently in the walls of the sacrotubular system. The precipitate corresponding to the Z-disk and the hypercontracted area supports only the view that at sites not the amount of calcium but the lead-binding capacity has increased, due probably to local pH changes and the accumulation of lead-reactive groups.

Hence, it is assumed that in carbon monoxide poisoning the calcium turnover is damaged. However to prove this hypothesis the present calcium detection methods are not satisfactorily sensitive or specific.

REFERENCES

1. Astrup, P.: (1972) Some physiological and pathological effects of moderate carbon monoxide exposure. Brit. med. J. **4,** 447. — 2. Anderson, R. F., Allenworth, D. C., Degroot W. J.: (1967) Myocardial toxicity from carbon monoxide poisoning. Ann. intern. Med. **67,** 1172. — 3. Ball, E. G. Strittmatter, F., Cooper, O.: (1951) The reaction of cytochrome oxidase with carbon monoxide. J. biol. Chem. **193,** 635. — 4. Bartók, I., Virágh, Sz., Menyhárt, J.: (1974) Gyors szerkezeti változások szomjazás után rehydrált patkányok májsejtjeiben. Morph. Ig. Orv. Szle. **14,** 81. — 5. Bové, J. L., Sienberg, S.: (1970) Airborne lead and carbon monoxide at 45th street, New York City. Science **167,** 986. — 6. Breckenridge, B.: (1953) Carbon monoxide oxidation by cytochrome oxidase in muscle. Amer. J. Physiol. **173,** 61. — 7. Brenner, G. M., Wenzel, D. G.: (1972) Carbon monoxide and cultured rat heart cells I. Inhibition of cell growth and maintenance of beating rate. Toxicol. Appl. Pharmacol. **23,** 251. — 8. Campbell, H. E.: (1969) Carbon monoxide, smoking in automobiles and in hay lofts. Amer. med. Ass. **207,** 951. — 9. Carasso, N., Favard, P.: (1966) Mise en évidence du calcium dans les myonemes pédonculaires de cilies peritriches. J. Microsc. **5,** 579. — 10. Coburn, R. F., Williams, W. J., Forster, R. E.: (1964) Effect of erythrocyte destruction on carbon monoxide production in man. J. clin. Invest. **43,** 1098. — 11. Colucci, J. M., Begman, C. R.: (1969) Carbon monoxide in Detroit, New York and Los Angeles air. Environ. Sci. Tech. **3,** 41. — 12. Constantin, L. L., Franzini-Armstrong, C., Podolsky, P. J.: (1965) Localization of calcium-accumulating structures in striated muscle fibres. Science **147,** 158. — 13. Diculescu, I., Popescu, L. M., Ionescu, N., Butvescu, N.: (1971) Ultrastructural study of calcium distribution in cardiac muscle cells. Z. Zelforsch. **121,** 181. — 14. Dutra, F. R.: (1952) Cerebral residua of acute carbon monoxide poisoning. Amer. J. clin. Path. **22,** 925. — 15. Dutra, F. R.: (1957) Carbon monoxide poisoning from the exhaust gases of motor vehicles. J. crim. Law **48,** 333. — 16. Ehrich, W. E., Bellet, S., Lewey, F. H.: (1944) Cardiac changes from CO poisoning. Amer. J. med. Sci. **208,** 511. — 17. Gilbert, G. J., Glaser, G. M.: (1959) Neurologic manifestations of chronic carbon monoxide poisoning. New. Engl. J. Med. **261,** 1317. — 18. Goldsmith, J. R., Cohen, S. I.: (1969) Epidemiological bases possible air quality criteria for carbon monoxide. J. Air. Pollut. Contr. Ass. **19,** 704. — 19. Göthe, C. S., Fristedt, B., Sundell, L., Kolmodin, B., Ehrner-Samuel, H., Göthe, K.: (1969) Carbon monoxide hazard in city traffic: an examination of traffic policemen in three Swedish towns. Arch. environ. Hth. **19,** 310. — 20. Hayes, J. M., Hall, G. V.: (1964) The myocardial toxicity of carbon monoxide. Med. J. Aust. **1,** 865. — 21. Jaffe, N.: (1965) Cardiac injury and carbon monoxide poisoning. S. Afr. med. J. **39,** 611. — 22. Kaminsky, L. S.: (1972) Carbon monoxide as a probe for conformation changes of ferrocytochrome C. Biochemistry **11,** 3702. — 23. Kerpel-Fronius, S., Hajós, F.: (1968) The use of ferricyanide for the light and electron microscopic

demonstration of succinic dehydrogenase activity. Histochemie **14,** 343. — 24. KJELDSEN, K., STRUP, P., WANSTRUP, J.: (1972) Ultrastructural intimal changes in the aorta after a moderate carbon monoxide exposure. Atherosclerosis. **16,** 67. — 25. KOMNICK, H.: (1969) Histochemische Calciumlokalisation in der Skelettmuskulatur des Frosches. Histochemistry **18,** 24. — 26. LANDAW, S. A.: (1969) Endogenous production of carbon monoxide: the human body as a cause of air pollution. Ann. intern. Med. **70,** 1275. — 27. LEGATO, M. J., LANGER, G. A.: (1969) The subcellular localization of calcium ion in the mammalian myocardium. J. Cell. Biol. **41,** 401. — 28. MANT, A. K.: (1960) A review of 100 cases of carbon monoxide poisoning. Med. -leg. J. **28,** 31. — 29. MCCALLISTER, L. P., HADEK, R.: (1973) The localization of calcium in skeletal muscle: its distribution in muscles in which the caffeine induced contracture was arrested. J. Ultrastruct. Res. **45,** 59. — 30. REITH, A.: (1973) The influence of triiodothyronine and riboflavin deficiency on the rat liver with special reference to mitochondria. Lab. Invest. **29,** 216. — 31. REITH, A., FUCHS, S.: (1973) The heart muscle of the rat under influence of triiodothyronine and riboflavin deficiency with special reference to mitochondria. A morphologic and morphometric study by electron microscopy. Lab. Invest. **29,** 229. — 32. SHEPHARD, R. J.: (1967) Normal levels of activity in Canadian citydwellers. Canad. med. Ass. J. **97,** 313. — 33. SELYE, H., BAJUSZ, E.: (1959) Sensitization by potassium deficiency for the production myocardial necrosis by stress. Amer. J. Path. **35,** 525. — 34. SIGGAARD-ANDERSEN, J., PETERSEN, F. B., HANSEN, T., MELLEMGAARD, K.: (1969) Vascular permeability and plasma volume changes during hypoxia carbon monoxide exposure. Angiology **20,** 356. — 35. SIMPSON, K.: (1955) Carbon monoxide poisoning: medico legal problems. J. forens. Med. **2,** 5. — 36. SOMOGYI, E., SÓTONYI, P., BUJDOSÓ, GY., NEMES, A.: (1971) Untersuchung von Herzmuskelveränderungen die unter dem Einfluss von Niederspannung-Schrittmacher-Strom (pacemaker) zustande gekommen sind. Z. Rechtsmed. **68,** 180. — 37. SUZUKY, T.: (1969) Effects of carbon monoxide inhalation on the fine structure of the rat heart muscle. Tohoku, J. exp. Med. **97,** 197. — 38. VARGA, T., SZINAY, GY., SOOS, J., KÖVES, I.: (1973) Heart muscle changes after open heart surgery. Acta. morph. Acad. Sci. hung. **21.,** 213. — 39. WENZEL, D. G., BRENNER, G. M.: (1973) Carbon monoxide and cultured rat heart cells. II. Interaction of carbon monoxide and hypoxia on growth and contractile activity. Toxicol. Pharmacol. **24,** 256.

UNTERSUCHUNG DER AUSWIRKUNGEN DER EXPERIMENTELLEN KOHLENMONOXYDVERGIFTUNG AN DER HERZMUSKULATUR

I. BALOGH, P. SÓTONYI und E. SOMOGYI

Mit Hilfe der Elektronenmikroskoptechnik der Verfasser war es nicht möglich ein ultrastrukturelle Veränderung festzustellen, die bei der Kohlenmonoxydvergiftung eine spezifischen Charakter aufweisen würde. Unter anderem behandeln die Autoren das Spezifikum der Kalziumdarstellung mit Bleisalzen.

ИССЛЕДОВАНИЕ ВЛИЯНИЯ ЭКСПЕРИМЕНТАЛЬНОЙ ИНТОКСИКАЦИИ ОКИСИ УГЛЕРОДА НА СЕРДЕЧНУЮ МЫШЦУ

И. БАЛОГ, П, ШОТОНЬИ и Э. ШОМОДЬИ

Специфические ультраструктурные изменения интоксикации окиси углерода не наблюдались применением электронно-микроскопической техники авторов. Исследовалось методики для демонстрации кальции с помощью свинцовой соли.

Dr. István BALOGH
Dr. Péter SÓTONYI
Dr. Endre SOMOGYI

Semmelweis Orvostudományi Egyetem,
Igazságügyi Orvostani Intézete,
1450 Budapest, Pf. 9. Hungary

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Central Municipal Hospital for Infectious Diseases of Budapest, National Institute of Hygiene, Budapest, Hungary

INTRANUCLEAR VIRUS-LIKE PARTICLES IN HB_sAG- AND IH_xAG-NEGATIVE ACUTE HEPATITIS (TYPE C?)

(PRELIMINARY REPORT)

G. KENDREY, I. HOLLÓS and B. LÁSZLÓ

(Received September 20, 1975)

Key-words

Virus-like particles, acute hepatitis, hepatitis type C.

Intranuclear virus-like particles were found by electron microscopy in liver cells of a woman suffering from mild HB_sAg- and IH_xAg-negative acute hepatitis. The particles encountered were morphologically different from those found in hepatitis B and hepatitis A respectively. Further studies are required to clarify whether the structures represent an incidental finding of a new human (passenger) virus or they may be related to the aetiological agent of the supposed hepatitis type C.

The discovery of Australia (i.e. HB_sAg) antigen [2] with three distinct types of particle in the serum using negative staining [1], electron microscopic demonstration of intranuclear virus-like particles (i.e. virus-cores) in liver cells of HB_sAg-positive patients [12], furthermore visualization of virus-like particles in faecal extracts of patients during the icteric phase of hepatitis A [4, 18], have given new impulse to hepatitis research [17]. There are serological tests for the differential diagnosis of hepatitis A and B, i.e. by complement fixation and reverse passive hemagglutination methods [6, 7].

Recent data imply that a substantial proportion of post-transfusion (i.e. inoculation type) hepatitis cases is caused neither by HB-virus nor by the agent of hepatitis A and suggest the existence of an additional virus(es), hepatitis type C [13].

Case report

A 24-year-old woman developed jaundice 2 months after delivery of a healthy child and at the end of the first month after taking an oral contraceptive. Clinical features and laboratory tests suggested hepatocellular injury and cholestasis. Repeated search for HB_sAg, anti-HB_s and IH-antigen during the acute icteric phase of the disease gave negative results. A liver biopsy 9 days after the onset of jaundice showed typical mild cholestatic hepatitis.

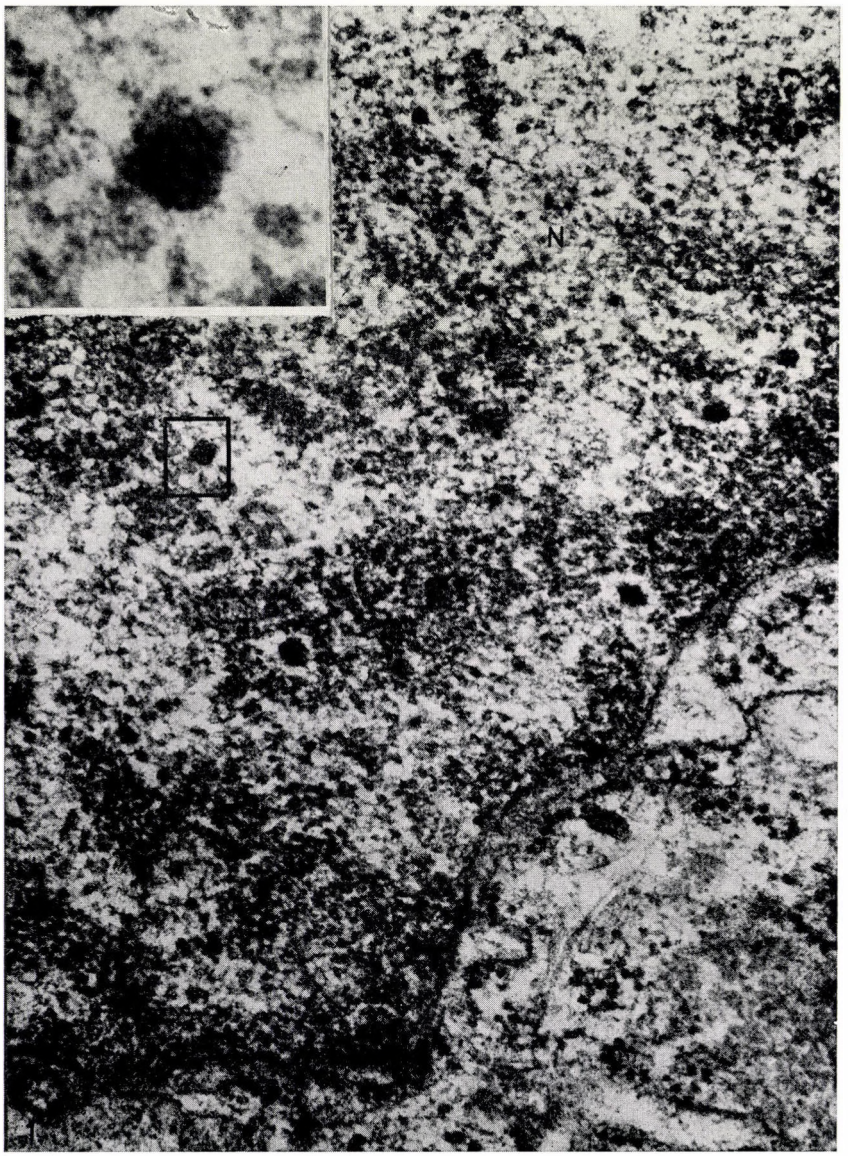

Fig. 1. Portion of liver cell nucleus (N) with round and oval osmiophilic particles, some of them surrounded by a clear halo ×81 000
Inset: A higher magnification of the virus-like particle outlined in Fig. 1. with tubular inner structure ×375 000
(Osmium fixation, uranyl acetate and lead citrate, JEM 6CM electron microscope)

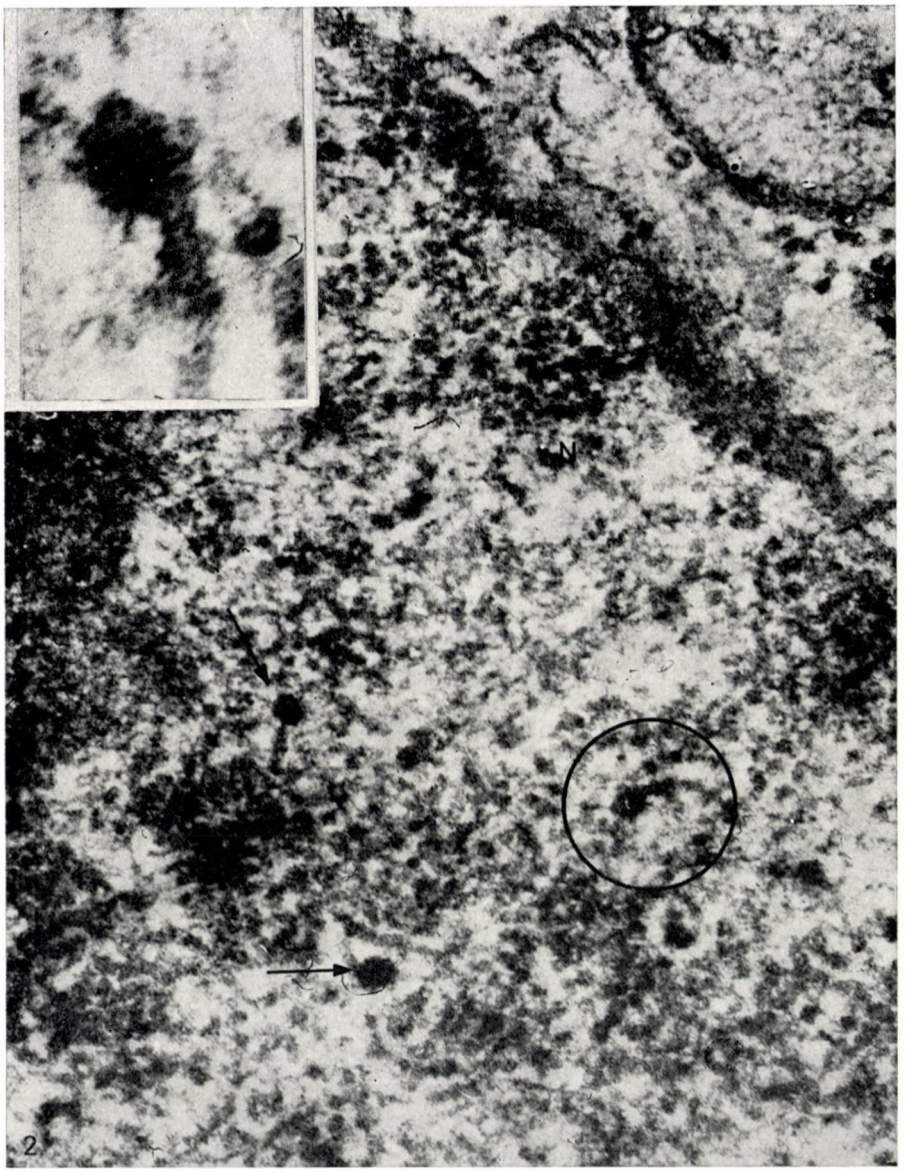

Fig. 2. Part of hepatic cell nucleus (N). Some particles have a tale-like structure (circle and arrows). ×102 000
Inset: Tubular inner structure and "tale" of the virus-like particle encircled in Fig. 2. ×378 000
(Osmium-fixation, uranyl-acetate and lead citrate, JEM 6CM electron microscope)

In ultra-thin sections many hepatocytes contained intranuclear, round or oval, highly osmiophilic particles of 30—40 nm in diameter, some of them surrounded by electron lucent halo (Fig. 1). At higher magnification a tubular inner structure could be demonstrated within the particles (Fig. 1. inset). In a small number of liver cell nuclei there were similar particles which had an osmiophilic, 28—37 nm, tale-like structure (Fig. 2). The latter appeared granular and had a denser outer layer (Fig. 2. inset).

The particles encountered had a virus-like morphology and were different from those found in liver cell nuclei of HB_sAg-positive patients either with acute or chronic liver disease [5, 8—11, 15], or in HBAg-carriers with or without liver involvement [3, 14, 16]. They had no morphological similarity to virus-like particles seen in faecal extracts of patients with hepatitis A [4, 18].

The exact nature and significance of these particles are not known. We would speculate that the structures we now describe represent an incidental finding of a new human (passenger?) virus or they may be related to the aetiological agent of hepatitis C.

REFERENCES

1. Almeida, J. D., Zuckerman, A. J., Taylor, P. E., Waterson, A. P.: (1969) Immune-electron microscopy of the Australia-SH (serumhepatitis) antigen. Microbios **1,** 117. — 2. Blumberg, B. S., Alter, H. J., Visnich, S. F.: (1965) A "new" antigen in leukaemia sera. JAMA **191,** 541. — 3. Caramia, F., De Bac C., Ricci, G.: (1972) Virus-like particles within hepatocytes of Australia antigen carriers. Amer. J. Dis. Child. **123.** 309. — 4. Feinstone, S. M., Kapikian, A. Z., Purcell, R. H.: (1973) Hepatitis A; detection by immune electron microscopy of a virus-like antigen associated with acute illness. Science **182,** 1026. — 5. Gudat, F., Bianchi, L., Sonnabend, W., Thiel, G., Aenishaenslin, W., Stalder, G. A.: (1975) Pattern of core and surface expression in liver tissue reflects state of specific immune response in hepatitis B. Lab. Invest. **32,** 1. — 6. Hilleman, M.: (1974) IABS Symposium on viral hepatitis. Milan. December 16—19. — 7. Hollós, I., László, B., Pálfy, Á., Szécsey, Gy., Novák, E.: Acta Microbiol. Acad. Sci. Hung. In press. — 8. Huang, S. N. (1971) Hepatitis-associated antigen hepatitis. An electron microscopic study of virus-like particles in liver cells. Amer. J. Path. **64,** 483. — 9. Huang, S. N., Groh, V.: (1973) Immunoagglutination electron microscopic study on virus-like particles and Australia antigen in liver tissue. Lab. Invest. **29.** 353. — 10. Kendrey, G., Szalka, A., László, B., Binder, L., Hollós, I.: (1975) Immunosuppressive therapy and virus-cores in HBAg-positive chronic hepatitis. Klin. Wschr.: **53,** 1081. — 11. Krawczynski, K., Nazarevicz, T., Brzosko, W. J., Nowoslawski, A.: (1972) Cellular localization of hepatitis-associated antigen in livers of patients with different forms of hepatitis. J. Infect. Dis. **126.** 372. — 12. Nowoslawski, A., Brzosko, W. J., Madalinski, K., Krawczynski, K.: (1970) Cellular localisation of Australia antigen in the liver of patients with lymphoproliferative disorders. Lancet **I,** 494. — 13. Prince, A. M., Grady, G. F., Hazzi, Ch., Brotman, B., Kuhns, W. J., Levine, R. W., Millian, S. J. : (1974) Long-incubation post-transfusion hepatitis without serological evidence of exposure to hepatitis B-virus. Lancet **II,** 241. — 14. Ricci, G., De Bac, C., Caramia, F.: (1972) Liver disease in Australia-antigen carriers. Lancet **I,** 494. — 15. Scotto, J. M., Homberg, J. Cl., Caroli, J.: (1972) Electron microscopy studies of severe virus hepatitis. Amer. J. Dis. Child. **123,** 311. — 16. Tapp, E., Jones, D. M., Anfield, C., Whittaker, J. S., Woolf, I. L., Dymock, I. W.: (1974) Intranuclear particles in hepatocytes of HBAg-positive blood-donors. Lancet **I,** 934. — 17. Virus hepatitis updated (Editorial) (1975) Lancet **I,** 1365. — 18. Zuckerman, A. J., Bird, R. G., Darnell, R., Farrow, L. J., Stewart, J. S., Lamb, S. G.: (1974) Hepatitis A virus-like particles. Brit. med. J. **1,** 453.

VIRUSARTIGE KÖRPERCHEN IN DEN KERNEN VON LEBERZELLEN BEI HB_sAg- UND IH_xAG-NEGATIVER AKUTER HEPATITIS (TYP C?)

G. KENDREY, I. HOLLÓS und L. BARNABÁS

In den Zellkernen der Leber einer an leichter HB_sAg- und IH_xAg-negativer Hepatitis leidenden Frau wurden bei der elektronenmikroskopischen Untersuchung virusartige Körperchen gefunden. Diese Partikelchen waren von den bei A- bzw. B-Virus-Hepatitis nachgewiesenen Körperchen morphologisch abweichend. Zur Klärung der Frage, ob die obigen Gebilde als Zufallsbefund zu betrachten sind und ein neues humanes (passenger?) Virus oder den Erreger der angenommenen Hepatitis-C darstellen, sind weitere Untersuchungen erforderlich.

ВИРУСОПОДОБНЫЕ ТЕЛЬЦА В ЯДРАХ ПЕЧЕНОЧНЫХ КЛЕТОК ПРИ HB_sAG- И IH_xAG-ОТРИЦАТЕЛЬНОМ ОСТРОМ ГЕПАТИТЕ (ТИП С?)

Г. КЕНДРЕИ, И.ХОЛЛОШ и Б. ЛАСЛО

В клеточных ядрах печени женщины, страдающей легкой формой HB_sAG- и IH_xAg-отрицательного гепатита, при электронномикроскопическом исследовании были обнаружены вирусоподобные тельца. Эти частицы морфологически отличались от телец, выявленных при вирус-А- или вирус-В-гепатите. Для уяснения вопроса о том, следует-ли рассматривать вышеуказанные вирусоподобные образования как случайную находку и являются-ли они новым человеческим (passenger?) вирусом или возбудителем предположенного гепатита С, необходимы еще дальнейшие исследования.

Dr. Gábor KENDREY
Dr. Barnabás LÁSZLÓ
Fővárosi László Kórház,
1097 Budapest, Gyáli út 5/7., Hungary

Dr. Iván HOLLÓS,
Országos Közegészségügyi Intézet,
1966 Budapest, Gyáli út 2—6., Hungary

RECENSIONES

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The RNA tumour viruses are able to induce tumours in animals. This class of viruses has several such biological, biochemical and structural properties, which distinguish it from the other RNA viruses.

Concerning their fine structure, these viruses may be classified into B and C type particles. Both have a RNA genome, which is replicated by an intermediary DNA.

Among the RNA tumour viruses the C-type has been studied most thoroughly. These viruses induce leukaemia, sarcoma and occasionally some other kind of tumour in numerous animal species. Under laboratory conditions viruses induce *in vitro* cellular transformation.

Normal cells — at least in some animal species — obviously possess such complete genetical information which is suited for building up C-type particles.

The function *in vitro* of this genetic material of the somatic cells is unknown; it seems to play a role in the development of "spontaneous" tumours.

The first paper published in the number at issue discusses the RNA and DNA components of the genetic material of the C-type viruses. It proposes a model, which may help to study the replication and interaction of the virus with chromosomes.

Some new experimental data are reported, which are in contrast with the former concept concerning the ultrastructure of C-type virus particles, the connection between virus and animal cells, the classification, cellular and molecular biology, and genetics, of RNA tumour viruses.

The first publication (RNA and DNA forms of the genetic material of C-type viruses and the integrated state of the DNA form in the cellular chromosome, by M. HILL and JANA HILLOVA) supplies the reader also with the necessary information by the hypothesis is offered to explain the replication cycle of the RNA tumour viruses, with the intermediary help of DNA. The interrelation of malignity is brought into connection with an evolutionary process or with the endogenous depression of the transferred genetic material coding the C-type viruses.

The second paper (Viruses as immunological adjuvants in cancer, by J. LIDENMANN) deals with tumour immunology. The homogenate of tumour cells infected with viruses is immunologically more effective than a similar extract of non-infected cells. This phenomenon has been studied chiefly with non-specific tumours and influenza viruses, but it was demonstrated using specific tumour filtrates and other viruses too. The hypothesis of a carrier — hapten type connection also is put forward; it may come into existence at various levels of virus development. Viruses, that seem suited in man for "virus-assisted immunotherapy" were adapted to human tissues. Further human studies are recommended.

The third paper (Transport of sugars in tumour cell membrane, by M. HATANAKA) discusses the transport of sugars across normal and tumour cell membranes. The previously observed differences, e. g. aerobic glycolysis of tumour cells, were explained by the rate of proliferation rather than by malignity. According to recent studies based on the connection of cellular density and proportion of glycolysis the changes of the rate of glycolysis are, however, not caused by proliferation, but by changes in the transformation of normal cells. The hypothesis is supported by a considerable number of experiments and cellular biological and biochemical examinations. Finally the fourth paper (Antigens of murine leukemia viruses, by F, LILLY and

R. STEEVES) discusses the knowledge concerning proteins with antigenic properties isolated from murine leukemia viruses. A genetical, biochemical and immunological survey is given with numerous references.

SZ. OTTÓ

H. JELLINEK: *Arterial Lesions and Arteriosclerosis.* Akadémiai Kiadó, Publishing House of the Hungarian Academy of Sciences, Budapest 1974. 331 pages, with 245 black and white and 188 coloured microphotograms, 3 figures, 2 tables

This book summarizes the results of recent researches on both the pathogenesis and the pathology of arterial changes and arteriosclerosis, based on the studies of a team of the Second Institute of Pathological Anatomy of Semmelweis University Medical School, Budapest. The main part of the book is written by Professor H. JELLINEK; in the authorship of the chapters his present and former collaborators, had an outstanding role.

Structurally, the book is divided into a preface, 11 chapters and an appendix. In the preface the Editor reports that intensive research performed during about 15 years on the vascular diseases occurring in man or induced in animal experiments allowed the conclusion that any vascular lesion is essentially identical and the advanced lesions remind in each case of the arteriosclerotic changes.

These studies support convincingly the similar conception of Gerő et. al. as well as of Hauss' school concerning the pathogenesis of arteriosclerosis. These authors regard as the decisive factor in the evolution of this polyaetiological disease the "nonspecific" stereotypic changes in the composition of the basic substance of vascular connective tissue, induced by various stress effects or the so-called "nonspecific" mesenchymal reaction.

The first chapter discusses the ultrastructure of the arterial wall of mammals, analysing in detail the structure of the intimal, medial and adventitial layers in the aorta, the large and small vessels as well as in the capillaries. References are supplied concerning the significant cellular elements detected here, the endothelial cells, smooth muscle cells and the fibroblasts.

The second chapter discusses on the basis of the authors' own studies the aortic reaction to various effects, which consists in a proliferation of the intima. Here are discussed both the development and the structure of the regenerative intimal proliferation, its morphogenesis, the origin of cellular components, the endothelial and smooth muscle cells, and the role of the smooth muscle cells in fibrillogenesis, further the intimal proliferation in vascular crafts as well as the vascular changes developing in experimentally produced hypertension.

The third chapter demonstrates the pathogenesis and ultrastructure of the arteriosclerotic changes, the fourth chapter presents observations of the large arteries of muscles. Chapter 5 discusses the morphological changes induced in the small arteries and arterioles by experimental hypertension, topical application of acid, noradrenaline treatment and hypersensitivity reaction elicited by horse serum. It is demonstrated that the morphological changes are similar in each case and differ from the lesions observed in the large vessels only in quantity of elastic elements.

Chapter 6 summarizes experimental observations on the development of subendothelial fibrinoid and on histochemical and enzymological changes in the connective tissue.

Chapter 7 demonstrates the chronic changes developing in the small vessels subsequent to simple injury or after an acute hypertensive crisis, further in benign hypertension, hypoxia and following the subsidence of elicited experimentally acute changes.

Chapter 8 discusses the therapy of changes in the small vessels, developing in experimental hypertension. In Chapter 9, a summary is given of the conclusions drawn from the experimental data.

Chapter 10 discusses the transport across the vascular wall, further the factors responsible for permeability, intramural diffusion of various plasma components, such as the proteins, lipides, lipoproteins, and ions, the role of vasoactive amines and hormones, and the effect of hypoxia and pH as well as hypertension, on transport mechanism. Special emphasis is laid on the role of permeability in arteriosclerosis.

Chapter 11 gives a survey of the experimental results, together with their critical evaluation.

The Appendix contains some points important for the investigator interested in the topic, e.g. a description of the methods of fixation and staining; a list of the publications of the author and his collaborators as well as a detailed list of more than thousand references, an author and a subject index. Finally, a collection of microphotographs assembled with utmost care is added, containing 245 black and white and 188 coloured plates.

The high level of the documentation material has to be emphasized. The first part of the figures on 60 pages consists of electronmicroscopic pictures. Their contrasting is excellent, the cutting is clear. It is regrettable that the light microscopic pictures are condensed on 8 pages; in spite of their excellent quality they are too small for demonstrating details.

The profound minuteness of detail well-proportioned and didactic in the work will fulfil the requirements of ever interested research worker. The bibliography meets the requirements of a source of references, concerning the aetiology, pathogenesis and pathology, of arteriosclerosis.

S. Gerő

INDEX

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Institute of Anatomy, Histology and Embryology, University Medical School, Debrecen, Hungary

STUDIES ON CARTILAGE FORMATION XX. HISTOCHEMICAL INVESTIGATION OF SOME ENZYMES OF GLYCOGEN METABOLISM IN REGENERATIVE ARTICULAR SURFACES

Cs. Hadházy, T. Glant, B. Mándi, S. Harmati, L. Bordán, and K. Balogh

(Received February 28, 1975)

In 28 dogs the distal articular cartilage of the femur was removed and the regenerating articular surface on the 70th postoperative day was studied histochemically for hexokinase, glucose-6-phosphatase, phosphohexose-isomerase, fructose-1, 6-diphosphatase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase, lactate dehydrogenase isoenzymes, phosphoglucomutase, phosphorylase, glycogen synthetase, UDP — glucose dehydrogenase, and UDP-glucuronic acid-4-epimerase.

The articular surface consisted of fibrous tissue and of cartilage islets. The latter contained cells differentiating into cartilage and young chondrocytes. The glycolytic enzymes reacted positively in the regenerative articular surface. Enzyme activities were higher in the cells (particularly the chondroblasts and young chondrocytes) of the cartilage islets than in the connective tissue. In the cells differentiations into cartilage, beside the LDH isoenzymes characteristic of glycolysis, a significant LDH_1 and LDH_2 activity was observed. At the same site the presence of fructose-1, 6-diphosphatase-activity could be assumed, but there was no glucose-6-phosphatase activity. Glycogen synthesis proceeded in the cells of the cartilage islets and UDP-glucuronic acid-4-epimerase activity was observed in the differentiated cells. UDP-glucose dehydrogenase activity was positive in every section of the articular surface.

Introduction

The glycolytic enzymes of cartilage and granulation tissue have been studied in detail by means of chemical methods. Among others Delbrück [6], Silberger et al. [31, 32], Kuhlman and McNamee [21, 22], Marcus and Srivastava [24] studied the cartilage and Woessner and Boucek [39, 40], and Hartman and Walpurger [17] the granulation tissue.

Probably because of technical reasons the study of cartilage differentiation has been somewhat neglected. We have studied the glycolytic enzymes of chondrification under regenerative conditions [14, 15, 27, 28], while histochemical studies have been performed by Balogh and Hajek [3], Raekallio et al. [29], Takada [33] and Wolfe and Cohen [41]. As a continuation of earlier studies [16] in the present paper we wish to report on results of enzyme histochemical tests in regenerating cartilage.

Material and method

In 28 dogs pretreated with morhine-atropine and anaesthesized with Thialbarbital the left knee joint was exposed and the distal articular cartilage of the femur removed together with a few mm of the spongiosa. Next, a new articular surface was formed from the spongiosa and the incision was closed (for details, see 13).

After 70 days the animals were killed and 3 × 5 mm specimens were obtained various parts of the regenerating articular surface by incisions penetrating into the spongiosa. The osseous part was thinned, the specimens were frozen in dry ice and 10 μm cryostat sectiones were prepared without fixation and decalcination. Of the glycolytic enzymes, hexokinase (MEIJER, [25]), glucose-6-phophatase (FOUQUET, WEGMANN, [8]), phosphohexose-isomerase (MEIJER, BLOEM [26]), fructose-1,6-diphosphatase (VERNE, WEGMANN [35]), aldolase (ABE, SHIMIZU [1]), glyceraldehyde-3-phosphate dehydrogenase (HIMMELOCK, KARNOVSKY [18]), lactate dehydrogenase (WEGMANN, SOTELO [37]), lactate dehydrogenase isoenzymes (GEREBTZOFF [9]) were studied and of other enzymes participating in glycogen metabolism, phosphoglucomutase (YANO [42]), phosphorylase (GOLDBERG et al. [10]), glycogen synthetase (WEGMANN et al. [38]), UDP-glucose dehydrogenase (BALOGH jr., COHEN [2]), UDP-glucuronic acid-4 (5)-epimerase (DICULESCO, ONICESKO [7]).

The problems of specificity necessitated the detection of non-specific phosphomonoesterases (acid and alkaline phosphatase). These were detected partly by the PB- i.e. by the Ca-Co method (GÖMÖRI [12]) and partly by the azo-dyes. Acid phosphatase (BURSTONE [5]) was detected with napthol As-Bi-Na-phosphate, 1.04. mM; "Edelrotamin ITR", 5 mM; in 50 mM acetate buffer pH 5.2; and alkaline phosphatase (BARKA, ANDERSON [4]) with naphthol As-Bi-Na-phosphate, 1.04 mM; Fast blue salt: 1 mM 50 mM veronal acetate buffer pH 9.2.

Each enzyme was tested in material from 4 dogs, all with positive and negative controls. The simultaneously processed liver of positive control dogs was treated like the material under investigation. The articular surfaces used as positive controls were incubated without substrate. In the reactions involving glucose-6 phosphatase and fructose-1,6-diphosphatase 10 mM NaF was used as inhibitor. After incubation the slides were rinsed and fixed in neutral formaldehyde diluted 1 : 10 and after repeated rinsing covered with Aquamount (Michrome, E. Gurr, London).

Results

The operated joint was bleeding and a haematoma and later granulation tissue was formed on the bone wound. Subsequently the granulation tissue became rich in fibres and after about a month small cartilage islets appeared in the vicinity of the spongiosa. After 70 days the regenerating cartilage surface consisted of connective tissue and of larger merging cartilage islets (Fig. 1). The connective tissue was poorly vascularized and there were fibroblasts between its fibres. Starting from the edge in the direction of the spongiosa, the cartilage islets consisted of cartilage precursors in various stages of maturity ("environmental fibroblasts", prechondroblasts, chondroblasts) and of young chondrocytes for details see references [13, 16].

Results are given in Table I where the estimated degree of positivity of the various reactions is marked from + to ++++; — stands for a negative reaction and ± for uncertain positivity.

The glycolytic enzymes reacted positively in the cells of the regenerating articular surface. Hexokinase (Figs 2, 3) and aldolase (Figs 6, 7) activities were substantially, phosphohexose-isomerase (Figs 4, 5) activity slightly lower in the connective tissue than in the cartilage islets. Glyceraldehyde-3-phosphate

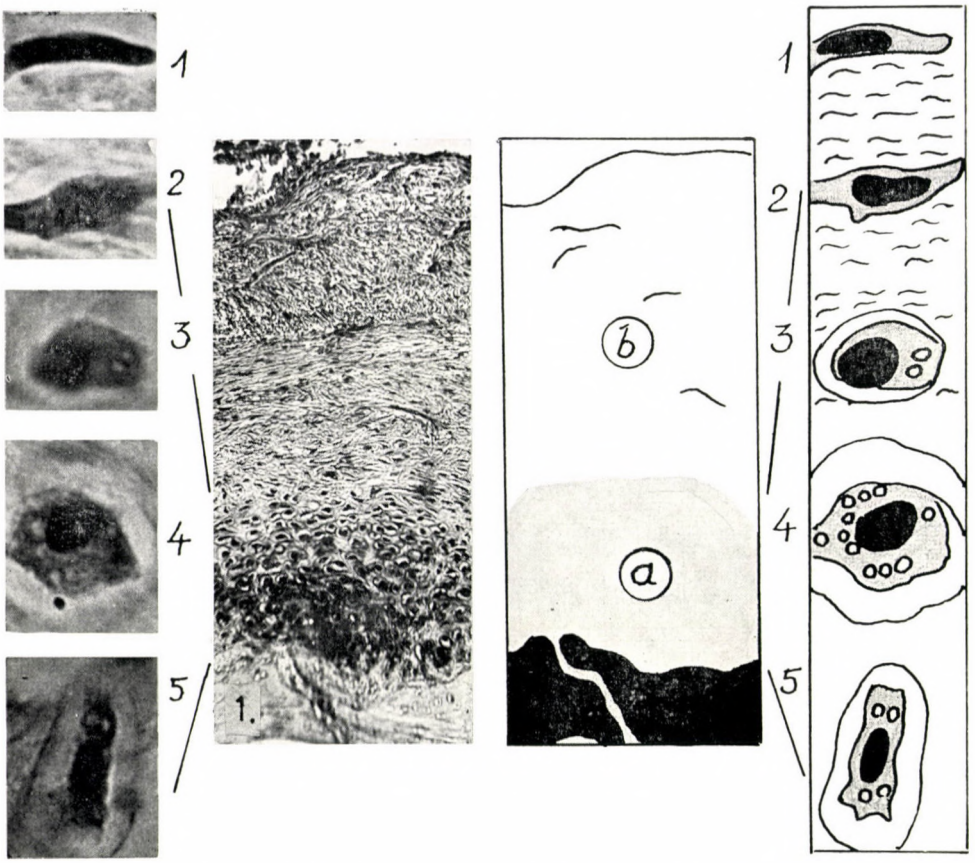
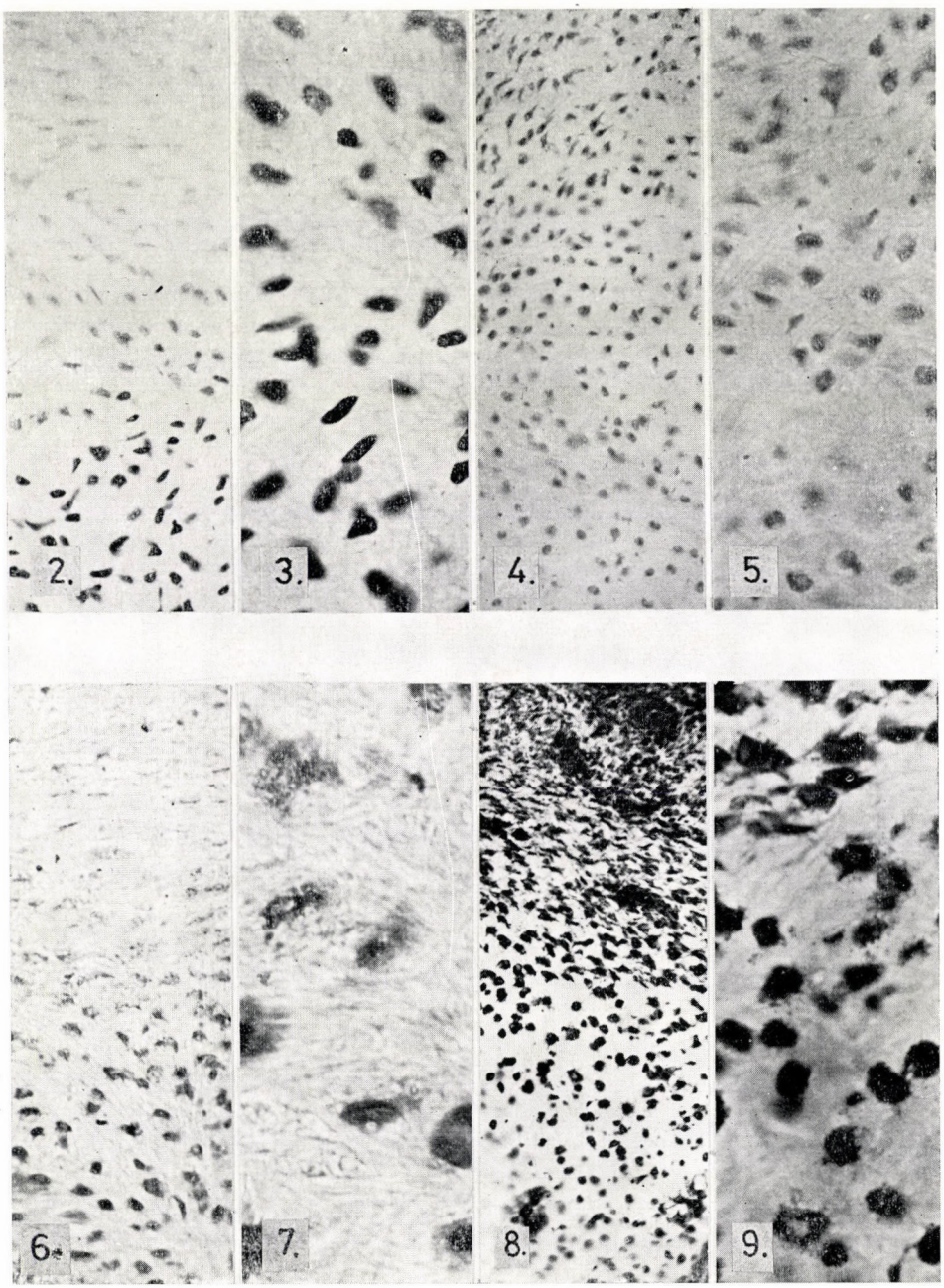
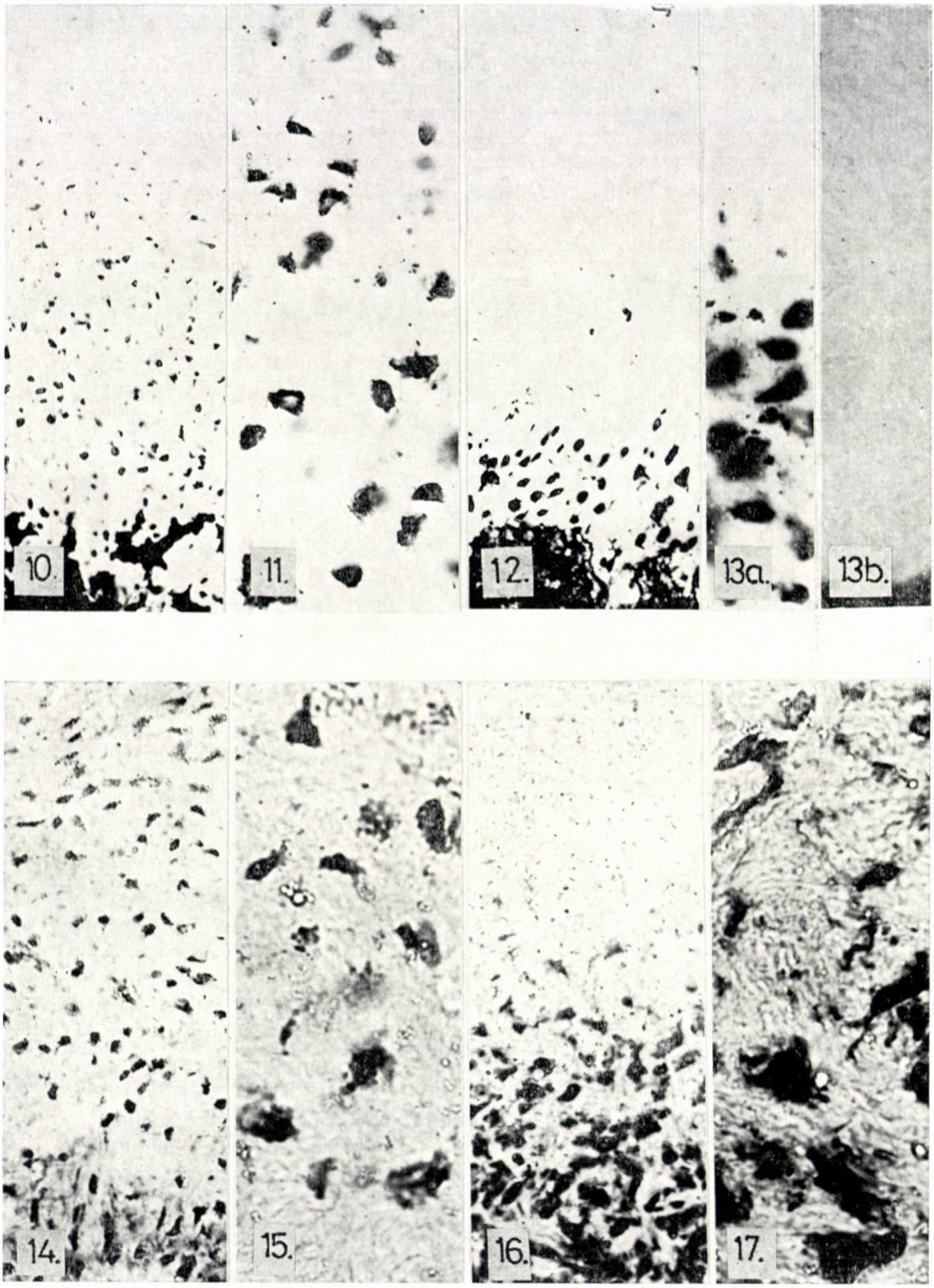


Fig. 1. 70 day old regenerating articular surface. Cartilage island on spongiosa trabecule (a). The rest of the articular surface consists of connective tissue containing constricted vessels (b). In the connective tissue fibroblasts, (1), in the cartilage islets environmental fibroblasts (2), prechondroblasts (3), chondroblasts (4) and young chondrocytes. Haematoxylin-eosin, ×95

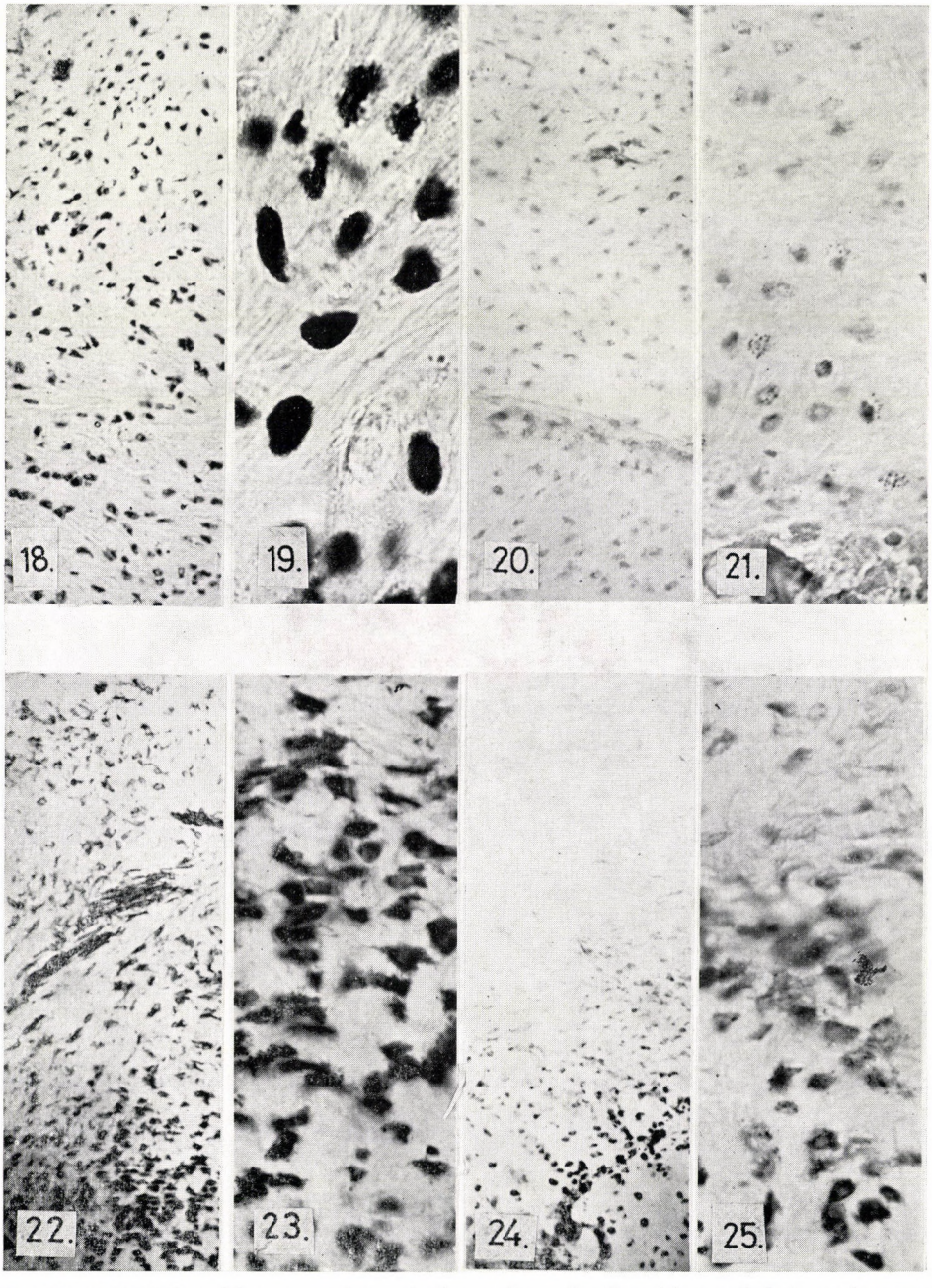
dehydrogenase (Figs 8, 9) and lactate dehydrogenase (Figs 18, 19) showed maximum positivity in all cell types. On the entire articular surface the activity of lactate dehydrogenase M-isoenzymes (LDH_5 and LDH_4) was dominating (Figs 22, 23). Activities of the H-isoenzymes (LDH_1, LDH_2), particularly in the connective tissue regions were weaker (Figs 24, 25). Positivity of glucose-6-phosphatase (Figs 10, 11) and fructose-1,6-diphosphatase (Figs 12, 13) were limited to the cartilage islets. The latter enzyme was detected only in chondroblasts and young chondrocytes. Evaluation of the results was made difficult by the simultaneous activity of non-specific phosphomonoesterase. With glucose-6-phosphatase acid phosphatase, with fructose-1,6-diphosphatase (Figs 14, 15) alkaline phosphatase is likely to interfer (Figs 16, 17). Sodium fluoride inhibits both these reactions (Fig. 13/b).



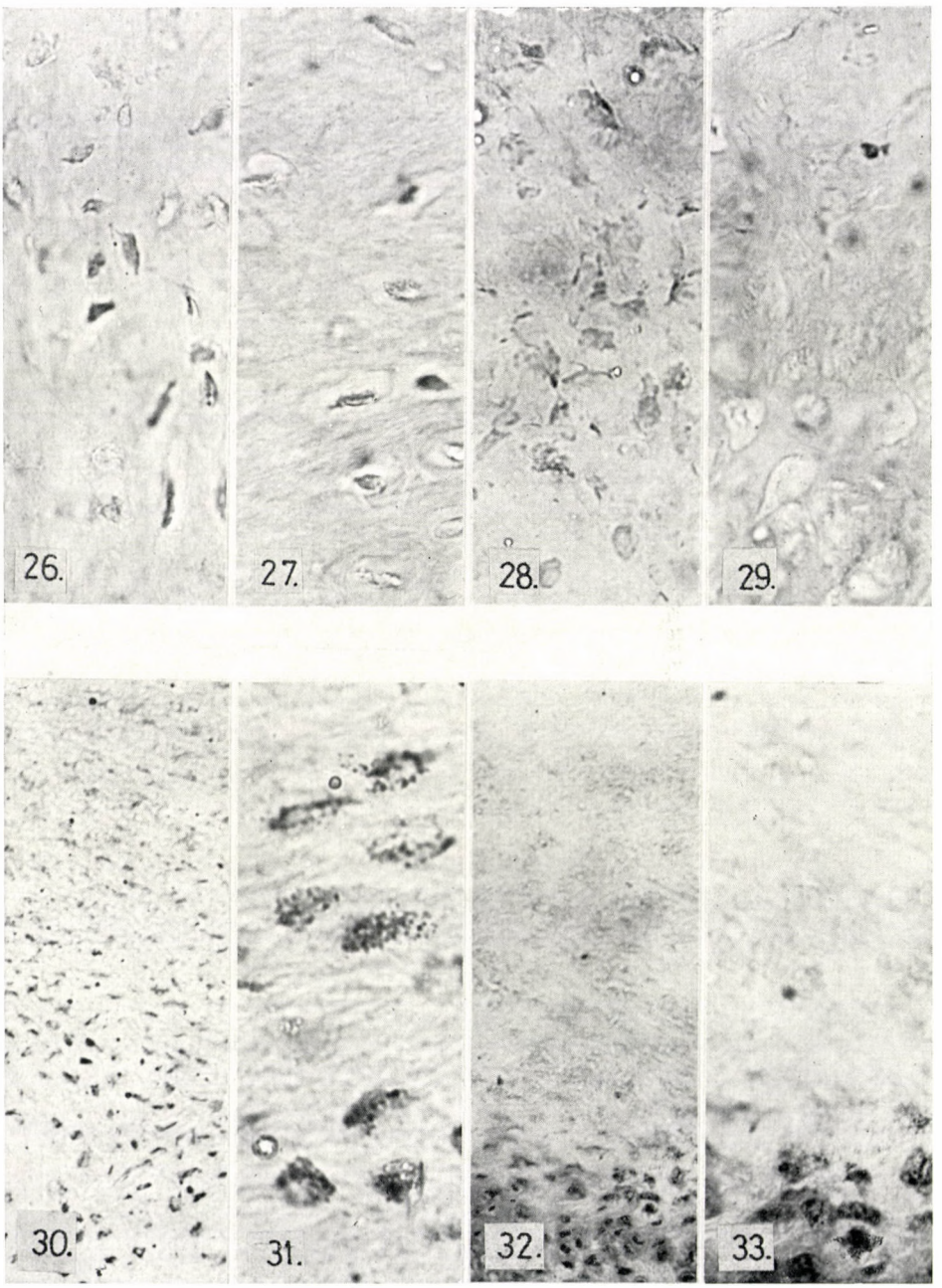
Figs 2 to 9: 70 day old regenerating articular surface. Under low power the cartilage islets and their environment, under higher power cells of cartilage islets are visible. Hexokinase (Figs 2, 3; × 110 and ×300, respectively) phosphonexose-isomerase (Figs 4 and 5; ×80 and ×250, respestively), aldolase (Figs 6 and 7; ×100 and ×340, respectively), glyceraldehyde-3-phosphate-dehydrogenase (Figs 8 and 9; ×100 and × 300, respectively) reactions



Figs 10 to 17: 70 day old regenerating articular surface. Under low power cartilage islets and their environment, under high power cells of cartilage islets are visible. Glucose-6-phosphatase (Figs 10 and 11; × 80 and × 300 respectively), fructose-1,6-diphosphatase (Figs 12 and 13/a; × 100 and ×300 fructose-1,6-diphosphatase in case of inhibition by NaF (Fig. 13/b, ×300), acid phosphatase (Figs 14 and 15; ×100 and ×340 respectively) and alkaline phosphatase (Figs 16 and 17; ×110 and ×340 respectively) reactions.



Figs 18 to 25: 70 day old regenerating articular surface. Cartilage islets and their environment are shown under low power, cells of cartilage islets under high power. Lactate dehydrogenase (Figs 18 and 19; ×100 and ×300, respectively), lactate dehydrogenase M-isoenzymes (Figs 22 and 23; ×80 and ×250 respectively), lactate dehydrogenase H-isoenzymes Figs 24 and 25; ×80 and ×250, respectively), phosphoglucomutase (Figs 20 and 21; ×100 and ×250, respectively), reactions.



Figs 26 to 33: 70 day old regenerative articular surface. Cartilage islands and their environment under low power, cells of cartilage islets under high power. Phosphorylase (Fig. 26, ×340), phosphorylase control (Fig 27, ×340), glycogen synthetase (Fig. 28, ×340), glycogen synthetase control (Fig. 29, ×340), UDPG dehydrogenase (Figs 30 and 31, ×100 and ×340, respectively), UDP glucuronic acid-4-(5)-epimerase (Figs 32 and 33; ×110 and ×280, respectively), reactions.

The other enzymes displayed a practically identical behaviour. Phosphoglucomutase activity was the same connective tissue and the cartilage islets (Figs 20, 21). The same was the case with UDP-glucose dehydrogenase (Figs 30, 31). UDP-glucuronic acid-4 (5)-epimerase activity was restricted to the chondroblasts and the young chondrocytes (Figs 32, 33). Phosphorylase (Figs 26, 27) was practically lacking while glycogen synthetase activity was present in the cartilage islets. (Figs 28, 29).

Discussion

Due to the lack of appropriate methods, earlier histochemical tests in the regenerating cartilage were limited to the detection of lactate dehydrogenase [3, 29, 33, 41] and glyceraldehyde-3-phosphate dehydrogenase [3]. An intensive lactate dehydrogenase activity was observed in the cells of the granulation tissue [29] and an even more intensive one in the differentiating cartilage where the activity of glyceraldehyde-3-phosphate dehydrogenase was also marked [3, 41]. With the use of up-to-date methods we have found in all cell types of the regenerating articular surface hexokinase, phosphohexose-isomerase, glyceraldehyde-3-phosphate dehydrogenase, aldolase and lactate dehydrogenase activity. Their differences reflect the activity conditions revealed by chemical methods [6]. The observed maximum lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase positivity did not allow to conclude to local differences, while the uneven distribution of the positivity of hexokinase, aldolase and, to a certain degree, also of phosphohexose-isomerase seems to indicate a more intensive glycolysis in the differentiating and young chondrocytes than in the connective tissue.

In the cartilage precursors, in agreement with other authors [3, 29, 33, 41] we too [16] observed the activity of enzymes of the citrate cycle, terminal oxidation and pentose-phosphate shunt the findings concerning LDH-isoenzymes offered some information about the intensity ratio of the glycolytic and the above aerobic processes. The H-isoenzymes LDH_1 and LDH_2, the dominating isoenzymes of tissues which obtain their energy from oxidative processes, while the M-isoenzymes LDH_5 and LDH_4 are characteristic of tissues with a glycolytic metabolism [34, 36]. On the basis of these observations, in the connective tissue regions of the regenerating articular surface glycolysis predominates, while in the cartilage islets anaerobic and aerobic glucose degradation occurs simultaneously.

The specificity of the glucose-6-phosphatase and fructose-1,6-diphosphatase reaction is a difficult problem because of the presence of non-specific phosphomonoesterases. Fructose-1, 6-diphosphatase was investigated at pH 8.6 when the results might be influenced by the alkaline phosphatase activity.

This latter is not, or only very slightly inhibited by NaF [30] and differs considerably from fructose-1, 6-diphosphatase both with respect to liability to inhibition and substrate specificity (the second hydrolyzes fructose-1,6-diphosphate and fructose-1-phosphate) [20]. Since NaF inhibits the reaction, it seems likely that fructose-1,6-diphosphatase is present in the cells of the cartilage islets. Glucose-6-phosphatase was estimated at pH 6.7, thus an interference by acid and perhaps even by alkaline phosphatases might have occurred. Inhibition by NaF practically excludes the possibility of alkaline phosphatase activity, but offers no means for a differentation between acid phosphatase and glucose-6-phosphatase, both enzymes being sensitive to NaF [19]. Thus, the probability of a glucose-6-phosphatase activity was supported only by the pH unfavourable to acid phosphatase [11] and by a somewhat different localization (glucose-6-phosphatase does not hydrolyze Na-beta-glycerophosphate [23]), but this difference was not well-defined. It must be added that the problem could not be decided by chemical tests either.

Certain enzymes of the glycogen metabolism of cartilage differentation were investigated by WOLFE and COHEN [41] under similar conditions. These authors found a slight phosphorylase and glycogen synthetase activity in the cartilage precursors. We have obtained similar results in that the activity ratio of the above enzymes indicated a preference for glycogen synthesis in cartilage precursors.

UDP-glucose dehydrogenase is known to catalyze the formation of UDP-glucuronic acid, while UDP-glucuronic acid-4-epimerase catalyzes the UDP-glucuronic acid $\rightleftarrows$ UDP-iduric acid conversion. The latter are the uronic acid components of glycosaminoglycanes. According to our results, UDP-glucuronic acid is formed in the entire area of the regenerating articular surface, while UDP-iduric acid mainly in the cartilage islets. (For detailed data on the UDP-glucose dehydrogenase activity of the regenerating articular surface, see reference [28].)

REFERENCES

1. ABE, T., SHIMIZU, N.: (1964) Histochemical method for demonstrating aldolase. Histochemie **4,** 209. — 2. BALOGH, K. jr., COHEN, R. B.: (1961) Histochemical localization of uridine diphosphoglucose dehydrogenase in cartilage. Nature (Lond.) **192,** 1199. — 3. BALOGH, K. jr., HAJEK, J. V.: (1965) Oxidative enzymes of intermediary metabolism in healing bone fractures. A histochemical study. Amer. J. Anat. **116,** 429. — 4. BARKA, T., ANDERSON, P. J.: (1962) Histochemical methods for acid phosphatase using hexasonium pararosanilin as coupler. J. Histochem. Cytochem. **10,** 741. — 5. BURNSTONE, M. S.: (1958) Histochemical demonstration of acidic phosphatases with naphthol AS phosphates. J. nat. Cancer Inst. **21,** 523. — 6. DELBRÜCK, A.: (1970) Enzyme activity determinations in bone and cartilage. Enzym. biol. clin. **11,** 130. — 7. DICULESCO, I., ONICESCO, D.: (1965) La démonstration histochimique des épimerases de l'acide uridine diphospho-D-glucuronique. Histochimie **5,** 450. — 8. FOUQUET, J. P., WEGMANN, R.: (1964) Le métabolisme du fructose séminal. Étude histoenzymologique chez le rat normal, castré et injecté á la testostérone. Ann. Histochim. **9,** 115—123. — 9. GEREBTZOFF, M. A.: (1968) Contribution histochimique á l'étude de la lactate déshydrogénase et de ses isoenzymes. Path. et Biol. **16,** 601—608. — 10. GOLDBERG, B., WADE, O. R., JONES, H. W. jr.: (1952) Polysaccharide synthesis in frozen tissue sections as

a histochemical method for phosphorylase. J. nat. Cancer Inst. **13,** 543—577. — 11. GOODLAD, G. A. J., MILLS, G. T.: (1957) The acid phosphatases of rat liver. Biochem. J. **66,** 346—354. — 12. GOMORI, G.: Microscopic histochemistry. Principles and Practice. The University of Chicago Press, Chicago 1953. — 13. HADHÁZY, Cs., OLÁH, É.: (1958) Untersuchungen über die Knorpelbildung. I. Problemstellung und allgemeine histologische Untersuchungen unter Neodifferenzierungs-Verhältnissen. Acta biol. Acad. Sci. hung. **9,** 139—158. — 14. HADHÁZY, Cs., OLÁH, É.: (1967) Studies on cartilage formation. X. Phosphoglucose isomerase activity of the chondrifying articular surface in various stages of regeneration. Acta biol. Acad. Sci. hung. **18,** 47—51. — 15. HADHÁZY, Cs., OLÁH, É., KISS, É.: (1971) Studies on cartilage formation. XIII. Phosphofructokinase activity of regenerating articular cartilage. Acta biol. Acad. Sci. hung. **22,** 43—52. — 16. HADHÁZY, Cs., GLANT, T., MÁNDI, B., TÓTH, Z., VERECKEI, L., BOROSS, A.: (1973) Studies on cartilage formation. XV. Enzyme histochemical investigation of Krebs cycle, terminal oxidation and hexose monophosphate shunt. Acta morph. Acad. Sci. hung. **21,** 13—25. — 17. HARTMANN, F., WALPURGER, G.: (1967) Der Energiestoffwechsl im Carrageenin-Granulom. Ein Beitrag zur Biochemie der Entzündung. II. Der Gehalt an Enzymen. Z. Rheumaforsch. **26,** 12—20. — 18. HIMMELOCK, S. R., KARNOVSKY, M. J.: (1961) The histochemical demonstration of glyceraldehyde-3-phosphate DH activity. J. biophys. biochem. Cytol. **9,** 573—581. — 19. HOFFMANN-OSTENHOFF, O., EHRENREICH, R.: Phosphatester-hydrolasen (Phosphoesterasen). In: Hoppe-Seyler, Thierfelder: Handbuch der physiologisch- and pathologisch-chemischen Analyse. X. Aufl. Bd. VI. B. Lang, K., Lehnartz, E., Hoffmann-Ostenhoff, O., Siebert, G. (eds.) 10th ed. Vol 6B. Springer Verlag, Berlin—Heidelberg—New York 1966. — 20. HOLLMANN, S.: Non-glycolytic pathways of metabolism of glucose. Academic Press, New York 1964. — 21. KUHLMAN, R. E.: (1960) A microchemical study of the developing epiphyseal plate. J. Bone Jt Surg. **42 A,** 457—466. — 22. KUHLMAN, R. E., MCNAMEE, M. J.: (1970) The biochemical importance of the hypertrophic cartilage cell area to enchondral bone formation. J. Bone Jt Surg. **52 A,** 1025—1032. — 23. LANGDON, R. G., WEAKLEY, D. R.: (1957) Preparation and some properties of soluble glucose-6-phosphate. Fed. Proc. **16,** 208. — 24. MARCUS, R. E., SRIVASTAVA, V. M. L.: (1973) Effect of low oxygen tension on glucose-metabolising enzymes in cultured articular chondrocytes. Proc. Soc. exp. Biol. (N. Y.) **143,** 488—491. — 25. MEIJER, A. E. F. H.: (1967) Histochemical method for the demonstration of the activity of hexokinase and glucokinase. Acta histochem. (Jena) **28,** 286—290. — 26. MEIJER, A. E. F. H., BLOEM, J. H.: Histochemical method for the demonstration of the activity of glucosephosphate isomerase. Acta histochem. (Jena) **32,** 110—116. — 27. OLÁH, É., HADHÁZY, Cs., ANDRÁSSY, K.: (1966) Studies on cartilage formation. XI. Phosphoglucomutase and glucose-6-phosphatase activities of articular surface in various stages of regeneration. Acta biol. Acad. Sci. hung. **19,** 65—74. — 28. PRANDINI, B., OLÁH, É., HADHÁZY, Cs.: (1971) Studies on cartilage formation. XIV. Uridine diphosphoglucose dehydrogenase activity of the regenerating articular cartilage. Acta biol. Acad. Sci. hung. **22,** 59—66. — 29. RAEKALLIO, J., KOVÁCS, M., MAKINEN, P. L.: (1970) The appearance of oxidoreductases in healing fractures. Acta path. microbiol. scand. Sect. A. **78,** 658—664. — 30. ROCHE, J.: Phosphatases. In: The Enzymes. SUMNER, J. B., MYRBÄCK, K., (eds.) Vol. 1. Part 1., Academic Press, New York 1950. — 31. SILBERGER, R., STAMP, W. G., LESKER, P. A., HASLER, K.: (1970) Aging changes in ultrastructure and enzymatic activity of articular cartilage of guinea pigs. J. Geront. **25,** 184—198. — 32. SILBERBERG, R., LESKER, P.: (1971) Enzyme activity in aging articular cartilage. Experientia (Basel) **27,** 133—135. — 33. TAKADA, K.: (1966) Enzyme histochemistry in bone tissue. II. Histochemical detection of hydrolytic and oxidative enzymes in callous tissue. Acta histochem. **23,** 53—70. — 34. TUSHAN, F. S., RODNAN, G. P., ALTMAN, M., ROBIN, E. D.: (1969) Anaerobic glycolysis and lactate dehydrogenase (LDH) isoenzymes in articular cartilage. J. Lab. clin. Med. **73,** 649—656. — 35. VERNE, J., WEGMANN, R.: (1958) Activité fructose-1-6-phosphatasique. Variations expérimentales. Ann. Histochim. **3,** 187—198. — 36. VESELL, E. S.: (1966) pH-Dependence of lactate dehydrogenase isoenzyme inhibition by substrate. Nature (Lond.) **210,** 421—422. — 37. WEGMANN, R., SOTELO, C.: (1962) Aspects cytoenzymologiques du métabolisme des glucides de la cellule de Purkinje. Ann. Histochim. **7,** 61—81. — 38. WEGMANN, R., VERNE, J., GUHA, S.: (1960) Activité de l'uridine diphosphoglucose transférase dans le muscle. Ann. Histochim. **5,** 253—258. — 39. WOESSNER, J. F. jr., BOUCEK, R. J.: (1959) Enzyme activities of rat connective tissue obtained from subcutaneously implanted polyvinyl sponge. J. biol. Chem. **234,** 3296—3300. — 40. WOESSNER, J. F. jr., BOUECK, R. J.: (1961) Connective tissue development in subcutaneously implanted polyvinyl sponge. II. Enzymic changes during development. Arch. Biochem. **93,** 95—109. — 41. WOLFE, H. J., COHEN, R. B.: (1963) Histochemical studies on the regenerating urodele limb. I. Oxidative and glycogenic enzymes. Develop. Biol. **8,** 48—66. — 42. YANO, Y.: (1968) Acta histochem. cytochem. Japon. **1,** 186. cit.: PEARSE, A. G. E. (ed.): Histochemistry. Theoretical and Applied. Vol. 3. Churchill, London 1972.

UNTERSUCHUNG DER KNORPELBILDUNG. XX. HISTOCHEMISCHE UNTERSUCHUNG DER GLYKOLYSE UND EINZELNER ENZYME DES GLYKOGENSTOFFWECHSELS IN DER REGENERIERENDEN GELENKFLACHE

CS. HADHÁZY, T. GLANT, B. MÁNDI, S. HARMATI, L. BORDÁN und KLÁRA BALOGH

Die distale Gelenkfläche des Femurs von 28 Hunden wurde entfernt und am 70. Tag nach dem Eingriff die Aktivität der Hexokinase, Glukose-6-phosphatase, Phosphohexoseisomerase, Fruktose-1,6-diphosphatase, Aldolase, Glyceraldehyd-3-phosphat-Dehydrogenase, Milchsäuredehydrogenase, der Isoenzyme der Milchsäuredehydrogenase, der Phosphoglukomutase, Phosphorilase, Glykogensynthetase, UDP-Glukose-Dehydrogenase, UDP-Glukuronsäure-Epimerase mit histochemischen Methoden untersucht.

Die regenerierende 70tägige Gelenkfläche besteht aus fibrösem Gewebe sowie aus Knorpelinseln. Die letzteren enthalten sich zu Knorpel differenzierende Zellen und junge Chondrozyten. Die untersuchten glykolytischen Enzyme geben in der regenerierenden Gelenkfläche eine positive Reaktion. Die Enzymaktivität der Knorpelinselzellen (insbesondere die der Chondroblasten und der jungen Knorpelzellen) übertraf die Aktivität des Bindegewebes. In den sich zu Knorpel differenzierenden Zellen ist neben der für die Glykolyse charackteristichen Aktivität der Isoenzyme der Milchsäuredehydrogenase auch eine erhebliche LDH_1- und LDH_2-Aktivität nachweisbar. In den gleichen Zellen kann eine Fruktose-1,6-phosphatase-Aktivität angenommen werden, während sich die Aktivität der Glukose-6-phosphatase nicht beweisen ließ. In den Knorpelinselzellen erfolgt eine Glykogensynthese, und in den höher differenzierten Zellen läßt sich eine Aktivität der UDP-glukuronsäure-4-epimerase aufzeigen. In sämtlichen Teilen der Gelenkfläche erwies sich die Reaktion der UDP-glukose-Dehydrogenase als positiv.

ИССЛЕДОВАНИЯ ПО ОБРАЗОВАНИЮ ХРЯЩА. XX. ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ГЛИКОЛИЗА И ОТДЕЛЬНЫХ ЭНЗИМОВ ОБМЕНА ГЛИКОГЕНА В РЕГЕНЕРИРУЮЩЕЙСЯ СУСТАВНОЙ ПОВЕРХНОСТИ

Ч. ХАДХАЗИ, Т. ГЛАНТ, Б. МАНДИ, Ш. ХАРМАТИ, Л. БОРДАН и К. БАЛОГ

Авторы удалили дистальный суставной хрящ бедренной кости 28 собак и на 70-ый день после операции изучали гистохимическими методами в регенерирующейся суставной поверхности активность гексокиназы, глюкоза-6-фосфатазы, фосфорексозизомеразы, фруктоза-1,6-дифосфатазы, альдолазы, глицеральдегид-3-фосфат-дегидрогеназы, лактат-дегидрогеназы, изоэнзимов лактат-дегирогеназы, фосфоргютамазы, фосфорилазы, гликоген синтетазы, KDP-глюкоза-дегидрогеназы и UDP-глюкуроновой кислоты-4-эпимеразы.

70-дневная регенерирующая суставная поверхность состоит из фиброзной ткани и из хрящевых островков. Последние содержат клетки, дифференцирующиеся в хрящ, и молодые хондроциты. Исследовавшиеся гликолитические энзимы давали в регенерирующейся поверхности положительную реакцию. В клетках хрящевых островков (в частности в хондробластах и молодых хрящевых клетках) энзиматическая активность превышает актиность соединительной ткани. В клетках, дифференцирующихся в хрящ, наряду с активностью изоэнзимов лактатдегидрогеназы, характерной для гликолиза, наблюдается также значительная актиность $ЛДГ_1$ и $ЛДГ_2$. В этих же клетках можно полагать активность фруктоза-1,6-дифосфатазы; активности глюкоза-6-фосфатазы не удалось доказать. В клетках хрящевых островков происходит синтез гликогена; в более дифференцированных клетках выявляема активность UDP-глюкуроновая кислота-4-эмимеразы. Реакция UDP-глюкоз-дегидрогеназы во всех частях суставной поверхности оказалась положительной.

Cs. Hadházy
T. Glant
B. Mándi
S. Harmati
L. Bordán
Klára Balogh

Institute of Anatomy, Histology and Embryology
University Medical School
4012 Debrecen, Pf. 12., Hungary

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Department of Anatomy, Histology and Embryology, and Central Research Laboratory, University Medical School, Debrecen

THE EFFECT OF GLUCOSE-1 PHOSPHATE CALCIUM ON THE EPIPHYSEAL CARTILAGE OF THE RAT

I. Földes, S. Varga and J. Laczkó

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Electron microscopic investigations were carried out on the proximal tibial epiphyseal cartilage of albino rats treated with 2 mg G-1-P-Ca daily for one and three weeks. The treatment

— enhanced the secretory activity of the upper cells of the zone of maturing cartilage with a consequential enlargement of the Golgi region, depletion of secretory products, and an increase in the number of microtubuli.

— In addition, the treatment increased the number of matrix vesicles in the extracellular space showing incipient calcification. This findings confirm the earlier light micriscopic observation of the calcification stimulating effect of G-1-P-Ca.

Introduction

Previous investigations by means of histological and histochemical methods and isotopic tracers revealed that some phosphate esters (G-1-P, G-6-P, ATP) caused increased calcification and enchondral ossification [9]. ATP and G-1-P-Ca also stimulated mucopolysaccharide production and calcification [11, 14, 15].

Anderson [2] and Bonucci [6] found by electron miroscopy membrane corpuscles termed vesicles or globules, in the cartilage matrix. Subsequently, the existence of these globules has been confirmed by numerous authors [1, 3, 28, 31, 32]. According to Thyberg and Friberg the matrix vesicles containing acid mucopolysaccharides are the centres of hydroxyapatite crystal formation.

Matsuzawa and Anderson [20] demonstrated alkali phosphatase, ATP-ase and acid phosphatase in the vesicles of the epiphyseal cartilage. Similar vesicles showing pyrophosphatase, ATP-ase, alkali phosphatase and 5'AMP-ase activities were isolated in the fifth fraction of the epiphyseal cartilage of calf embryos [1, 3]. An increased local concentration of calcium by means of active transport through the cell membrane may occur in many tissues by the site of calcification. Anderson and Sajdera [4] suppose that

Abbreviations:

G-1-P	glucose-1-phosphate
G-1-P-Ca	glucose-1-phosphate calcium
G-6-P	glucose-6-phosphate
ATP	adenosinetriphosphate

the matrix vesicles are also involved in binding Ca ions. According to ANDERSON [2] the electron density of granules in the cells of the epiphyseal cartilage of the normal rat is proportional to the rate of calcification of the matrix vesicles. In the vicinity of the zone of calcification in rachitic rats ANDERSON found few cells containing granules whereas the number of vesicles was approximately the same as in non-rachitic controls. In most matrix specimens no apatite crystals were found but a few crystals were present in the vesicles of the hypertrophic zone.

The Golgi apparatus and the rough surface endoplasmic reticulum of the chondrocytes are known to be involved in the secretion of cartilage substance ground [16, 22, 29, 33].

We have therefore studied the effect of G-1-P Ca on the epiphyseal cartilage with special attention to secretory granules and matrix vesicles.

Material and method

Investigations were carried out on the proximal epiphyseal cartilage of the tibia in 12 albino rats weighing 100 g. Eight rats were given 2 mg of G-1-P-Ca (SPA, Milano) daily intraperitoneally for one and three weeks. Four rats served as untreated controls. After the completion of treatment the animals were killed under ether anaesthesis and the proximal epiphyseal cartilages were removed and cleansed from the soft parts. The material was minced finely and fixed in 3% glutaraldehyde solution adjusted to pH 7.2 with 0.1 M cacodylate buffer at room temperature for 4 hours. Following fixation the specimens were washed in buffered solution and post-fixed in 2% osmium tetroxide buffered with 0.1 M cacodylate at room temperature for 2 hours, dehydrated in graded alcohol series and embedded in Araldite. The sections were contrasted with uranyl acetate and lead citrate and examined with a JEM 100B electron microscope.

Results and discussion

After one and three weeks of G-1-P-Ca treatment alike, the region of Golgi vesicles appeared to be larger in the proliferative, and especially in the hypertrophic zone (Figs 1 and 2). The upper part of the latter zone correspond to the so-called "zone of transition" seen under the light microscope. The enlarged Golgi vesicles delimited by smooth membranes contained a granular substance of moderate electron density (Fig. 3). Part of the Golgi apparatus, mainly in the more distal cells, was localized in the peripheral zone, in the vicinity of the cytomembrane (Fig. 4). No appreciable differences were noted between treated and control rats as regards the extension of the granular reticulum, number and size of cisterns, and nature of the substance present in them (Fig. 5). Owing to increased secretion there was an increased depletion of substances from the cell. Consequently, various formations due to extrusion of substances into the extracellular space could be seen: vacuoles, vesicles opening in the extracellular space, presecretory granulas penetrating through

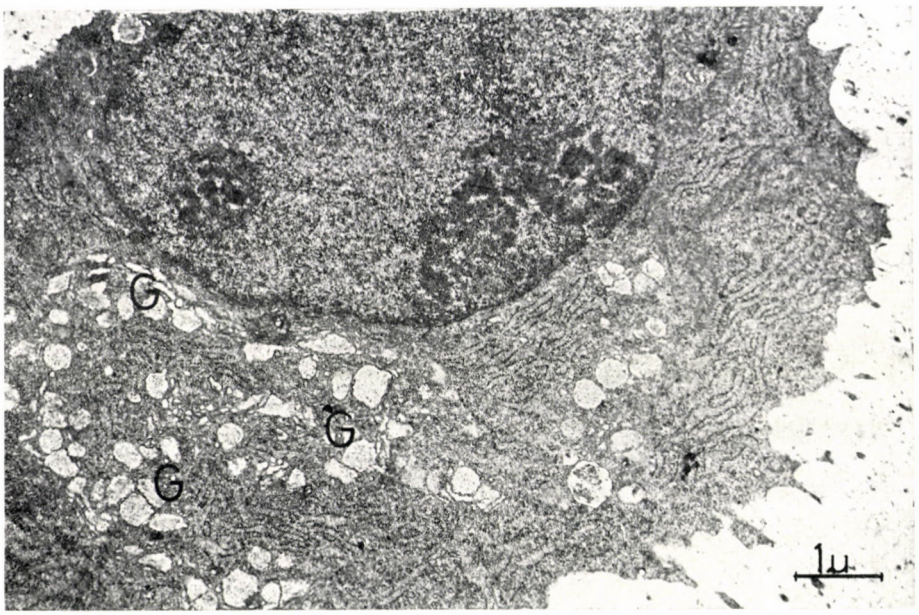

Fig. 1. Upper part of hypertrophic zone of epiphyseal cartilage in the rat after one week of G-1-P-Ca treatment. Extended territory of perinuclear Golgi vesicles (G)

the cell membranes or cell processes (Fig. 6). Intensive calcification occurred in the extracellular space, in the longitudinal intercolumnar septum (Fig. 7). In the hypertrophic zone, two types of matrix vesicles could be distinguished. One type, a lysosome-like vesicle surrounded a triple membrane corresponded to the corpuscle described by THYBERG and FRIBERG [32], these structures of varying size and density were found in great numbers in the matrix (Fig. 8). The second type was represented by matrix vesicles localized likewise among the fibres in the ground substance. These vesicles contained apatite crystals displaying initial calcification (Fig. 9). Numerous such matrix vesicles were found in the epiphyseal cartilage of rats treated with G-1-P-Ca.

In the matrix the amount of interfibrillar substance containing mainly MPS-s seemed to have increased as compared to the number of fibres.

The present electron microscopic findings have confirmed our previous results obtained with the light microscope. On G-1-P-Ca treatment enlargement of the Golgi region and an increase in size and number of Golgi vesicles were noted. Increased secretion of MPS seemed to be due to the probably enhanced activity of the Golgi apparatus. Recently, the role of the Golgi apparatus in the production of MPS-s has been the subject of numerous studies [5, 7, 16, 22, 25].

Enlargement of Golgi region was conspicuous in the upper part of the hypertrophic zone where under normal conditions the endoplasmic reticulum

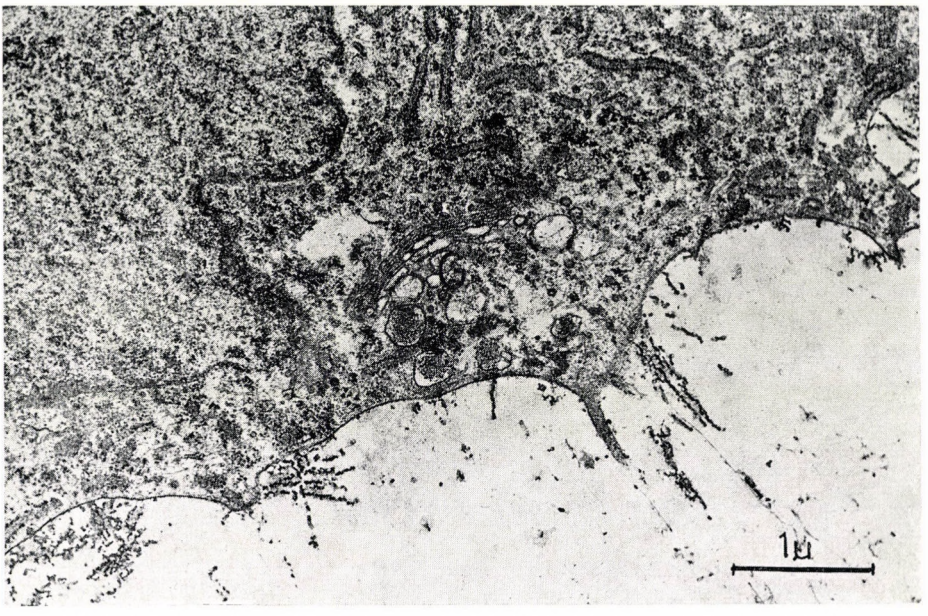

Fig. 2. Epiphyseal cartilage from rat treated with G-1-P-Ca for one week. The Golgi apparatus (G) is localized at the periphery of the cell

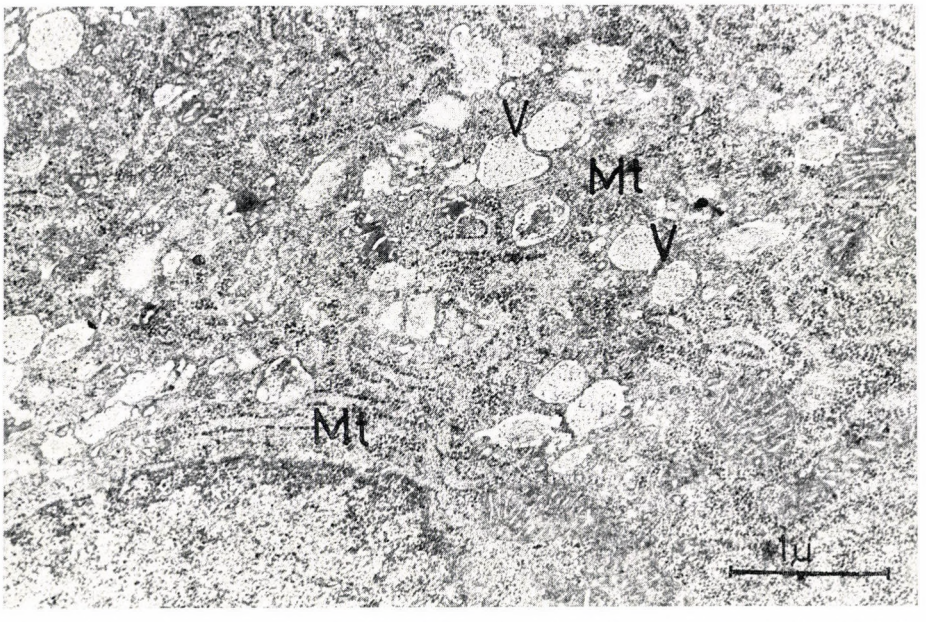

Fig. 3. Upper zone of hypertrophy of epiphyseal cartilage from rat treated with G-1-P-Ca for 3 weeks. Golgi vesicles (V) contain a granular substance. In the proximity of the nucleus and among the Golgi vesicles microtubuli (Mt) are visible

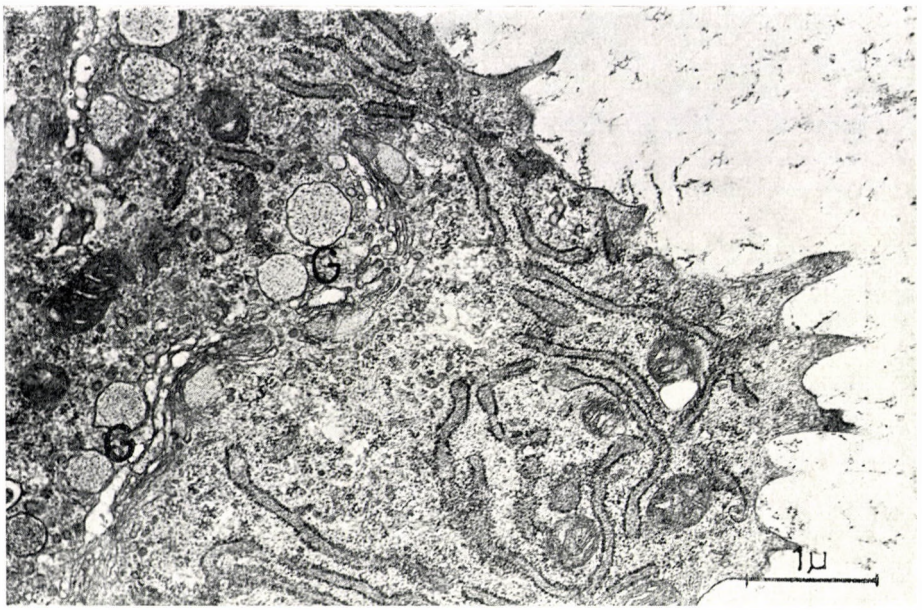

Fig. 4. Zone of hypertrophy of epiphyseal cartilage from rat treated with G-1-P-Ca for 3 weeks. The Golgi zone is well visible in the cell

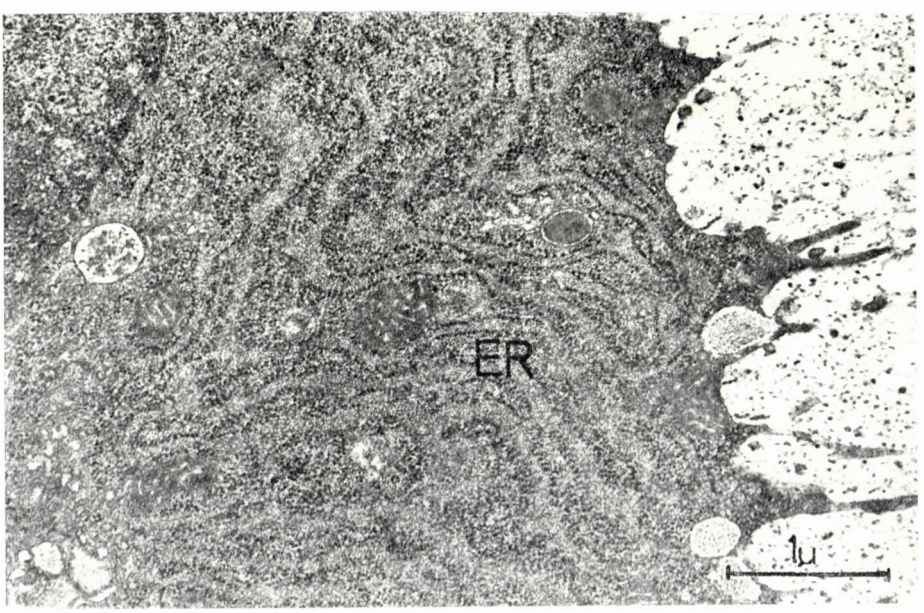

Fig. 5. Zone of hypertrophy of the epiphyseal cartilage from rat treated with G-1-P-Ca for one week. Normally developed endoplasmic reticulum (ER) and vesicles extending into the extracellular space

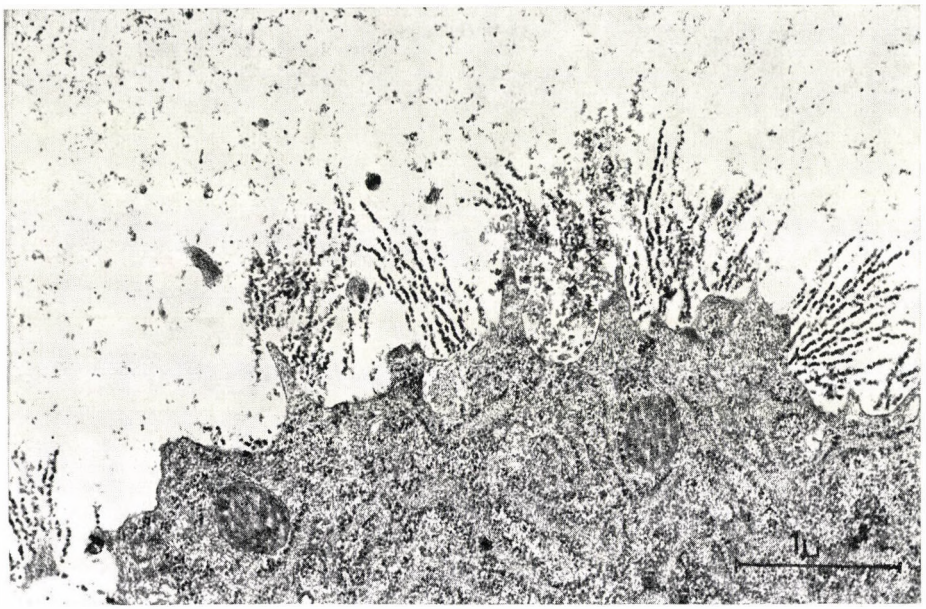

Fig. 6. Presecretory granules extruded through the cell process and membrane

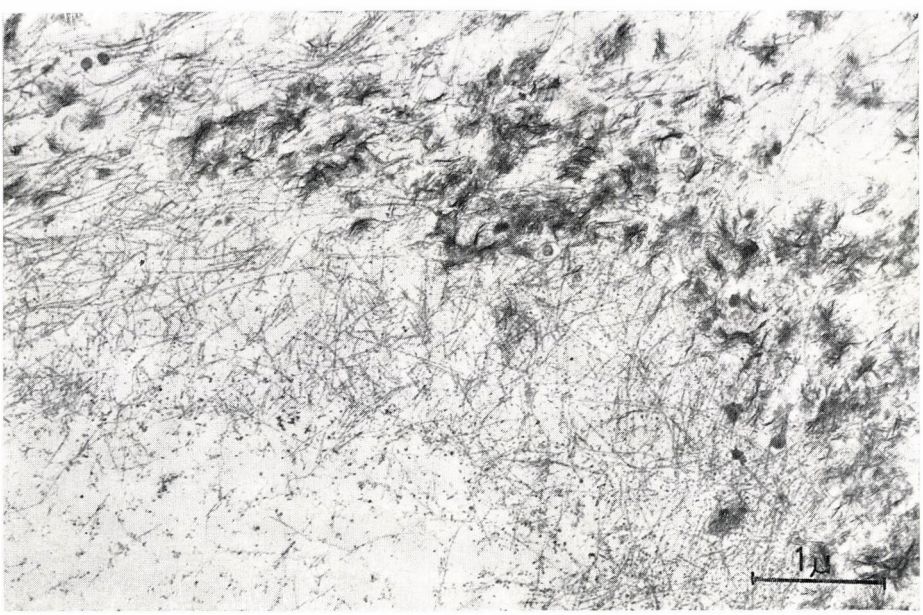

Fig. 7. Distal part of epihyseal cartilage of rat after G-1-P-Ca treatment for one week Intensive calcification in longitudinal intercolumnar septum

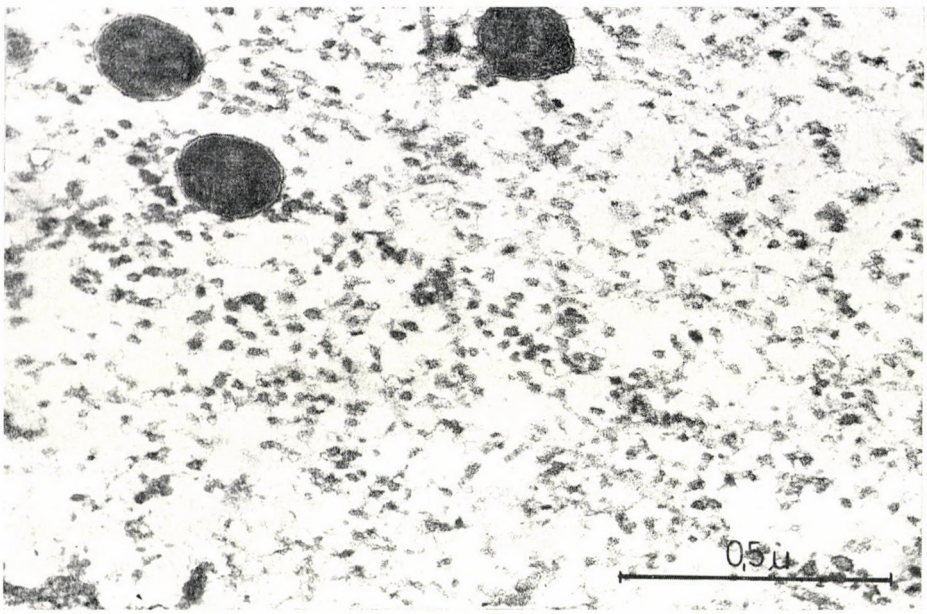

Fig. 8. Epiphyseal cartilage of rat after G-1-P-Ca treatment for 3 weeks. Numerous lysosomelike matrix vesicles in the extracellular space

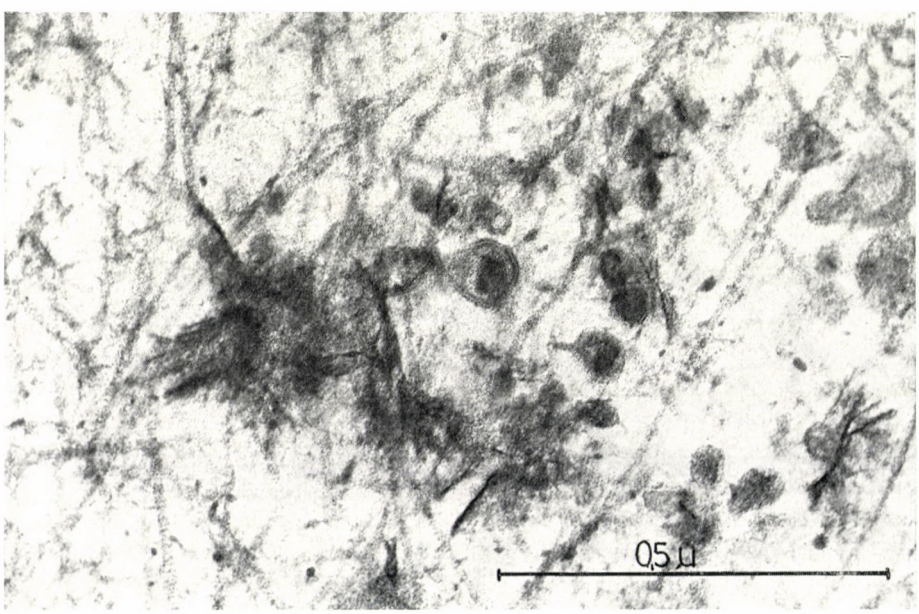

Fig. 9. Epiphyseal cartilage of rat after G-1-P-Ca treatment for one weak. In the extra cellular space, large numbers of matrix vesicles showing incipient calcification

is the characteristic organelle. A similar extension of the Golgi region was noted in the hypertrophic cells after vitamin D treatment but here the main effect was seen mainly in the cells of the proliferation zone [12]. There is an evident parallelism between the results obtained with vitamin D and G-1-P-Ca treatment as shown electron microscopically (increase in number of Golgi vesicles) and histochemically (increase in amount of acid MPS-s). No changes due to G-1-P-Ca were noted in the endoplasmic reticulum as demonstrated by the structure of the extracellular substance, displaying an increased amount of interfibrillar matrix among a few mature collagen fibres.

On G-1-P-Ca treatment microtubules appeared in the cells of the epiphyseal cartilage of adult rats. SLAUTTERBACK [30] was the first to describe microtubules in osmium-fixed specimens obtained from Hydra. We have observed microtubules at the periphery of cells of the costal cartilage [24]. SANDBORN et al. [27] found microtubules in material fixed in glutaraldehyde. PALFREY and DAVIES [23] described microtubules in articular cartilage, LÉVAI and MARX [17] in embryonic epiphyseal cartilage. Microtubules are usually located perinuclearly, or in the peripheral part of the cell. In our specimens microtubules were found among the Golgi vesicles, in the vicinity of the nucleus (Fig. 3). Though their function has not been clarified, it they seem to play a role in transport connected in some way with the Golgi apparatus.

In the epiphyseal cartilage two types of matrix vesicles or corpuscles could be distinguished. MATUKAS and KRIKOS [21] suggested that the matrix vesicles are lysosomal dense bodies originating from the chondrocytes as these components show great morphological similarities. THYBERG and FRIBERG [32] speak of two types of matrix vesicle: one is a lysosomal dense body, while the other, containing a cytoplasm-like substance, may have originated from detached cytoplasmic membrane. In the first type of vesicles THYBERG and FRYBERG [32] demonstrated acid phosphatase but this has not been confirmed by MATSUZAWA and ANDERSON [20]. These authors too mention the possibility of a lysosomal enzyme effect but they could not obtain evidence of the presence of the enzyme.

Evidently, the two types show different morphological features and probably their functions are also different. In the corpuscles of lysosomal character (first type) no signs of initial calcification were found. This may have been due to technical shortcomings or, more probably, to the involvement of these bodies in the preparation of the matrix. The second type of vesicle in which incipient calcification was noted, are probably related to cell destruction. According to some authors, [2, 6] the vesicles originate from the cells in the zones of resting and proliferating cartilage from where together with the matrix, they pass in the zones of hypertrophic and calcifying cartilage where calcification begins. The vesicles are, however, less numerous in the upper zones.

In our material the first type of vesicle was more numerous in the upper

part of the zone of maturation, while the second type mainly in the distal part of the cartilage.

The present findings seem to support and explain the increase in amount and the changes of MPS under the effect of G-1-P-Ca treatment.

REFERENCES

1. Ali, S. Y., Sajdera, S. W., Anderson, H. C.: (1970) Isolation and characterization of calcifying matrix vesicles from epiphyseal cartilage. Proc. nat. Acad. Sci. hung. **67,** 1513. — 2. Anderson, H. C.: (1969) Vesicles associated with calcification in the matrix of epiphyseal cartilage J. Cell Biol. **41,** 59. — 3. Anderson, H. C., Matsuzawa, T., Sajdera, S. W., Ali, S. Y.: (1970) Membranous particles in calcifying cartilage matrix. Trans. N. Y. Acad. Sci. **32,** 619. — 4. Anderson, H. C., Sajdera, S. W.: (1970) Extraction as a technique for the electron microscopic study of protein polysaccharides and collagen in cartilage matrix. Fed. Proc. **29,** 554. — 5. Bennett, G., Leblond, C. P., Haddad, A.: (1974) Migration of glycoprotein from the Golgi apparatus to the surface of various cell types as shown by radioautography after labelled fucose injection into rats. J. Cell Biol. **60,** 258. — 6. Bonucci, E.: (1970) Fine structure and histochemistry of calcifying globules in epiphyseal cartilage Z. Zellforsch. **103,** 192. — 7. Doty, S. B., Schofield, B. H.: (1972) Electron microscopic localization of hydrolytic enzymes in osteoclasts. Histochem. J. **4,** 245. — 8. Fewer, D., Threadgold, J., Sheldon, H: (1964). Studies on cartilage. Electron microscopic observations on the autoradiographic localisation of ^{35}S in cells and matrix. J. Ultrastruct. Res. **11,** 166. — 9. Földes,I: (1963) Data on enchondral ossification with special regard to phosphate esters and mucopolysaccharides. (*Hung.:* Adatok a chondralis csontfejlődéshez, különös tekintettel a foszfátészterekre és a mucopolysaccharidokra.) Kandidátusi értekezés, Debrecen. (Cand. dissertation) — 10. Földes, I:: (1967) Importance of phosphate esters in enchondral ossification. Stud. biol. hung. **6,** Akadémiai Kiadó, Budapest. (Acad. Publication House, Budapest.) — 11. Földes, I.: (1969) Autoradiographic studies on cartilage. (*Hung.:* Autoradiografiás vizsgálatok porcszövetekben.) — Magy. Tud. Akad. Biol. Oszt. Közl. **12,** 147. (Publ. of the Hung. Acad. of Sci., Biol. Dept.) — 12. Földes, I.: (1975) Study of the mechanism of action of calcification and mineralization in rats treated with vitamin D and phosphate ester. (*Hung.:* A kalcifikáció és a mineralizáció hatásmechanizmusának vizsgálata D vitaminnal és foszfátészterrel kezelt patkányokban.) Doktori értekezés, Debrecen. (Doctor' sdegree dissertation, 1975) — 13. Földes, I., Nagy, Zs., Benkő, K., Lévai, G., Ary-Balogh, P.: (1965) Elektronenmikroskopische Untersuchungen an postembryonalen Epiphysenknorpel der Albinoratte. Acta morph. Acad. Sci. hung. **13,** 283. — 14. Földes, I., Géhl, Á.: (1966) The effect of adenosinetriphosphate on closing of the epiphyseal cartilage. *(Hung.:* Adenozintrifoszfát hatása az epiphysis porc záródására.) Morph. Ig. Orv. Szle. **6,** 36. — 15. Földes, I., Petkó, M.: (1969) Effect of calcium glucose-1-phosphate on rickets. Panminerva med. **11,** 544. — 16. Godman, G. C., Lane, N.: (1964) On the site of sulfation in the chondrocyte J. Cell Biol. **21,** 353. — 17. Lévai, G., Marx, I.: (1969) Untersuchungen über Knorpelgewebe. I. Die Feinstruktur der Knorpelzellen Z. mikro-anat. Forsch. **80,** 177. — 18. Martin, J. H., Matthews, J. L.: (1969) Mitochondrial granules in chondrocytes. Calc. Tiss. Res. **3,** 184. — 19. Martin, J. H., Matthews, J. L.: (1970) Mitochondrial granules in chondrocytes, osteoblasts and osteocytes: An ultrastructural and microincineration study. Clin. Orthop. **68,** 273. — 20. Matsuzawa, T., Anderson, H. C.: (1970) Phosphatases of epiphyseal cartilage studied by electron microscope histochemistry. Proc. 7th Congr. Electron Microscopy, Grenoble, **2,** 529. — 21. Matukas, V. J., Krikos, G. A.: (1968) Evidence for changes in protein polysaccharide associated with the onset of calcification in cartilage. J. Cell Biol. **39,** 43. — 22. Neutra, M., Leblond, C. P.: (1966) Radiographic comparison of the uptake of galactose ^{3}H and glucose ^{3}H in the Golgi region of various cells secreting glycoproteins or mucopolysaccharides. J. Cell Biol. **30,** 137. — 23. Palfrey, A. J., Davies, D. V.: (1966) The fine structure of chondrocytes. J. Anat. **100,** 213. — 24. Palfrey, A. J., Földes, I.: (1971) Ultrastructure of rat costal cartilage. J. Anat. **110,** 132. — 25. Peterson, M., Leblond, C. P.: (1964) Synthesis of complex carbohydrates in the Golgi region, as shown by autoradiography after injection of labelled glucose. J. Cell Biol. **21,** 143. — 26. Rohr, H., Walter, S.: (1966) Die Mukopolysaccharidsysteme in ihrer Beziehung zur submikroskopischen Struktur der Knorperzelle. Acta anat. (Basel) **64,** 223. — 27. Sandborn, E., Koen, P. F., McNabb, J. D., Moore, G.: (1964) Cytoplasmic microtubules in mammalian cells. J. Ultrastruct. Res. **11,** 123. — 28. Schenk, R. K., Miller, J.,

ZINKERNAGEL, R., WILLENEGGER, H.: (1970) Ultrastructure of normal and abnormal bone repair. Calc. Tiss. Res. **4,** (Suppl.) 110. — 29. SCHMALBECK, J., ROHR, H.: (1967) Die Mukopolysaccharid-Synthese in ihrer Beziehung zur Eiweiß-Synthese in der Brunnerschen Drüse der Maus (Elektronenmikroskopisch-autoradiographische Untersuchung mit ^{3}H Glucose.) Z. Zellforsch. **80,** 329. — 30. SLAUTTERBACK, D. B.: (1963) Cytoplasmic microtubules. I. Hydra. J. Cell Biol. **18,** 367. — 31. SPYCHER, M. A., MOOR, H., RUETTNER, J. R.: (1969) Electron microscopic observations on aging and osteoarthritic human articular cartilage. II. The fine structure of freeze-etched aging hip joint cartilage. Z. Zellforsch. **98,** 512. — 32. THYBERG, J., FRIBERG, U.: (1970) Ultrastructure and acid phosphatase activity of matrix vesicles and cytoplasmic dense bodies in the epiphyseal plate. J. Ultrastruct. Res. **33,** 554. — 33. WEINSTOCK, M., LEBLOND, C. P.: (1974) Synthesis, migration and release of precursor collagen by odontoblasts as visualized by autoradiograph after ^{3}(H) proline administration. J. Cell Biol. **60,** 92.

ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNG DER WIRKUNG VON GLUKOSE-1-PHOSPHATKALZIUM AUF DIE EPIPHYSENKNORPEL VON RATTEN

Von

I. FÖLDES, S. VARGA und J. LACZKÓ

Etwa 100 g schwere Ratten wurden mit 2 mg G-1-P-Ca (SPA-Milano) pro die behandelt und am proximalen Epiphysenknorpel der Tibia der Tiere wurden elektronenoptische Untersuchungen durchgeführt. Aufgrund der Versuchsergebnisse übt die G-1-P-Ca -Behandlung eine doppelte Wirkung aus, indem sie

— einerseits die sekretorische Tätigkeit in den oberen Zellen der Reifungszone anregt. Infolge dieser Stimulierung vergrößert sich der Umfang des Golgi-Apparates, und es erhöht sich auch die Entleerung der sezernierten Produkte sowie die Zahl der Mikrotubuli:

— andererseits zeigt der Behandlung die Zahl der eine initiale Kalzifikation zeigenden Matrix-Vesikel im extrazellulären Raum einen Anstieg. Durch den letzteren Befund wird die frühere lichtmikroskopische Beobachtung der Verfasser bestätigt, wonach das G-1-P-Ca eine kalzifikationssteigernde Wirkung ausübt.

ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ ДЕЙСТВИЯ ГЮКОЗ-1--ФОСФАТА КАЛЬЦИЯ НА ЭПИФИЗАРНОМ ХРЯЩЕ КРЫСЫ

И. ФЁЛДЕШ, Ш. ВАРГА и Й. ЛАЦКО

Белым крысам весом приблизительно в 100 г авторы давали 2 мг глюкоз-1 фосфат кальция (SPA-Milano) в день и после этого они проводили на проксимальном эпифизарном хряще большеберцовой кости животных электронномикроскопические исследования. Согласно результатам опытов дача глюкоз-1-фосфата кальция оказывает двойное действие, а именно

— повышает скереторную функцию верхних клеток зоны созревания. В результате этого увеличивается размер аппарата Гольджи, выделение секреционных продуктов, и увеличивается число микротрубок;

— в внеклеточном пространстве дача препарата повышает число матрикс-пузырьков, показывающих начальную кальцификацию. Послеоним результатом подкрепляется прежнее наблюдение авторов под оптическим микроскопом, согласно которому глюкоз-1--фосфат кальция вызывает повышение кальцификации.

Dr. István FÖLDES DOTE Anatómiai Szövet- és Fejlődéstani Intézet 4012 Debrecen, Pf. 14, Hungary

Dr. Sándor VARGA, Dr. Jenő LACZKÓ | DOTE Központi Kutató Laboratórium 4012 Debrecen, Pf. 3, Hungary

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Second Department of Anatomy, Semmelweis University Medical School, Budapest, Hungary

EFFECT OF NEONATAL THYMECTOMY ON RABBIT TONSILS

I. Oláh and I. Törő

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An intensive lymphocyte migration takes place through the venules lined with high endothelium and situated in the extrafollicular areas of the tonsils. After thymectomy the migration of lymphocytes is less marked and in the areas surrounding the vessels lymphocyte depletion, connective tissue proliferation and a disintegration of the intimate contact between reticular cells and reticular fibres can be observed. A similar lymphocyte depletion appears also in the epithelium. Similarly as the deep cortical substance of the lymph nodes the extrafollicular regions of the tonsils belong to the peripheral lymphoid organs.

Introduction

An interaction between the tonsils and the thymus has been suggested at the end of the last century. Retterer [16] believed to have discovered epithelial reticulum in the subepithelial region of the tonsils. Apart from this error, he has to be given the credit for recognizing in the tonsile that the epithelium-lymphocyte interaction topographically looks to be identical with that of the thymus. This was supported by Ewing [5] who claimed a similarity in structure between thymomas and tonsillar lymphoepitheliomas. According to Jolly [9] a functional interaction develops between the tonsillar epithelium and the lymphocytes. He called the thymus and the bursa of Fabricius "organes lymphoepitheliaux" and assumed a close relationship between these organs and the lymph organs associated with the intestine.

After the development of the modern immunology arose the question whether the tonsils were central lymphoid organs like the thymus and the bursa of Fabricius or peripheral lymph organs like the spleen and the lymph nodes.

Since Parrott et al. [15] had performed neonatal thymectomy in mice it has been known that the peripheral lymphoid organs (lymph nodes, spleen) during a certain stage of their development are under the influence of the central lymphoid organs (thymus). After thymectomy the deep cortical substance of the lymph nodes is underdeveloped and cytopenic. Normally this area is characterized by the presence of venules with a high endothelium and an intensive lymphocyte migration, through the wall of the venules while

after thymectomy the endothelium becomes flat and lymphocyte migration diminishes or ceases.

In view of these findings we have studied the role of the thymus as of a central lymphoid organ, in the development of the tonsils and the changes occurring in the structure after thymectomy.

Material and method

Thymectomy was performed on a total of 51 newborns of 8 mother rabbits. After operation the baby rabbits were given back to the mothers so that feeding difficulties should not interfere with the effect of thymectomy. Two animals of each litter (a total of 16) were shamoperated and these animals then served as controls. Thirtyfive to forty days after thymectomy the tonsils were removed fixed by perfusion with a 4.5% buffered solution of glutaraldehyde then after rinsing with buffer solution post-fixed in 1% osmium tetroxide solution dehydrated in an alcohol series and embedded in Araldite. Semi-thin and thin sections were prepared with Reichert OMU-2 ultramicrotome. Semi-thin sections were stained with azure II and toluidine blue, the thin sections with uranyl acetate and Pb-citrate, and studied by JEM 6C electron microscope.

Results

In the rabbit, peripheralization of lymphoid cells from the thymus occurs mainly before birth [1], and only if few individuals is the intensity of this process shifted to the perinatal period. Hence, only in the latter animals the effect of neonatal thymectomy on the peripheral lymphoid organe can be demonstrated. Tissue changes after thymectomy reach only in the extrafollicular substance a degree which might be assessed morphologically.

After thymectomy a conspicuous transformation takes place in the supporting system and lymphocyte composition of the extrafollicular substance of the tonsils.

No morphological changes occur in the development and structure of the follicules. Next well-developed cellrich follicules the cytopenia of the extrafollicular substance is striking (Fig. 1).

The venule lined with high endothelium which were detected in the thymus dependent regions of the lymph nodes were present in the extrafollicular substance of the tonsils too. It was a lymphocyte migration through these vessel-walls (Fig. 2). After thymectomy the subendothelial connective tissue around these vessels broadened and lymphocyte migration was less frequent (Fig. 3). The flattening of the endothelial cells described by PARROTT et al. [15] for mouse lymph nodes, was only moderate. In the accumulated connective tissue of the extrafollicular substance mainly lymphoblast II. [14] and medium size lymphocytes were observed (Fig. 4), while compared to the controls there was a significant decrease in the number of small lymphocytes (Figs 4, 5).

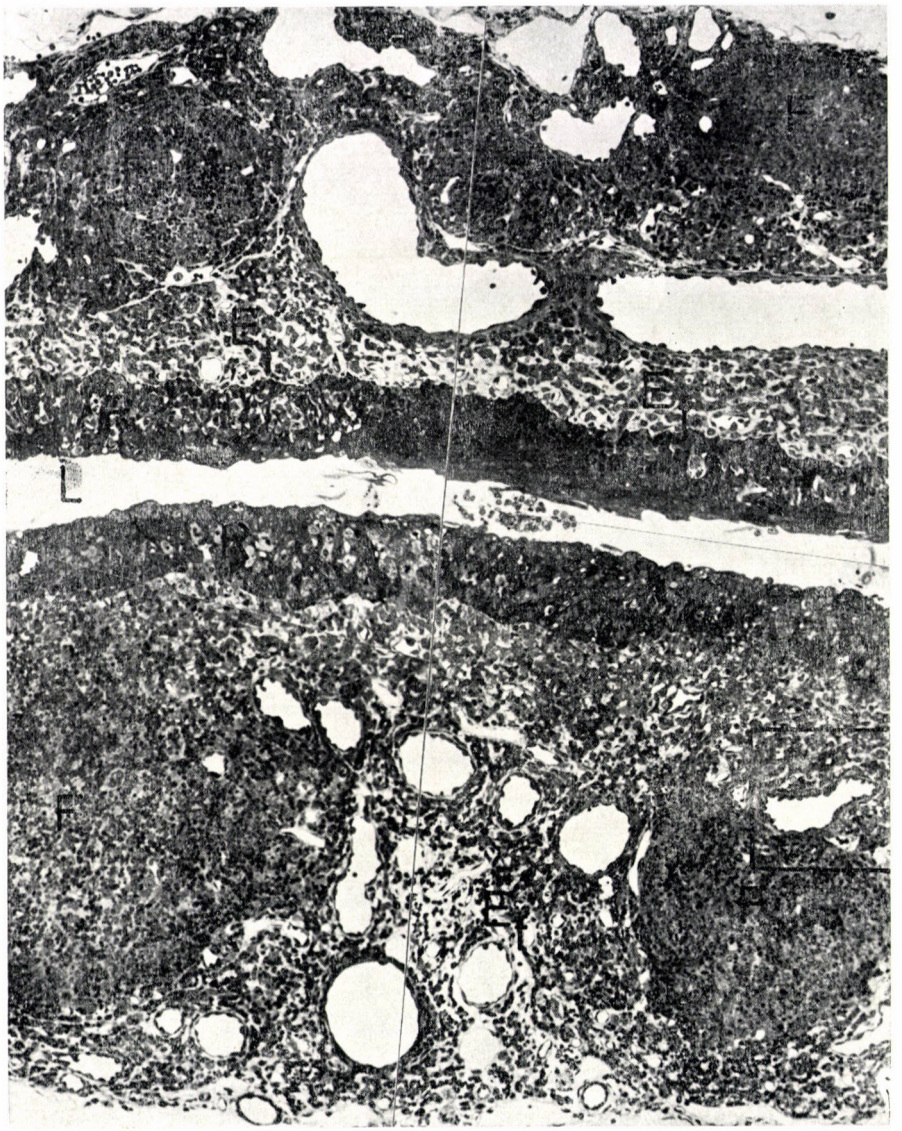

Fig. 1. Tonsil of a thymectomized animal. The follicules (F) are well-developed, rich in lymphoid cells while there are few cells in the extrafollicular region (Ef). The framed area can be seen under high power in Fig. 4 L: crypt lumen, R: reticular epithelium, S: stratified squamous epithelium ×280

In the extension of the reticulated epithelial regions (epithelial area infiltrated with lymphoid elements) no changes were observed, though the cellular composition of the lymphoid elements which migrated into the epithelium had undergone a change. There was a slight decrease in lymphocyte

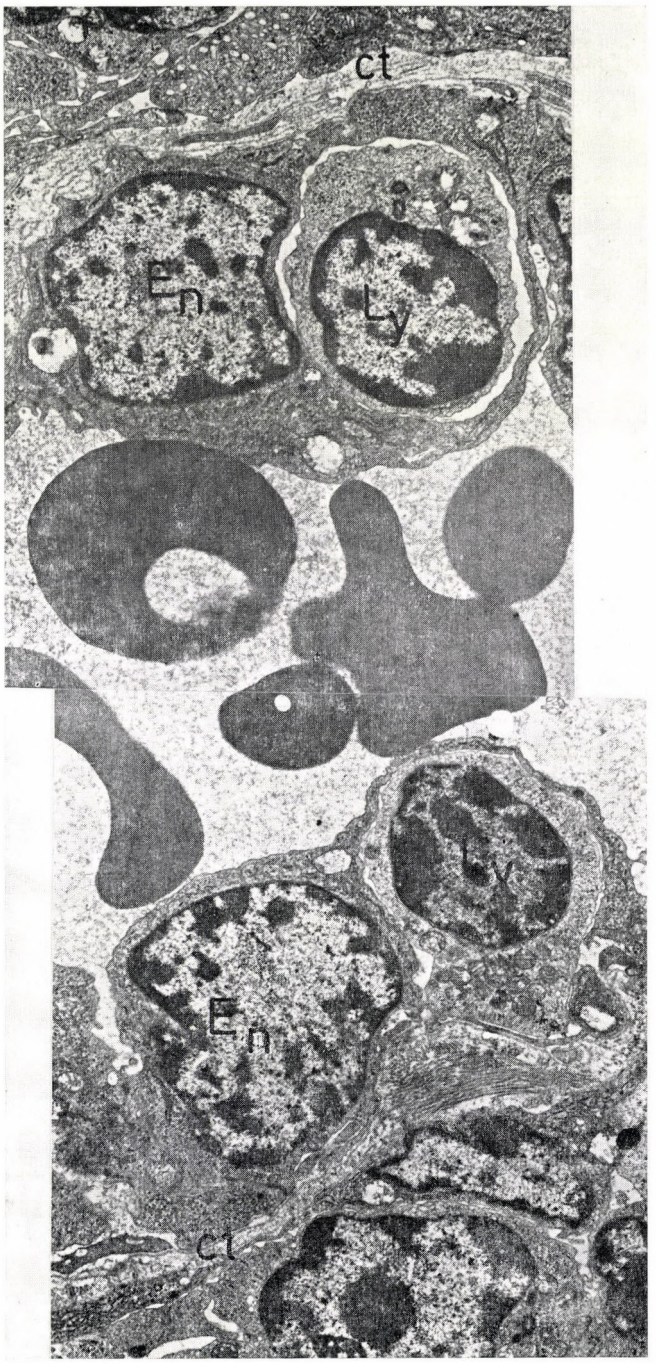

Fig. 2. High endothelial venule of control animal. The endothelial cells (En) bulge into the lumen, under them lymphocytes (Ly) migrating through the wall of the vessel. Thin subendothelial connective tissue (Ct) ×4900.

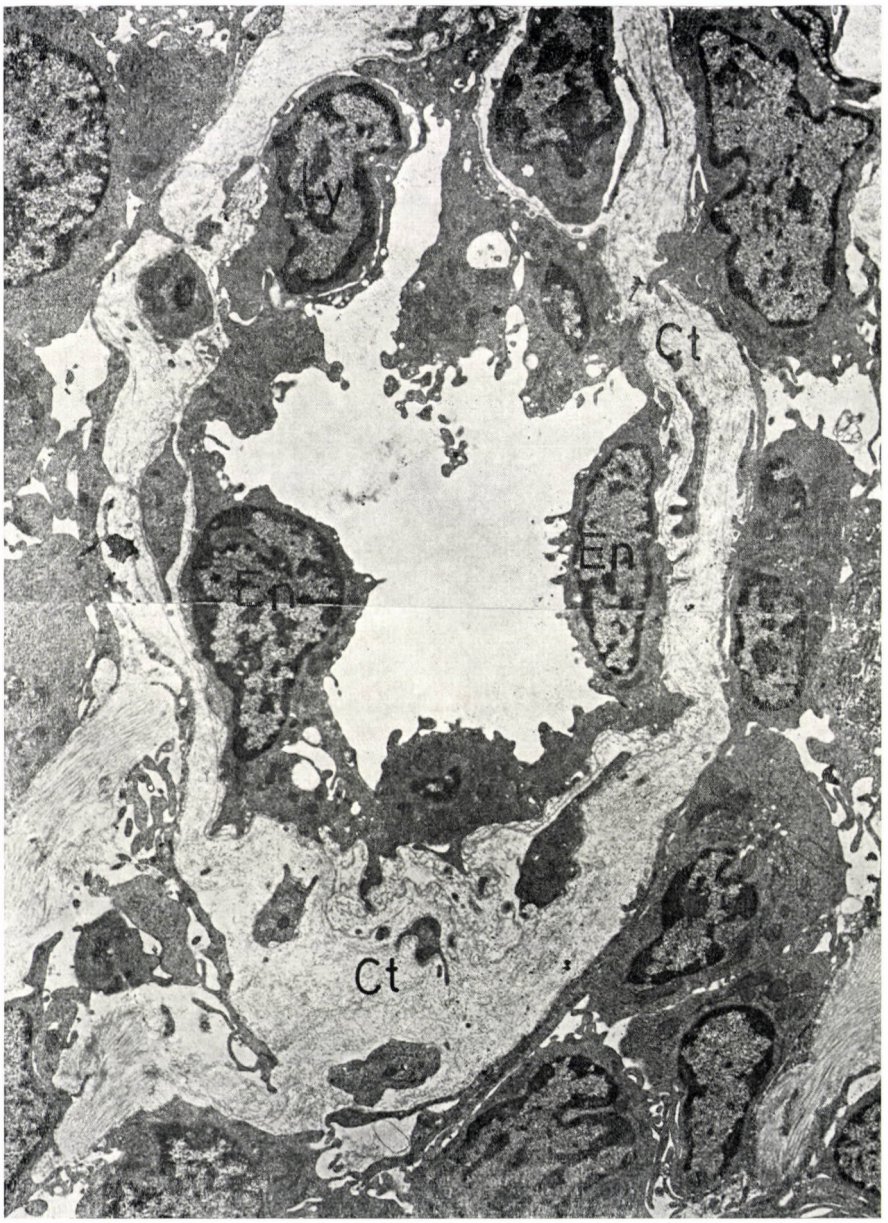

Fig. 3. High endothelial venule of thymectomized animal. The endothelium (En) is somewhat flatter, the subendothelial connective tissue widened (Ct). Lymphocytes (Ly) migrate through the vascular wall. ×5300

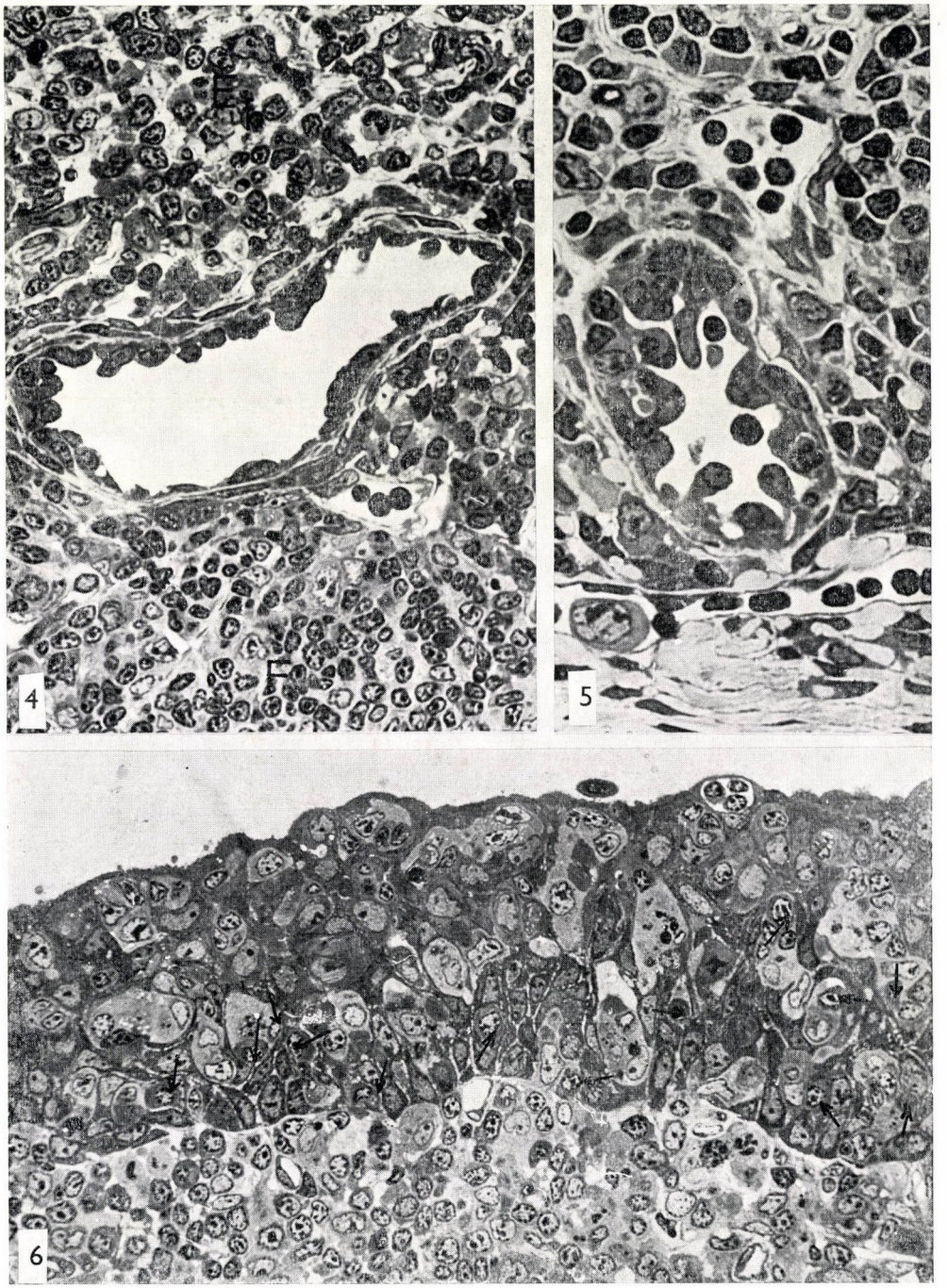

Fig. 4. Detail of Fig. 1. In the extrafollicular region (Ef) above the venule, extensive light areas containing collagen are visible among the loosely situated lymphoid cells. Beside a few mature lymphocytes mainly medium size lymphocytes and lymphoblasts are visible. Slight lymphocyte migration through the endothelium. Under the venule a cell-rich follicule (F) can be seen. ×780

Fig. 5. High endothelial venule of control animal. Compare with Fig. 4. ×1600

Fig. 6. Epithelium of crypt in thymectomized animal. In the basal part of the epithelium an unusually large number of plasmocytes (arrow) are visible. In the subepithelial extrafollicular region majority of lymphoid cells is young. ×900

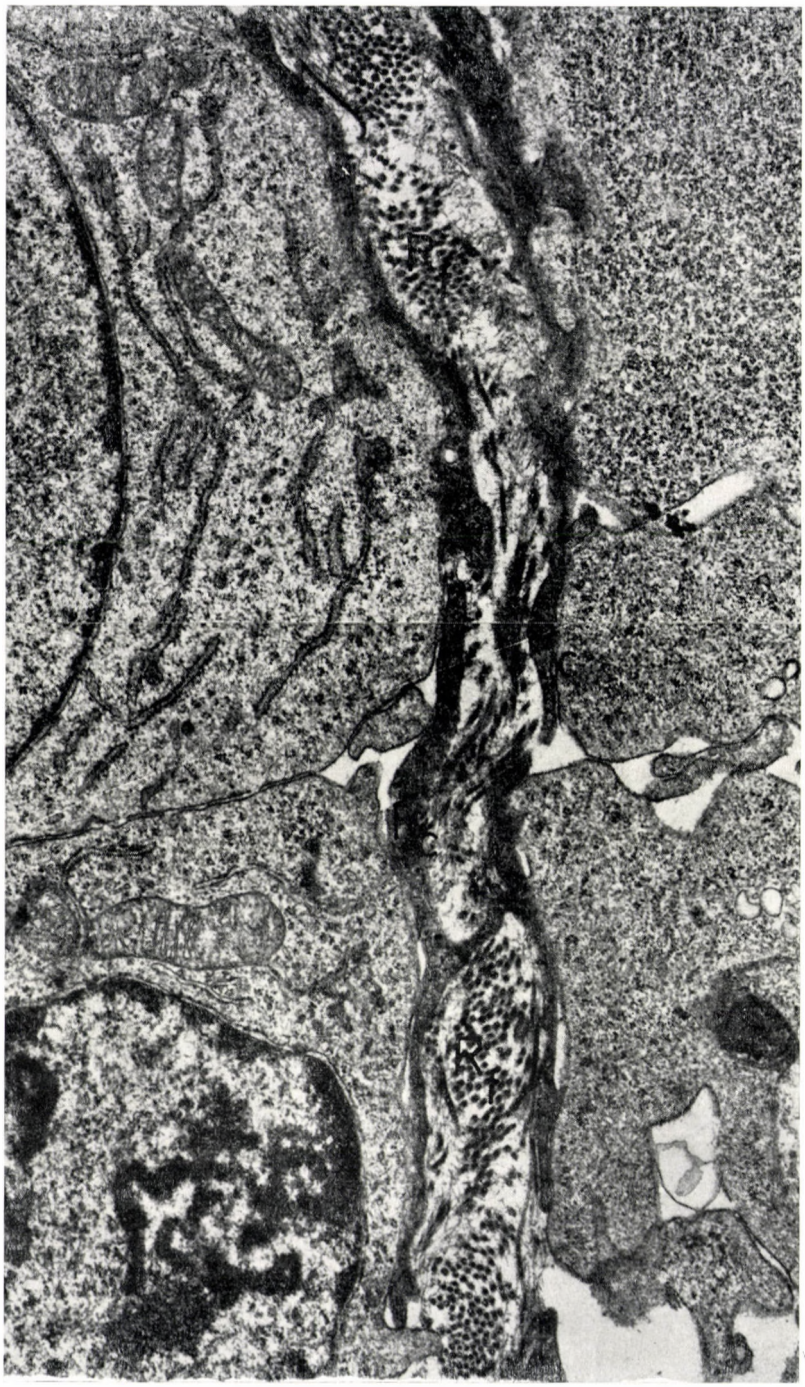

Fig. 7. Reticular fibres (Rf) of a normal animal. They surrounded by a reticular cell sheath (Rc), isolating the fibrillar substance of the fibres from the lymphoid parenchyma, ×18,200

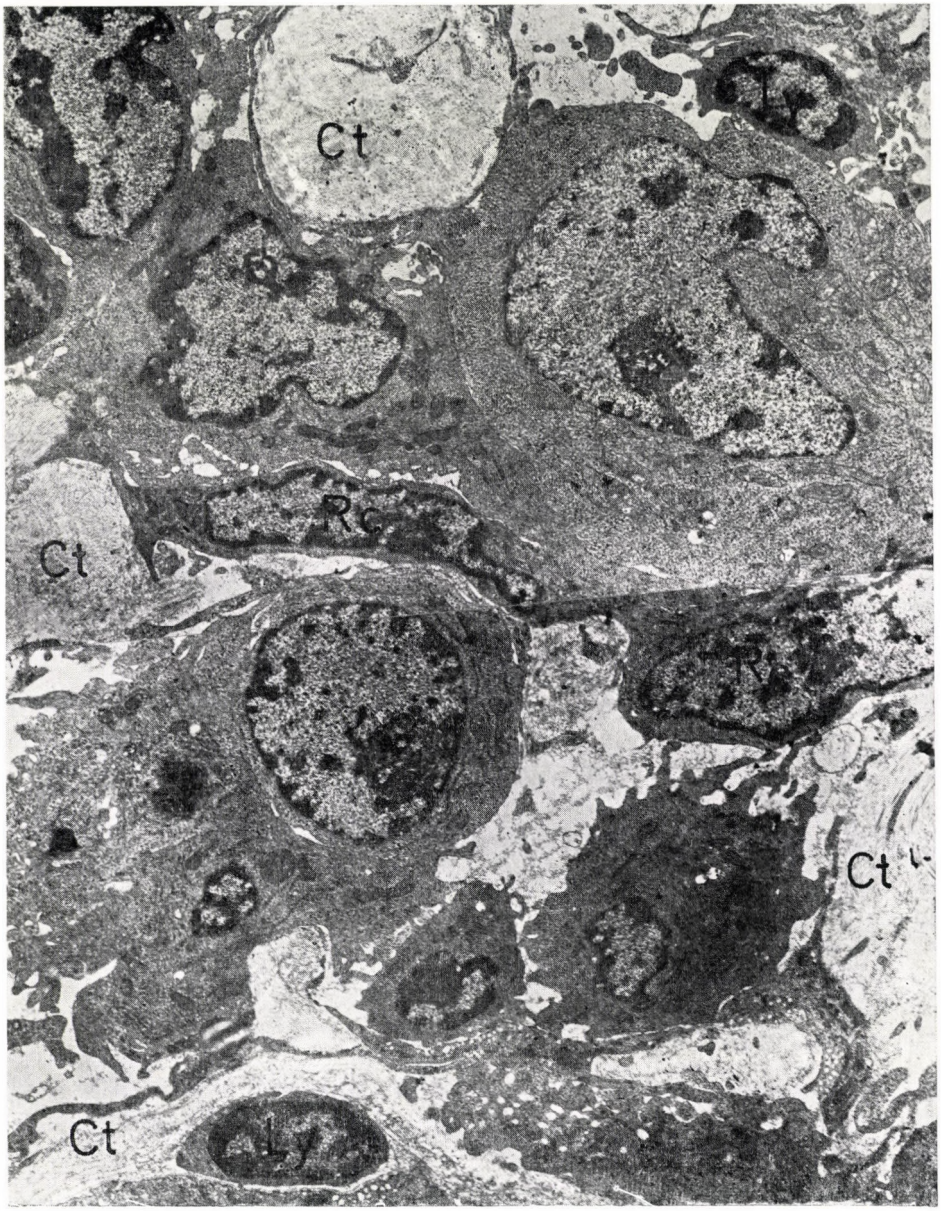

Fig. 8. Extra-follicular substance of a thymectomized animal. Mainly young lymphoid cells with some lymphocytes (Ly) are visible. In the connective tissue mass (Ct) reticular cells (Rc) appear like fibrocytes. ×6400

count parallel to a rise in the number of plasma cell limited mainly to the basal part of the epithelium (Fig. 6).

In normal animals the reticular fibres consisting of collagenous microfibrils are surrounded by a continuous reticular cell sheath (Fig. 7) which separates the lymphoid elements of the parenchyma from the reticular fibres. After thymectomy the characteristic link between reticular fibres and reticular cell was upset. Forming large quantities of collagen the reticular cells are unable to cover the reticular fibres and to isolate their collagenous substance from the lymphoid parenchyma. Among the scattered collagen fibres and lymphoblasts II the reticular cells appear like fibrocytes (Fig. 8).

Discussion

The peripheral lymphoid organs divide into compartments T and B. After neonatal thymectomy mainly compartment T is depleted of lymphocytes [15, 23]. This finding is supported by the observations of de SOUSA et al. [19] on congenitally hypothymic mice. As in mice and rats only the lymph nodes, spleen and Peyer's patches had been studied, in the lack of observations concerning the tonsils their classification has been based on indirect functional data.

A number of authors has proved the specific antibody production of the tonsils in vivo and in vitro [2—4, 8, 17, 18, 21] a function characteristic of the peripheral lymphoid organs.

FICHTELIUS [6] studied the number of mitoses and DNA synthesis of lymphocytes in the tonsillar epithelium and in the epithelium of the gut associated lymphoid organs and found that these are identical with those of the thymus. This author therefore assumed that the tonsils belong to the central lymphoid organs. Later, however, FICHTELIUS [7] told about mixed: central and peripheral immune-organ and considered the epithelium to represent a central and the subepithelial tissue a peripheral lymphoid organ. The existense of mixed immune organs has been suggested also by STRAMIGNONI et al. [20], who believe on grounds of experiments with PHA on the rabbit appendix that in the first days of postnatal life the appendix functions as a central lymphoid organ and becomes only later of a peripheral nature.

Recently, SUZUKI and INO [22] have suggested the existence of a thymus–tonsil interaction since ^{3}H-thymidine uptake by the tonsils is lower after thymectomy.

In an earlier study [13] we found in the extrafollicular substance of the tonsils venules with a high endothelial lining which by their structure and intensive lymphocyte flow might be analogous to those found in the thymus dependent deep cortical substance of mouse lymph nodes. It was therefore

assumed that the surrounding tonsillar region is analogous to compartment of the lymph nodes and might stand under the influence of the thymus.

The validity of this assumption has been confirmed by changes in the supporting system of the tonsils and in the lymphoid cellular composition of the parenchyma after thymectomy. The former showed a certain lack of organization: the characteristic links of the reticular cells with the reticular fibres were upset. It seems that of the dual function of reticular cells that is production of the ground substance of reticular fibres (fibrocyte type function) remained intact and ensheathing of the reticular fibre (reticular cell function) which is characteristic for lymphoid organs has been damaged. It is not clear whether the disorganization of the supporting system occurs after the lesion of the lymphoid parenchyma or the transformation of the supporting system can be attributed directly to the influence of the thymus.

Changes in the lymphoid parenchyma are related to lymphocyte depletion which was conspicuous around the venules lined with high endothelium in the extrafollicular substance. In these tonsillar regions instead of small lymphocytes lymphoblasts II dominate, while in normal animals lymphoblasts I are in preponderance in the extrafollicular substance. Lymphoblasts II are the characteristic cells of the germinal centres.

A certain relationship seems to exist between the increase in number of lymphoblasts II accumulating in the extrafollicular substance and of the similar cells in the epithelium. The large number of young and mature plasma cells in the epithelium suggests that lymphoblasts II which had migrated into the epithelium have differentiated in loco into plasma cells and may have originated from the follicules.

The reduction of the lymphocyte is not limited to the extrafollicular substance, but might be observed in the epithelium too. KOBURG [10, 11] and MEYER zum GOTTESBERGE and KOBURG [12] found with tests ^{3}-H-thymidine that the lymphoid elements in the epithelium originate from the germinal centres which is bursa-dependent.

The reduction in the number of lymphocytes appearing in the epithelium after thymectomy suggests, however, the migration of a considerable number of T lymphocytes beside B lymphocytes into the epithelium from the extrafollicular region which is under the influence of the thymus.

Conclusions

1. In the extrafollicular substance of the tonsils there are many venules lined by high epothelium through which an intensive lymphocyte migration takes place between circulation and tonsillar substance.

2. After neonatal thymectomy in these regions a lymphocyte depletion

can be observed. This also manifests in a reduced lymphocyte migration through the venules lined with high endothelium.

3. A considerable accumulation of connective tissue appears in the extrafollicular substance and the intimate link between reticular cells and reticular fibres is impaired.

4. The number of lymphoblasts is higher in the extrafollicular region.

5. In the epithelium there is an increased number of plasma cells which had probably differentiated from lymphoblasts.

6. The characteristic changes following thymectomy suggest that the extrafollicular substance of the tonsils is under the influence of the thymus and should therefore be considered thymus-dependent region.

REFERENCES

1. Cooper, M. D., Perey, D. Y., McKneally, M. F., Gabrielson, A. E., Sutherland, D. E. R., Good, R. A.: (1969) A mammalian equivalent of the avian bursa of Fabricius. Lancet **1,** 1388. — 2. Diamanstein, T., Hafferberth, J.: (1969) Nachweis spezifischer 19-S antikörperbildener Zellen in der Tonsille. Arch. Ohr-, Nas.-u. Kehlk-Heilk. **194,** 432. — 3. Drabe, J.: (1957—58) Immunisierungsversuche an Kaninchen bei nasaler Bakterienzufuhr. Arch. Ohr-, Nas-u. Kehlk.-Heilk. **171,** 287. — 4. Drabe, J.: (1961) Experimentelle Untersuchungen zur Frage der Antikörperbildung in den Tonsillen. Fortsch. Hals-Nas.-Ohrenheilk. **7,** 217. — 5. Ewing, J.: (1929) Lymphoepithelioma. Amer. J. Path. **5,** 99. — 6. Fichtelius, K. E.: (1967) The mammalian equivalent to bursa of Fabricius birds. Exp. Cell Res. **46,** 231. — 7. Fichtelius, K. E., Sundstrom, C., Kullgren, B., Linna, J.: (1969) The lympho-epithelial organs of Homo sapiens revisited. Acta path. microbiol. cand. **77,** 103. — 8. Ishikawa, T., Widner, K., Arbesman, C. E.: (1972) Distribution of immunoglobulins in palatine and pharyngeal tonsils. Int. Arch. Allergy **43,** 801. — 9. Jolly, J.: (1913) Sur les organes lympho-épitheliaux. C. R. Soc. Biol. (Paris) **74,** 540. — 10. Koburg, E.: (1963) Autoradiographische Untersuchungen über die Zellenbildungsvorgänge im Lymphknoten und Tonsillen. Arch. klin. exp. Ohr-, Nas- u. Kehlk. Heilk. **182,** 135. — 11. Koburg, E.: (1970) Die Tonsille in immunologischen Geschehen. Arch. klin. exp. Orh-, Nas- u. Kehlk. Heilk. **196,** 65. — 12. Meyer zum Gottesberge, A., Koburg, E.: (1964) Zellproliferation und Zellwanderung in den Tonsillen. Acta otolaryng. (Stockh.) **57,** 383. — 13. Oláh, I., Surján, L. jr.: (1973) Light and electron microscopical observation of the tonsillar epithelium and of post-capillaries with thick endothelium. Xth World Congress of Otorhinolaryngology, Venice. — 14. Oláh, I. Röhlich, P., Törő, I.: (1975) Ultrastructure of lymphoid organs. An electron microscopic atlas. Akadémiai Kiadó, Budapest. — 15. Parrott, D. M. V., de Susa, M. A. B., East, J.: (1966) Thymus-dependent areas in the lymphoid organs of neonatally thymectomized mice. J. exp. Med. **123,** 191. — 16. Retterer, E.: (1886) Disposition et connections du réseau lymphatique dans les amygdales. C. R. Soc. Biol. (Paris) **8,** 27. — 17. Schürmann, F.: (1954) Ein Beitrag zur Frage der Antikörperbildung in den Tonsillen. Pract. oto-rhino-laryng. **16,** 153. — 18. Skoog, T.: (1936) To the question of the physiological significance of the tonsils, an animal-experimental study. Acta otolaryng. (Stockh.) **23,** 63. — 19. de Sousa, M. A. B., Parrott, D. M. U., Pantelouris, E. M.: (1969) The lymphoid tissues in mice with congenital aplasia of the thymus. Clin. exp. Immunol. **4,** 637. — 20. Stramignoni, A., Molló, F., Bussolati, G., Navone, R., Monga, G.: (1970) Morphological and biological properties of rabbit's appendiceal lymphocytes. Acta anat. (Basel) **76,** 579. — 21. Surján, L. jr., Surján, M.: (1970) The antibody production of tonsils. Arch. Ohr-, Nas- u. Kehlk.-Heilk. **195,** 331. — 22. Suzuki, M., Ino, H.: (1973) Relation between thymus and tonsils. Arch. Ohr-, Nas- u. Kehlk.-Heilk. **204,** 249. — 23. Wakeman, B. H., Arnason, B. G., Jankovic, B. D.: (1962) Role of the thymus in immune reactions in rats. III. Changes in the lymphoid organs of thymectomized rats. J. exp. Med. **116,** 187.

DIE WIRKUNG DER NEONATALEN THYMEKTOMIE AUF DIE KANINCHENTONSILLEN. EINE LICHT- UND ELEKTRONENMIKROSKOPISCHE STUDIE

I. OLAH und I. TÖRŐ

In der extrafollikulären Substanz der Tonsillen ist eine intensive Lpmphozytenmigration über die mit hohem Endothel ausgekleideten Venulae wahrnehmbar. Nach der Thymektomie nimmt die Lpmphozytenmigration ab, während in der Gefäßumgebung Lymphozyten-Depletion Bindegewebsvermehrung und die Lösung der inningen Bindung zwischen den Retikulumzellen und den Gitterfasern wahrnehmbar ist. Die Lymphozyten-Depletion läßt sich auch im Epithel beobachten. Aufgrund der Untersuchungen darf angenommen werden, daß die extrafollikulären Tonsillenbereiche, ähnlich der tiefen Rindensubstanz der Lymphdrüsen, unter dem Einfluß des Thymus stehen und somit zu den peripheren Lymphorganen gehören.

ДЕЙСТВИЕ НЕОНАТАЛНОЙ ТИМЕКТОМИИ НА МИНДАЛИНЫ КРОЛИКОВ. МИКРОСКОПИЧЕСКОЕ И ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ

И. ОЛА и И. ТЁРЁ

Через выстланные высоким эндотелием малые вены, находящиеся во внефолликулярном веществе миндалин, наблюдается интенсивная миграция лимфоцитов. После тимектомии миграция лимфоцитов уменьшается, причем вокруг сосудов наблюдается уменьшение количества лимфоцитов, накопление соединительной ткани и прекращение тесной связи между ретикулярными клетками и решетчатими волокнами. Уменьшение количества лимфоцитов выявляемо также в эпителии. На основе исследований можно полагать, что внефолликулярные зоны миндалин — подобно глубокому корковому веществу лимфатических узлов — находятся под влиянием зобной железы, и что они, следовательно, относятся к периферическим лимфатическим органам.

Dr. Imre OLÁH
Dr. Imre TÖRŐ
Semmelweis Orvostudományi Egyetem II. sz. Anatómiai Intézet
1094 Budapest, Tűzoltó u. 58., Hungary

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Institute of Anatomy, Histology and Embryology, University Medical School, Debrecen

STRUCTURE OF THE OSTEOCYTE CAPSULE IN RATS WITH HYPERVITAMINOSIS-D AND RICKETS

Klára MATESZ, I. FÖLDES and L. MÓDIS

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A histochemical and topo-optical study was made of the changes of the osteocyte capsule (OC) of the cortical and spongious bone layers of the tibia and vertebra of rats with hypervitaminosis-D and rickets.

In hypervitaminosis-D the number of stained OCs was found to decrease. After treatment with high doses of vitamin D, a broadening of the OCs was noted in the area of demineralizing bone. Under the polarization microscope a decrease occurred in the proportion of birefringent OCs, more marked in birefringence than in staining. Since bone demineralization due to vitamin D was not followed by a rise in the number of osteoclasts, it is concluded that osteocytic activity has an important role in demineralization as shown by the broadened OC. In rachitic rats considerably fewer OC took the stain and showed birefringence than in the controls. Thus, alteration of the OC structure disturbs the nutrition of bone tissue and thereby aggravates the pathological process.

Introduction

The osteocyte capsule (OC) described by Virchow is a zone of special ground substance surrounding the osteocyte and the processes. The structure is termed "Grenzscheide", "Knochenkapsel", or "Randzone" in the German, "gaine limitante", or "rebord lacuno canaliculaire" in the French, and "osteocyte capsule" in the English literature. These terms correspond to Rouget-Neuman's sheath of the classical histological literature. Since its first description, the OC has been the subject of numerous investigations.

For a long time the OC was considered homogenous in nature. WEIDENREICH [27] was the first to suggest its fibrillar structure. The first detailed histochemical observations are known from LORBER's [18] work who demonstrated the presence of acid mucopolysaccharides (MPS), lipides and alkaline phosphatase in the OC. LIPP [16] found PAS positivity in this zone. HARRIS and HAM [13] were the first to suggest that the OC may play a functional role in diffusion between bone cells and capillaries.

SCHMIDT [23] observed birefringent OCs in non-decalcified bone sections stained with Congo red. By topo-optical reactions using gold chloride, SCHMIDT demonstrated that the OC contains collagen fibres running parallel to the surface. The electron microscopic structure of the OC has been studied by BAUD [1], BAUD and DUPONT [2], BAUD and MORGENTHALER [3]. These authors consider the OC as an amorphous substance rich in MPSs, in which collagen

fibrils are present. BAUD distinguished two types of OC, an osteoblastic and an osteoclastic type. According to his measurements the OC (termed by him lacuno-canalicular system) has a surface of 250 mm^2/mm^3 interstitial bone tissue. BÉLANGER [4] suggested that osteocytes are non-resting cells capable of building up and destroying ground substance.

Similar changes were noted by REMAGEN et al. [21] after administration of dihydrotachysterol. These authors demonstrated calcium and phosphate crystals in the OC. Then MÓDIS [19] demonstrated that the OC was not amorphous but actually a structured pericellular layer in which the MPS chains are situated parallel to the cell surface showing an orientation of higher order than that of the bone matrix. The structure was found to change with advancing age and damaging factors affect the staining and briefringence of the OC [19].

Histological examinations using haematoxylin chromotrope have revealed that bone demineralization due to vitamin D was not associated with a numerical increase of osteoclasts and BÉLANGER [4] described the process of osteocytic osteolysis. These observations have made us to assume that in addition to osteoclastic resorption some other factors may also be involved in the demineralization due to hypervitaminosis D and we have started studies on the structure of the osteocyte capsule.

Material and methods

In the experiments, 12 albino rats of 100 g weight of both sexes were used. The animals were divided in 3 groups of 4 rats each Group I was treated with doses of 10 000 I. U. vitamin D_3 in oil administered orally through a stomach tube daily for 14 days. The rats of Group II received 40 000 I. U. vitamin D_3. Group III included the control.

After killing the rats by exanguination the tibia and three thoracic vertebrae were removed for examination. Half of the material was fixed in formol and the other half fixed and decalcified in Susa's fluid, then embedded in paraffin and sections 10—12 μ thick were cut and kept in xylol overnight at 37 °C. The sections were stained with 0.1% toluidine blue solution (Chroma) at pH 3.5 [22].

For evaluation, in each section 500 osteocytes were counted both in the compact and in the spongious layer, and the proportion of stained OC was established. The visual fields examined were then examined in polarized light, using the same microscope. Here we have also counted the osteocytes surrounded by birefringent capsules. Then we calculated:

— the percentage of stained OCs around the osteocytes, as seen in the light microscope;
— the percentage of stained OCs showing polarizing activity.

The data were illustrated graphically. The same calculations were performed for the controls.

In addition, the effect of hypovitaminosis D on the number and structure of the OCs has also been studied in 8 young albino rats of 25—30 g weight, these animals were fed Mc Collum's rachitogenic diet for 8 weeks, while the other 4 served as controls. The material was processed and evaluated in the same way as in the first experiment.

In every specimen the alcian blue—PAS reaction and in the non-decalcified sections, Kossa's reaction was performed.

Results

On vitamin D treatment the OCs became thicker under the effect of 10,000 I. U. vitamin D_3, the thickening was restricted to small, well delimited area of the spongiosa. After administration of such small doses, the OCs gave

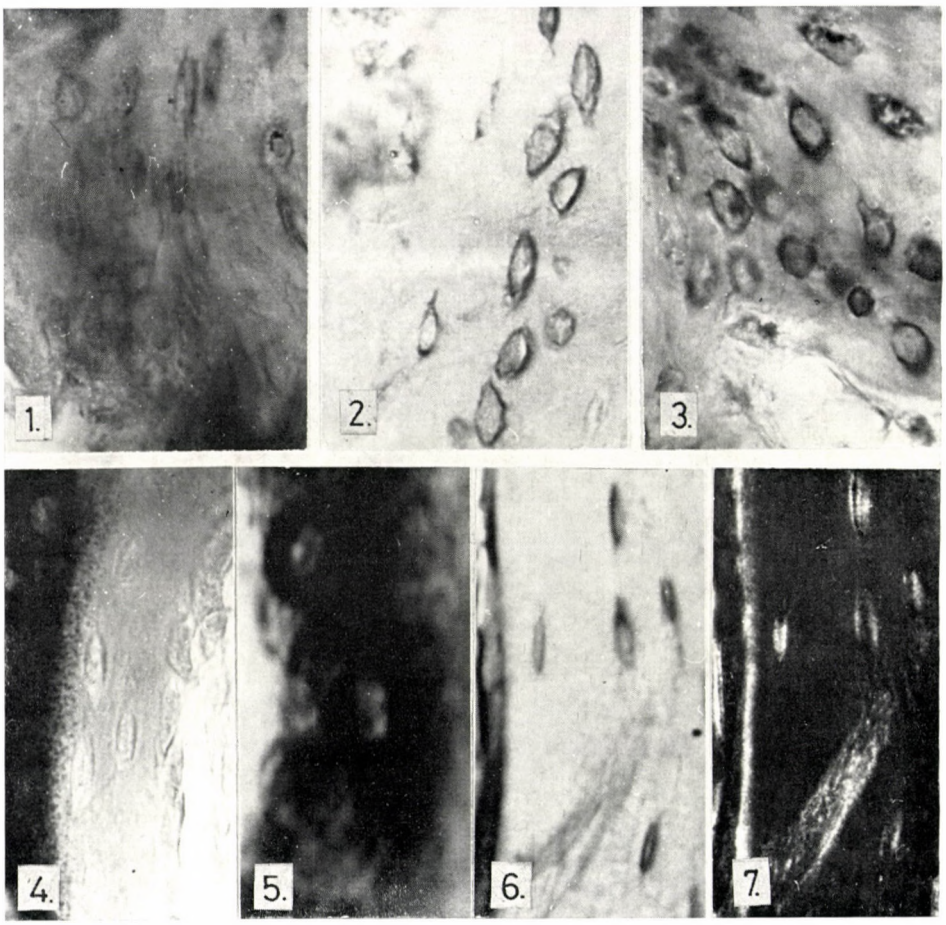


Fig. 1. Cortical bone from tibia of control rat. Light photomicrograph. Alcian blue — PAS reaction. Magnification: 40 × 6.3 × 0.5

Fig. 2. Cortical bone from tibia of rat treated with 10 000 I. U. vitamin D_3. Light photomicrograph. Alcian blue — PAS reaction. Magnification: 40 × 6.3 × 0.5

Fig. 3. Cortical bone from tibia of rat treated with 40 000 I. U. vitamin D_3. Light photomicrograph. Alcian blue — PAS reaction. Magnification: 40 × 6.3 × 0.5

Fig. 4. Cortical bone from tibia of rat treated with 10 000 I U. vitamin D_3. Light photomicrograph. Kossa reaction. Magnification: 63 × 6.3 × 0.5

Fig. 5. Cortical bone from tibia of rat treated with 40 000 I. U. vitamin D_3. Light photomicrograph. Kossa reaction. Magnification: 63 × 6.3 × 0.5

Fig. 6. Cortical bone (tibia) of control rat. Light photomicrograph. Toluidine blue pH 3.5. Magnification: 40 × 6.3 × 0.5

Fig. 7. Cortical bone from tibia of control rat. Polarization micrograph. Toluidine blue pH 3.5. Magnification: 40 × 6.3 × 0.5

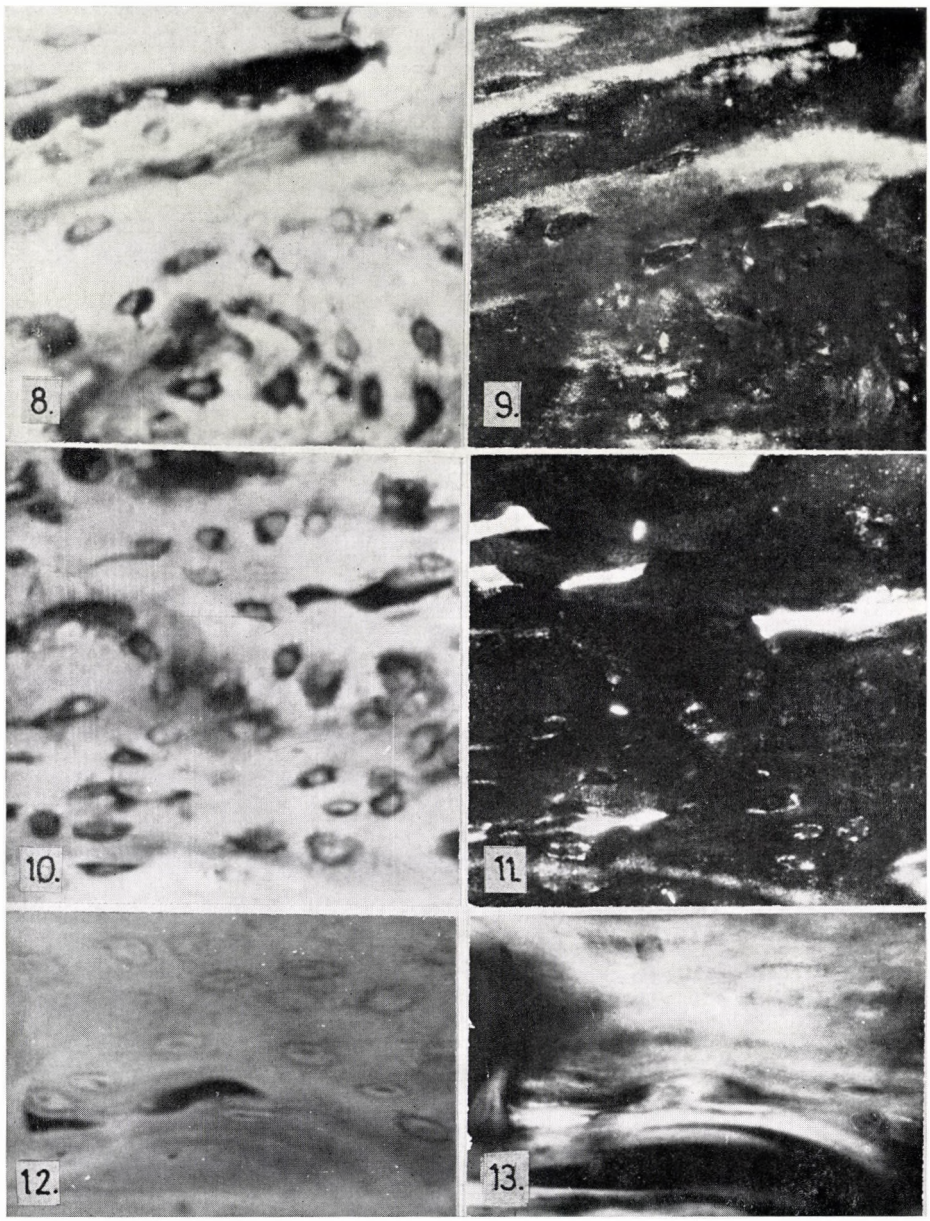


Fig. 8. Spongious layer from tibia of rat treated with 10 000 I. U. vitamin D_3. Light photomicrograph. Toluidine blue pH 3.5. Magnification: 40 × 6.3 × 0.5

Fig. 9. Spongious layer from tibia of rat treated with 10 000 I. U. vitamin D_3. Polarization micrograph. Toluidine blue pH 3.5. Magnification: 40 × 6.3 × 0.5

Fig. 10. Spongious layer from tibia of rat treated with 40 000 I. U. vitamin D_3. Light photomicrograph. Toluidine blue pH 3.5. Magnification: 40 × 6.3 × 0.5

Fig. 11. Spongious layer from tibia of rat treated with 40 000 I. U. vitamin D_3. Polarization micrograph. Toluidine blue pH 3.5. Magnification: 40 × 6.3 × 0.5

Fig. 12. Cortical layer from tibia of rachitic rat. Light photomicrograph. Toluidine blue pH 3.5. Magnification: 40 × 6.3 × 0.5

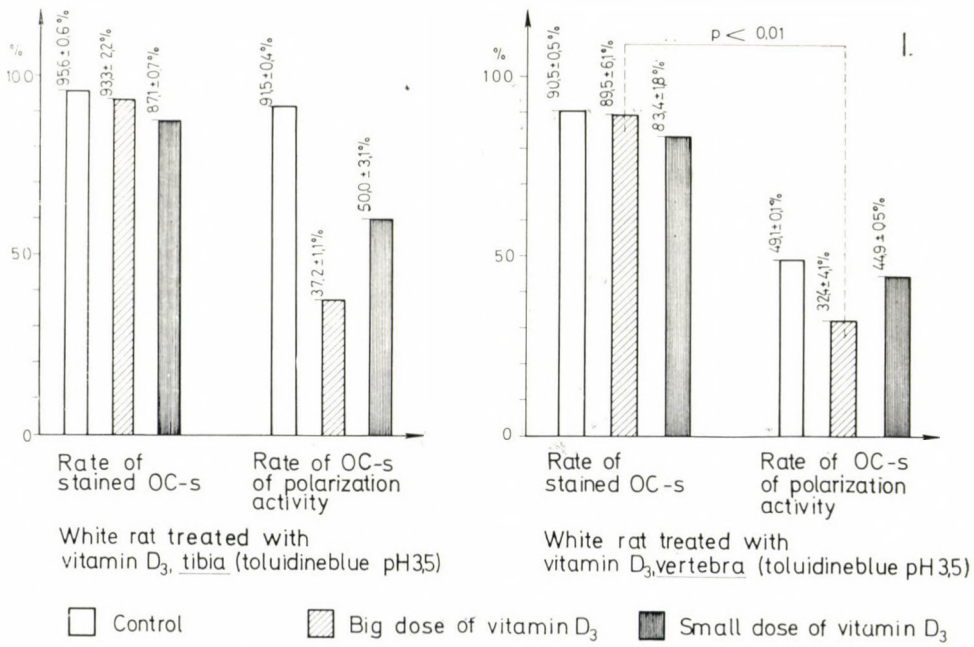
Fig. 13. Cortical layer from tibia of rachitic rat. Polarization micrograph. Toluidine blue pH 3.5. Magnification: 40 × 6.3 × 0.5

a positive alcian blue or PAS reaction in the area of demineralization of the cortical bone (Figs 1 and 2). The broadened capsules were positive for calcium. After large doses, demineralization became more conspicuous and more extensive, accompanied by a marked enlargement of the capsules, particularly in the spongiosa (Fig. 3).

While in the demineralizing cortical bone the OC were broad, they were less broad in the demineralized bone where they exhibited an equally intense alcian blue, PAS and Kossa positivity (Figs 4, 5).

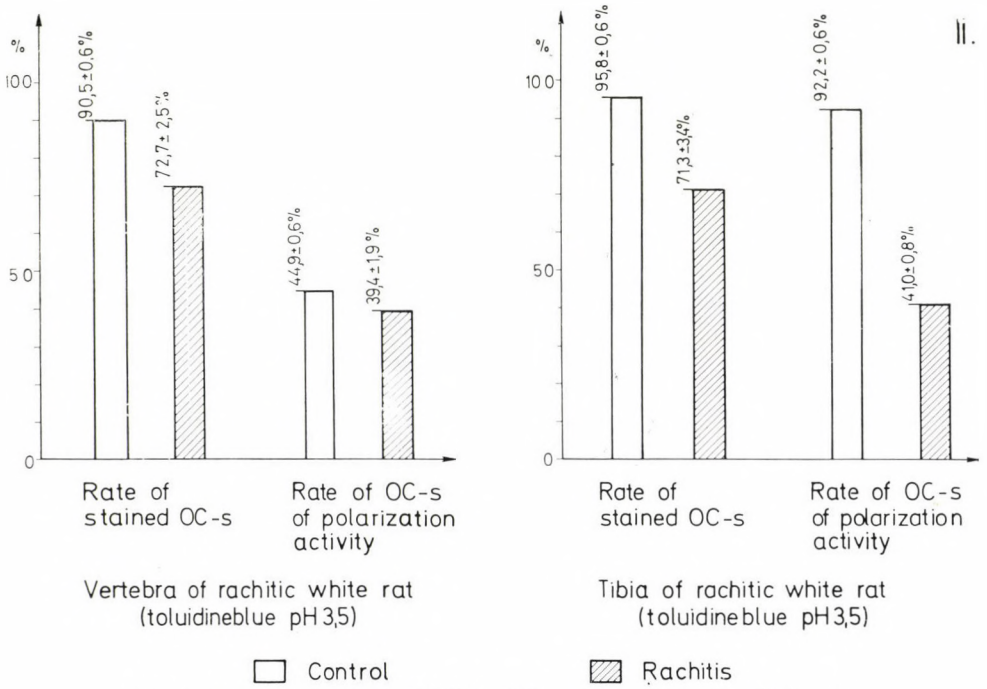
On treatment with large doses of vitamin D the number of stained capsules decreased in the spongious and cortical layers of the vertebra and tibia as compared to the controls (Figs 6 and 10). In both bones a marked reduction of birefringence was noted in the OCs (Figs 7 and 11). As shown in Graph I, in the case of the vertebra the difference was significant ($p < 0.01$).

Small doses of vitamin D caused a somewhat greater reduction in staining intensity than did large ones (Figs 8, 10). In polarized light the decrease was not so marked as with large doses (Figs 9, 11) in both the tibia and the vertebra. With regard to the latter bone the difference was highly significant ($p < 0.001$) as seen in Graph I.



Graph I Measurements of OC in tibia and vertebra of rat treated with vitamin D_3.

In the light microscope the sections obtained from rachitic animals showed less stained OCs than did the controls (Fig. 12). In polarized light change was of the same direction but the decrease of the number of birefringent OCs was more marked (Fig. 13; Graph II). In rachitic bone preparations the ground substance exhibited a lively dichroism.



Graph II Measurements of OC in tibia and vertebra of rachitic rat.

Discussion

The results have confirmed the statement that in the OC the MPSs are oriented parallel to the surface, [19].

1. On vitamin D treatment, especially if high doses are given, the OCs become considerably broader. Owing their varying shape, this could not be verified by measurements.

The thickness of the OCs is not uniform even under physiological conditions. In newly formed spongious bone the lacunae are large, while in lamellar bone and mature matrix, narrower [12].

According to Liu et al. [17] in hypervitaminosis-D the volume of the lacuno-canalicular system in the rat bone increases by 7%. On the basis of microradiographic investigations, Duriez and Cauchoix [9] concluded that

parathyroid hormone and hypervitaminosis-D are responsible for osteocytic osteolysis. As evidenced by light microscopic examinations, vitamin-D plays a role in osteocytic resorption. The latter process is entirely absent in the bones of chicken fed a diet low in Ca, and deficient in vitamin. After vitamin D treatment, incipient osteolysis can be noted which increases in intensity on the combined administration of vitamin D and parathyroid hormone [5]. TRUMMEL et al. [25] observed a parallel loss of the matrix and mineral salts in the bone under the effect of 25 HCC treatment.

By means of alpha radiography BÉLANGER and RASMUSSEN [7] and BÉLANGER et al. [6] demonstrated enlarged OC in the resorbing bone. In secondary hyperparanthyroidism, increased lacunar diameters were found in the phase of resorption [8]. After administration of parathyroid hormone an intense PAS positivity was noted in the matrix around the osteocyte [12]. Stimulation of osteocytic resorption is accompanied by increased metachromasia and protease activity of the OC [5]. A similar change was noted in endogenous parathyroid hyperactivity in animals fed a diet rich in P or poor in Ca [10]. Enlarged capsules are always present in the demineralizing bone. This finding seems to indicate that under certain conditions the osteocyte is able to resorb bone ground substance.

2. Regarding the structure of the OC we refer to the results of MÓDIS [19] who demonstrated that the OC appears in the rat bone on the 42nd postnatal day. The oriented MPS chains of the capsule correspond to chondroitin sulphate A and C, and they increase with age. According to JANDE and BÉLANGER [15] and JANDE [14] osteocyte capsules rich in oriented MPS are characteristic of resorptive osteocytes rather than of formative ones. These authors suggest that during resorption the transport paths of the OCs are under increased demand. This suggestion has been supported by WASSERMAN and YAEGER [26], BAUD and MORGENTHALER [3], DUPONT [8], and REMAGEN et al. [21]. The OC might have a role in matrix mineralization too. Similarly as REMAGEN et al. [21], we have also demonstrated Ca salts in the OC. LORBER [18] found alkaline phosphatase in the OC.

According to our findings, the number of birefringent OCs decrease considerably during demineralization due to hypervitaminosis-D. The capsules become broader but less birefringent. This phenomenon may be explained in several ways. Altered homeostasis may a priori change the synthesis of MPS. Enchanced bone resorption when not associated with the presence of large numbers of osteoclasts leads to increased esteocytic osteolysis. The presence of broadened, highly basophilic osteocyte capsules may be a sign of this process. The large amount of Ca accumulated in the OC may have a desorienting effect on the MPS chains.

In rachitic rats, the OCs likewise displayed a decrease in polarizing activity. STEENDIJK et al. [24] found that vitamin D deficiency was associated

with secondary hyperparathyroidism. DURIEZ and CAUCHOIX [9] described increased osteocytic activity in rickets.

Our results allowed to conclude that hypervitaminosis-D brings about a well-visible thickening of the OC which contains an alcian blue — PAS, and Kossa positive substance. This enlarged lacuno-canalicular system which even in its normal state amounts to double the osteoclastic Howship's lacunar system [11] is capable of removing the resorption products resulting from the effect of hypervitaminosis-D. Demineralization might thus be the result of active osteocytic activity or a consequence of the structural change taking place in the bone ground substance under the effect of vitamin D. The latter hypothesis seems to be supported by the change in structure of the OC. In fact, the number of birefringent OCs decreases markedly both in the tibia and in the vertebra in hypervitaminotic animals.

The altered OC structure may impair the nutrition of the bone tissue which in turn, may further aggravate the pathological condition.

REFERENCES

1. BAUD, C. A.: (1962) Morphologie et structure inframicroscopique des osteocytes. Acta anat. **51**, 209—225. — 2. BAUD, C. A., DUPONT, D. H.: (1962) The fine structure of the osteocytes in the adult compact bone. Proc. Fifth International Congress for Electron Microscopy, Philadelphia 1962. Ed. S. S. Breese, Jr. Vol 2. P. 10 Academic Press, New York. — 3. BAUD, C. A., MORGENTHALER, P. W.: (1963) Structure submicroscopique du rebord lacuno-canaliculaire osseux. Morph. Ib **104**, 467—486. — H. BÉLANGER, L. F.: (1969) Osteocytic Osteolysis. Calc. Tiss. Res. **4**, 1—12. — 5. BÉLANGER, L. F.: (1971) Osteocytic resorption. In: The Biochemistry and Physiology of Bone. Development and growth. **3**, 239. Ed.: Bourne, G. H. Vol 3, P 239. Academic Press, New York. — 6. BÉLANGER, L. F., JARRY, L., UHTHOFF, H. K.: (1968) Osteocytic osteolysis in Paget's disease. Rev. canad. Biol. **27**, 37. — 7. BÉLANGER, L. F., RASMUSSEN, P.: (1968) Inhibition of osteocytic osteolysis by thyrocalcitonin and some antigrowth factors. In: Parathyroid Hormone and Thyrocalcitonin (calcitonin). Eds.: Talmage, R. V., Bélanger, L. F. 156. Excerpta Med. Found. Amsterdam. — 8. DUPONT, D. A.: Contribution à l'étude de la croissance et du remainement osseux chez la souris. Thèse de Genève Albert Kundig Genève. 1964. — 9. DURIEZ, J., CAUCHOIX, J.: (1967) Le rôle des ostéocytes dans la résorption du tissu osseux. Presse méd. **75**, 1297. — 10. DURIEZ, J., GHOSEZ, J. É., FLAUTRE, B.: (1965) La resorption ou lyse périostéocytaire et son rôle possible dans la destruction du tissu osseux. Presse méd. **73**, 2581. — 11. FROST, H. M.: (1962) Specific surface and specific volume of normal human lamellar bone. Henry Ford Hosp. med. Bull. **10**, 35. — 12. HANCOX, N. M.: (1972) Biology of bone. Biological structure and function. The University Press, Cambridge. — 13. HARRIS, W. R., HAM, A. W.: (1956) The mechanism of nutrition in bone and how it affects its structure, repair and fate of transplantation. Ciba Foundation Symposium on Bone Structure and Metabolism. Ed.: Wolstenholme G. E. W., O'Connor C. M. Churchill, London, Pp. 135—147. — 14. JANDE, S. S.: (1971) Fine structural study of osteocytes and their surrounding bone matrix with respect to their age in young chicks. J. Ultrastruct. Res. **37**, 279—300. — 15. JANDE, S. S., BÉLANGER, L. F.: (1971) Electron microscopic of osteocytes and the pericellular matrix in rat trabecular bone. Calc. Tiss. Res. **6**, 280—289. — 16. LIPP, W.: (1954) Neuuntersuchungen des Knochengewebes. Acta anat. (Basel) **20**, 162—200. — 17. LIU, CH.-CH., BAYLINK, D. J., WERGEDAL, J.: (1974) Vitamin D- enhanced osteoclastic bone resorption at vascular canals. Endocrinology **95**, 1011—1018. — 18. LORBER, M.: (1951) A study of the histochemical reactions of the dental cementum and alveolar bone. Anat. Rec. **111**, 129—144. — 19. MÓDIS, L.: Topo-optical investigations of mucopolysaccharides (Acid glycosaminoglycans). Handbuch der Histochemie. Graumann, W., Neumann, K. Vol. II/4. Gustav Fischer, Stuttgart. 1974. — 20. RASMUSSEN, P.: (1969) The action of vitamin D deficiency on bone tissue and the epiphyseal plate in rats given adaequate

amounts of calcium and phosphorus in the diet. Arch. oral Biol. **14,** 1293. — 21. Remagen, W. Höhling, H. J., Hall, T. A., Ceasar, R.: (1969) Electron microscopical and microprobe observations on the cell health of stimulated osteocytes. Calc. Tiss. Res. **4,** 60—68. — 22. Romhányi, G.: (1963) Über die submikroskopische strukturelle Grundlage der metachromatischen Reaktion. Acta histochem. (Jena) **15,** 201—233. — 23. Schmidt, W. J.: (1959) Grenzscheiden der Lakunen und Kittlinien des Knochengewebes. Polarisationsoptische Analyse kollagenfreier kongorotgefärbter Schliffe. Z. Zellforsch. **50,** 275—296. — 24. Steendijk, R., Nielsen, H. K., Krhai, A.: (1968) Osteotomy, vitamin D and the metabolism of calcium and inorganic phosphate in vitamin D resistant rickets and osteomalacia. Helv. paediat. Acta **23,** 627. — 25. Trummel, C. L., Raisz, L. C., Blunt, J. W., Deluca, H. F.: (1969) 25-hydroxycholecalciferol stimulation of bone resorption in tissue culture. Science **163,** 1450. — 26. Wasserman, F., Yaeger, Y. A.: (1963) Fine structure of the osteocyte capsule and of the wall of the lacune in bone. Z. Zellforsch. **67,** 636—652. — 27. Weidenreich, F.: Das Knochengewebe. In: Handbuch der Mikroskopischen Anatomie des Menschen. Ed. by W. von Möllendorf, Vol. 2. Springer, Berlin. 1930.

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STRUKTURELLE UNTERSUCHUNG DER OSTEOZYTEN-KAPSEL BEI RATTEN MIT D-HYPERVITAMINOSE UND RACHITIS

Von

K. MATESZ, I. FÖLDES und L. MÓDIS

In der Kortikalis und in der Spongiosa der Tibia und der Wirbelknochen von Ratten mit D-Hypervitaminose und Rachitis wurden die Veränderungen der Osteozyten-Kapseln (OC) mit histochemischen und topooptischen Reaktionen untersucht.

Bei D-Hypervitaminose ist eine Abnahme des prozentuellen Anteils der sich färbenden OC wahrnehmbar. Nach Behandlung mit hohen D-Vitamindosen erfolgt im Bereich des sich demineralisierenden Knochens eine Verbreiterung der OC. Im Polarisationsmikroskop sieht man eine Abnahme des Anteils der eine Doppelbrechung aufweisenden OC. Die Doppelbrechung nimmt in viel größerem Maße ab als die Färbung. Aufgrund der Beobachtung, daß nach der unter D-Vitamin-Einfluß eintretenden Demineralisation kein Anstieg der Osteoklastenzahl erfolgt, wird angenommen, daß die Demineralisation die Folge einer aktiven Osteozytentätigkeit darstellt. Diese Aktivität wird durch die Verbreiterung der OC repräsentiert. Die durch die strukturelle Veränderung der OC bedingte Ernährungsstörung des Knochengewebes ruft eine weitere Verschlechterung des pathologischen Zustandes hervor.

ИЗУЧЕНИЕ СТРУКТУРЫ КАПСУЛЫ ОСТЕОЦИТОВ У КРЫС С ГИПЕРВИТАМИНОЗОМ D И РАХИТОМ

К. МАТЕС, И. ФЁЛДЕШ и Л. МОДИШ

В корковом слое и губчатом веществе большеберцовой кости и позвонка крыс с гипервитаминозом D и рахитом авторы изучали при помощи гистохимических и топооптических реакций изменения капсулы остеоцитов.

Было установлено, что при гипервитаминозе D уменьшается доля окрашенных капсул остеоцитов. После дачи больших доз витамина D в области деминерализации кости наблюдается расширение капсулы остеоцитов. В поляризационном микроскопе видно уменьшение доли капсул остеоцитов, поазывающих двойное преломление. Уменьшение двойного преломления гораздо больше, чем уменьшение окрашивания. Из факта, что после деминерализации, возникающем под влиянием витамина D, не происходит увеличения числа остеокластов, авторы сделают вывод, что деминерализация является результатом активности остеоцитов, проявляющейся в расширении капсулы остеоцитов. Измененная структура капсулы остеоцитов вызывает путем нарушения питания костной ткани дальнейшее ухудшение патологического состояния.

Dr. Klára Matesz
Dr. István Földes
Dr. László Módis
DOTE Anatómiai Szövet- és Fejlődéstani Intézet, 4012 Debrecen, Pf. 14., Hungary

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Institute of Biology, Semmelweis University Medical School, Budapest

DEMONSTRATION OF THE HETEROGENEITY OF THE MAST CELL POPULATION ON THE BASIS OF THE MUCOPOLYSACCHARIDE CONTENT

G. Csaba and P. Kovács

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From the aspect of its mucopolysaccharide content the mast cell population is not homogeneous. The pulmonary and heart muscle mast cells of the rat are alcian blue positive, the mast cells of the thyroid gland, lymph nodes, subcutaneous connective tissue, mesentery and peripheral nerve are safranin positive, whereas among the mast cells of the peritoneal cavity and the thymus there are both alcian blue and safranin positive forms. The least acid mucopolysaccharides are in the mast cells of the peritoneal fluid, the mesentery and the lungs, whereas the most acid ones are in the mast cells of the lymph nodes, the subcutaneous connective tissue and the thyroid gland. There is a considerable difference between the two last mentioned organs. The mast cells of the subcutaneous connective tissue are end-product cells without amine or precursor turnover, whereas the mast cells of the thyroid gland incorporate and deliver amines, which may participate in the regulation of the host gland.

Ever since their description by Ehrlich [12], the mast cells were recognized by their metachromatic staining with basic dyes and therefore every metachromatic connective tissue cell was classified as a mast cell. The metachromasia is due to the acid polysaccharide content of the granules [15, 18]. When in these cells biogenic amines had also been demonstrated (17, 21] then certain differences have become obvious. Some cells contain histamine in addition to heparin, are included while some contain also serotonin, the mast cells of mice and rats, or dopamine, of the ungulates [18]. Nevertheless, acid mucopolysaccharides and histamine had seemed to be obligatory components of the granules together with some third one. Than it was detected that in the vertebrates belonging to a phylogenetically lower class than the reptiles the metachromatic cells contain serotonin [14] instead of histamine [16, 20]. Subsequent microcinematographic analyses [2, 4] showed that there are active forms as well as end product (cadaver) cells the latter being immobile and not displaying any flow of the granules. The latter cell type, which is chiefly characteristic of the subcutaneous connective tissue, could not be classified in any of the maturation categories differentiated on the basis of their alcian blue — safranin staining [3]. These cells were safranin positive but at some sites e. g. in the peritoneal fluid they proved considerably active. At that time was the role of basic proteins in the type of staining observed [6] and it has become clear that it depended on the presence of a mucopolysaccharide, two kinds of amine and a basic protein whether the cell should be considered a mast cell and if so, in which maturation stage it was.

Functional study later revealed that whereas the peritoneal mast cells incorporated biogenic amine precursors [11], the mast cells localized elsewhere in the body failed to do so. At the same time the mast cells of thyroid incorporated serotonin [1, 8, 9] which was not taken up by other e. g. peritoneal mast cells [11]. In addition only the mast cells of the thyroid can incorporate uridine [10]. In the light of these data it seemed interesting to study whether the mast cells of their mucopolysaccharide in the quality localized of the individual organs could be differentiated.

Materials and methods

The examinations were performed in adult male Wistar CB rats. Mast cells were studied in peritoneal fluid (thick drop) lymph node, thymus, subcutaneous connective tissue (membrane preparation), mesentery (into preparation), thyroid gland, lungs, peripheral nerve (sciatic) and heart muscle. If not slated otherwise, paraffin sections were examined. Staining of intact cells was only taken into consideration. The materials were fixed in neutral formaldehyde and the mucopolysaccharides were demonstrated by the following methods [15].

1. Stains referring to the presence of acid mucopolysaccharides:

a. metachromatic stains:
0.01% alcoholic azure A
0.01% alcoholic toluidine blue
1.0% aqueous azur A.

In the case of azur A a difference in staining intensity is manifest between aqueous and alcoholic solutions.

b. ortochromatic stains:
0.01% alcoholic methylene blue;
1.0% aqueous methylene blue;
alcian blue — PAS;
periodic acid — paradiamine;
colloidal iron.

The alcian blue —PAS as well as the periodic acid-paradiamine methods allow to differentiate between periodate reactive and non-reactive polysaccharides.

2. Alcian blue — CEC.

This method allows a differentation of the polysaccharides of various acidity.

In the presence of 01 M $MgCl_2$ hylauronic acid and the weakly sulphated mucins while with 1.0 $MgCl_2$ only heparin will be stained.

3. Alcian blue — safranin [4, 19] permits conclusions concerning the maturation stage of the mast cells.

Results and discussion

Results are shown in Table I. In the case of a positive result the intensity of the staining was estimated in several specimens.

The examined mast cell population was not homogeneous; either their mucopolysaccharide or its maturity as injudged from the degree of sulphation was different.

On the basis of the alcian blue-safranin staining of their mast cells the organs could be divided into three groups. The lungs and the heart muscle contained only alcian blue positive cells; the thyroid, lymph nodes, sub-

Table I

Histochemical reaction in intact mast cells of various organs

	Lymp node	Peritoneal fluid	Thyroid gland	Subcut. connective tissue	Mesentery	Lungs	Peripheral nerve/sciatic	Thymus	Heart
Alcian blue — safranin	A: — S: ++	A: ++ S: ++	A: — A: ++	A: — S: ++	A: —	A: ++	A: —+	gland A: ++ capsule: A: ++ S: ++	A: ++
Alcian blue — PAS	A: ++ PAS: ++	A: ++	A: +	A: ++	A: —	A: ++ PAS: +	A: ++ PAS: §	gland A: ++ capsulePAS:++	A: ++ PAS: —
Colloidal iron	++	?	++	—	§	—	++	++	—
0.01% alcoholic Azure A	++	++	++	++	§	—	++	—	—
0.01% alcoholic Toluidine blue	++	++	++	++	++	—	++	gland + capsule ++	++
0.01% alcoholic methylene blue	++	++	++	++	++	§	++	gland: + capsule: ++	++
1.0% aqueous methylene blue	++	++	++	++	++	++	++	gland: ++ capsule: ++	++
1.0% aqueous azure A, pH 1.8	++	++	++	++	++	—	++	gland: — capsule: ++	++
Alcian blue-CEC 0.1 M $MgCl_2$	++	§	++	++	++	++	++	gland: ++ capsule: ++	++
0.5 M $MgCl_2$	++	§	++	++	++	++	++	gland: ++ capsule: ++	++
1.0 M $MgCl_2$	++	§	++	++	+	+	++	gland: ++ capsule: ++	++
Periodic acid — — Paradiamine	++	++	++	++	§	++	++	gland: — capsule: ++	++

§ Occasional reaction in some cells + = positive ++ = strongly positive

cutaneous connective tissue, mesentery and the peripheral nerves contained only safranin positive cells. The peritoneal fluid and the thymus contained both alcian blue and safranin positive mast cells. According to COMBS et al. [3] the reaction of the cells to alcian blue — safranin is characteristic of their maturity. The alcian blue positive mast cells are the youngest; next follow those of mixed granulation; finally, the mature cells contain exclusively safranin positive granules. The last group includes only the cells of the peritoneal fluid and the thymus. This classification, as it has been shown preciously [7], is valid solely for two sites viz. the thymus and the peritoneal cavity, these being the only areas where alcian blue positive and safranin positive cells occur simultaneously. This means at the same time that it is at these sites that maturation of lymphoid cells into mast cells occurs and also, that alcian blue and safranin positivity cannot be accepted as an indicator of the maturation of polysaccharides contained in the granules. Staining of the material contained in the granules depends on the proportion of components, and this proportion changes in the course of maturation.

Alcian blue staining of the mast cells signifies either that the quantity of the acid mucopolysaccharides is proportionate to that of the amines or it indicates an excess of basic proteins over the mucopolysaccharide [6].

This would be characteristic of the cells of the lung and the heart muscle. At the same time this polysaccharide is of heparin nature, thus intensely sulphated, since it retains its alcian blue staining even when examined at the critical electrolyte concentration with 1.0 M $MgCl_2$. The specific binding conditions of the granule components are indirected also by the fact that the mast cells of the lung and heart do not stain with alcoholic azure A or toluidine blue, whereas the mast cells are demonstrated by these dyes at other sites where the granules are safranin positive. Thus, not even exclusively alcian blue positive cells are identical in mucopolysaccharide content. Whereas the pulmonary ones are PAS positive, those in the heart muscle PAS are negative and while the mast cells in the heart muscle stain intensively with alcoholic methylene blue, those in the lungs are left unstained. It would be of interest to clarify the connection between this specific staining and the function of the heart muscle mast cells. HEINE and FÖRSTER [13] already observed a connection of the mast cells of the heart muscle with the sarcoplasmatic tubular system and the nerve endings, but this does not yet explain the differences in staining and binding.

Among the organs which contain only safranin positive mast cells there are considerable differences concerning the mucopolysaccharides. According to our previous examinations [6] safranin positivity in itself indicates an excess of the biogenic amines over the mucopolysaccharides. Thus from this point of view the mast cell population of the thyroid gland, lymph nodes, subscutaneous connective tissue, mesentery and peritoneal cavity is homogeneous.

It appears at the same time that whether judged by the full picture or the critical electrolyte concentration method, the most acid mucopolysaccharides are in the mast cells of the thyroid gland.

The mast cells of the thyroid gland occupy a specific position. They participate in the regulation of function by delivering their serotonin content and are able to take up serotonin from the surroundings [1, 8, 9]. When the highest mucopolysaccharide content is viewed from this aspect, it may be presumed that heparing owing to its strong electronegativity would ensure the amine uptake even from the surroundings. Still all these observations fail to supply an adequate answer to the question why the mast cells of the subcutaneous connective tissue, which contain mucopolysaccharides of almost identical acidity, are end-product cells, without any amine or precursor turnover [11], when at the same time the thyroid mast cells, which are also safranin positive, demonstrate an active function [10]. Here the routine function of the genetic apparatus of the thyroid gland, or the irreversible inhibition occlusion [5] in case of the subcutaneous connective tissue mast cells may be involved and this would be directed chiefly by the basic proteins [5, 6].

The mucopolysaccharides in the safranin positive mast cells of the peritoneal fluid, when examined by the critical electrolyte method, did not prove to be strongly sulphated and gave the weakest reaction among all the mast cells studied. This refers to a change the proportion of the granule components in the course of maturation. This change results in the safranin positive mast cells, and the change in the degree of sulphation of the mucopolysaccharide induces the specialised function in the thyroid or the functional inability in the subcutaneous connective tissue. This is shown also by the lower degree of sulphation of the mucopolysaccharides in the safranin positive mast cell granules of the mesentery compared with other safranin positive mast cells. On the basis of this observation it seems possible that mast cells already mature from the aspect of the granule components would be transferred directly from the peritoneal cavity to this site [7]; in these, however, sulphation of the mucopolysaccharides is not yet complete.

In summary, it appears that the denomination mast cell is an overall definition of a heterogeneous cell population. The members of the population are starting from the lymphoid cells and undergo maturation processes; they are adapted to their surroundings in the individual organs according to their function and, accordingly, quality and proportions of their chemical components are different. The quantitative and qualitative differences among the mucopolysaccharides form a small part of this heterogeneity.

REFERENCES

1. BARÁTH, P., CSABA, G.: (1975) Biogenic amine and amine precursor uptake of thyroid mast cells. Acta morph. Acad. Sci. hung. In press. — 2. BURTON, A. L.: (1963) Studies on living normal mast cells. Ann. N. Y. Acad. Sci. **103,** 245—262. — 3. COMBS, J. W., LAGUNOFF, D., BENDITT, E. P.: Differentiation and proliferation of embryonic mast cells of the rat. J. Cell Biol. **25,** 577—592. — 4. CSABA, G., TÖRÖK, O.: (1970) The physiology of mast cells II. Effect of cortisone treatment on mast cell behaviour. Acta biol. Acad. Sci. hung. **21,** 149—156. — 5. CSABA, G., SURJÁN, L. jr.: (1970) Mechanism of the formation of mast cell granules V. Histochemical detection of histones in the mast cell granules. Acta biol. Acad. Sci. hung. **21,** 341—346. — 6. CSABA, G.: (1971) Mechanism of the formation of mast cell granules VII. Participation of amines and basic proteins in the formation of the mast cell granules. Analysis of the heterogeneity of mast cells. Acta biol. Acad. Sci. hung. **22,** 155—168. — 7. CSABA, G., TÖRÖK, O.: (1972) Physiology of mast cells IV. Role of blood transport in the transformation of mast cells. Acta biol. Acad. Sci. hung. **23,** 369—376. — 8. CSABA, G., BARÁTH, P.: (1973) Tritiated 5-hydrocytryptamine uptake of the mast cells in the rat thyroid gland. Neuroendocrinology **12,** 67—70. — 9. CSABA, G., BARÁTH, P.: (1974) Effect of pinealectomy on the ^{3}H-5HT uptake of mast cells in the thyroid gland of the rat. Acta anat. (Basel) **89,** 442—451. — 10. CSABA, G., BARÁTH, P.: (1975) ^{3}H-uridine incorporation by the mast cells situated in different organs. Acta biol. Acad. Sci. hung. In press. — 11. CSABA, G., BARÁTH, P.: (1975) Evidence on the heterogeneity of mast cell populations as emerging from studies with labeled amines and amine precursors. Acta biol. Acad. Sci. hung. In press. — 12. EHRLICH, P.: (1877) Beiträge zur Kenntnis der Anilinfärbungen und ihrer Verwendung in der mikroskopischen Technik. Arch. mikr. Anat. **13,** 263—290. — 13. HEINE, H., FÖRSTER, F. J.: (1974) Zur Morphologie der Beziehungen zwischen Mast- und Muskelzellen bei Säugetieren. Acta anat. (Basel) **89,** 387—400. — 14. KAPA, E., SZIGETI, M., JUHÁSZ, Á., CSABA, G.: (1970) Phylogenesis of mast cells I. Mast cells of the frog Rana esculenta. Acta biol. Acad. Sci. hung. **21,** 141—147. — 15. PEARSE, A. G. E.: (1968) Histochemistry. Little, Brown and Co., Boston. — 16. REITE, O. B.: (1965) A phylogenetical approach of the functional significance of tissue mast cell histamine. Nature (Lond.) **206,** 1334—1336. — 17. RILEY, J. F.: (1959) The mast cells. Livingstone, Edinburgh and London. — 18. SELYE, H.: (1965) The mast cells. Butterworths, Washington. — 19. SPICER, S. S.: (1960) A correlative study of the histochemical properties of rodent acid mucopolysaccharides. J. Histochem. Cytochem. **8,** 18—36. — 20. TAKAYA, K.: (1968) Mast cell and histamine in the newt, Triturus pyrrhogaster Boié. Experientia (Basel) **24,** 1053—1055. — 21. WEST, G. B.: (1959) Tissue mast cells and tissue amines. J. Pharm. Pharmacol. **11,** 513—518.

NACHWEIS DER HETEROGENITÄT DER MASTZELLENPOPULATIONEN AUFGRUND DES MUKOPOLYSACHARIDENGEHALTS

Von

GY. CSABA und P. KOVÁCS

Die Mastzellenpopulationen sind hinsichtlich des Mukopolysacharidengehalts nicht einheitlich. Bei Ratten sind die Mastzellen der Lunge und des Myokards Alzyanblau-positiv, die Mastzellen der Schilddrüse, des Lymphknoten, des subkutanen Bindegeenwebes, des Mesenteriums und der peripheren Nerven sind Safranin-positiv, während unter den Mastzellen der Peritonealhöhle und des Thymus sowohl Alzyanblau- als auch Safranin-positive, Formen vorkommen. Die am weningsten sauren Mukopolysacharide finden sich in den Mastzellzellen der peritonealen Flüssigkeit, des Mesenteriums und der Lungen, die sauersten in den Mastdellen der Lymphknoten, des subkutanen Bindegewebes und der Schilddrüse. Auch zwischen den Mastzellen der beiden letzteren Organe lassen sich erhebliche Unterschiede aufzeigen. Die Mastzellen des subkutanen Bindegewebes sind Endproduktzellen, besitzen keinen Amin oder Amin-Präkursor-Stoffwechsel. während in den Mastzellen der Schilddrüse Aminaufnahme und -abgabe vor sich geht. Dieser Umstand dürfte in der Steuerung der Wirtsdrüse Rolle spielen.

ДОКАЗАТЕЛЬСТВО РАЗНОРОДНОСТИ ПОПУЛЯЦИЙ ТУЧНЫХ КЛЕТОК НА ОСНОВЕ СОДЕРЖАНИЯ МУКОПОЛИСАХАРИДОВ

Д. ЧАБА и П. КОВАЧ

В отношении содержания мукополисахаридов популяции тучных клеток не являются однородными. У крыс тучные клетки легких и миокарда дают положительную реакцию с алциановой синькой, тучные клетки шитовидной железы, лимфатических узлов, подкожной клетчатки, брыжейки и периферических нервов являются сафранин-положительными, в то время как в перитонеальной жидкости и зобной железе встречаются как альцианблау-положительные, так и сафранин-положительные формы тучных клеток. Наименее кислые мукополисахариды наблюдаются в тучных клетках перитонеальной жидкости, брыжейки и легких, а наиболее кислые — в тучных клетках лимфатических узлов, подкожной клетчатки и щитовидной железы. Тучные клетки двух последних органов также показывают существенные разницы. Тучные клетки подкожной клетчатки представляют собой клетки конечного продукта, без обмена амина и обмена прекурсоров аминов, в то время как в тучных клетках щитовидной железы осуществляется поглощение и отдача аминов. Последнее обстоятельство — быть может — играет роль в регулировании железы хозяина.

Dr. György Csaba, Dr. Péter Kovács
Semmelweis Orvostudományi Egyetem, Biológiai Intézet,
1450 Budapest, Tűzoltó u. 58., Hungary

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Institute of Biology, Semmelweis University Medical School, Budapest

SELECTIVE 5-HT UPTAKE BY EPITHELIAL CELLS OF THE RAT LUNG

G. Csaba and P. Kovács

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Some of the epithelial cells of the rat lung take up serotonin from the circulation whereas the precursor of serotonin, 5 — HTP, or histamine and histidine are not taken up. The observations raise the possibility that serotonin accumulation could be a manifestation of the general potentials of the entoderm.

Some sections of the intestinal tract possess the capacity to produce biogenic amines. Thus the intestinal enterochromaffine cells produce serotonin, the gastric epithelial cells, histamine [9]. This ability is not restricted to definite sections of the gut but a common property, which manifests itself in the endocrine glands of entodermal origin as well as in the epithelium of the trachea among specific conditions. In a previous study we could demonstrate with or without hormonal provocation the presence of histamine and serotonin in the thymus [3] and in the embryonal trachea [5]; whereas a considerable part of the tissues of entodermal origin are classified by Pearse [12] into the Apud series, thus they are containing amines or taking up amine precursors.

In the present work we have studied the lung as an organ of entodermal origin, to elucidate the question whether its epithelium would take up amine precursors or biogenic amines.

Materials and methods

Male Wistar CB rats of 80 g mean weight received 20 mg nialamide (Nuredal-United Pharmacological Products, Budapest) intraperitoneally, then after 30 minutes 250 $\times\mu$Ci ^{3}H-histidine (SCR-CEN. Belgium) spec. act. 42 Ci(mMol), ^{3}H-5-hydroxytrytophan (Amersham, U. K.) spec. act. 8.2 Ci(mMol), ^{3}H-histamine (SCR-Cen., Amersham, U. K.) spec.act. 10.7 Ci(mMol). One hour later the animals were killed with an overdose of ether specimens of their lungs were fixed in 3% glutaraldehyde and OsO_4 and embedded in Durcupan (Fluka). The semi-thin sections were covered with Ilford K5 emulsion and after exposition for 29 days developed in ORWO R9. The preparations were counterstained with basic toluidine blue.

Results

The epithelial cells of the lungs showed no uptake of either 5-HTP or histidine and histamine but in the serotonin-treated animals there was a high activity over some of the cells of the epithelial lining (Fig. 1). Only some scat-

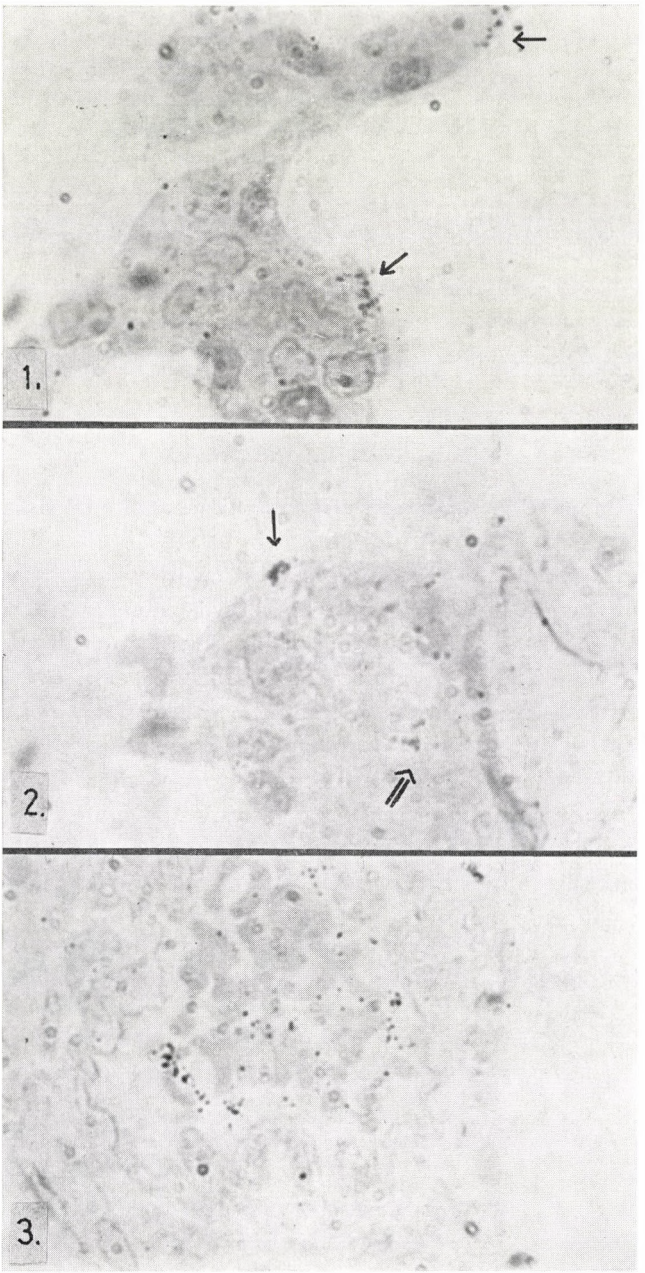

Fig. 1. Numerous grains above pulmonary alveolar epithelial cells (arrow). ×1000
Fig. 2. Serotonin in a single epithelial cell (arrow). Many grains above the capillary (double arrow). ×1000
Fig. 3. Cross section of vessel. The grains cannot be localized to cells. No grains above the endothelium of the vascular wall. ×1000

tered epithelial cells contained serotonin (Fig. 2). Over the vessels numerous extracellular granules were present (Fig. 3).

Discussion

On the basis of the experiments it seems that the epithelial cells of the lung are not forming, but taking up serotonin. If the cells would have any serotonin-producing capacity, then they should have taken up 5-HTP, but this failed to occur. Formerly ALABASTER and BAKHLE [1] as well as GILLIS [10] studied the amine uptake of the rat lung by biochemical methods and observed an uptake of 5-HT and noradrenaline. In their opinion the affinity of the lung is much stronger to 5-HT than to noradrenalin.

Our experiments have proved that for the serotonin uptake a few individual epithelial cells are responsible. The uptake occurs selectively, since histamine was not taken up. This uptake of 5-HT by the epithelial cells contradicts the supposition of ALABASTER and BAKHLE [1] who assumed that the capillary endothelium and the septal cells were taking up the substance. Their hypothesis was probably based on the experiments of HUGHES et al. [11] who demonstrated noradrenaline uptake in these cells by autoradiography. Since it follows from our experiments that serotonin is localized in the epithelial cells and cannot be found in the endothelium of the capillaries or of any other vessel, it is assumed that the uptake of serotonin and noradrenaline occurs either at different sites or else the in vitro perfusion method applied by the quoted authors [11] gave erroneous results.

It seems difficult to provide a functional explanation for the serotonin uptake by the lung. It may be presumed that the task of the cells would be the elimination of serotonin from the circulation. It is also possible that serotonin is metabolized in the cells and participates in the synthesis of some substance, similarly as the cells of the intestinal tract produce melatonin from serotonin [12]. Or, else, the epithelial cells taking up serotonin might be different from the respiratory epithelial cells also of entodermal origin and present at an identical site. When we studied the general iodine accumulating capacity of the entoderm [7] we could observe that individual cells of the atopically transplanted tracheal epithelium took up iodine from the circulation in the same scattered way observed in the present experiments. Comparing the results it seems that the specific endocrine properties of the entodermal epithelium manifest in some of these cells and same would be indicated by the serotonin uptake by the pulmonary epithelium.

The circumstance that some cell type in the present case the entodermal cells, which have preserved their endocrine properties, would take up the precursor and are preparing the hormone from the latter, whereas in other cases

they do not take up the precursor only the hormone itself, cannot be considered an exceptional phenomenon. The peritoneal mast cells take up amine precursors only [8], whereas the mast cells of the thyroid can take up serotonin, but not its precursors [2, 4, 6]. In the present case a similar phenomenon may have been observed.

REFERENCES

1. ALABASTER, V. A., BAKHLE, Y. S.: (1970) Removal of 5-hydroxytryptamine in the pulmonary circulation of rat isolated lung. Brit. J. Pharmacol. **40,** 468—482. — 2. BARÁTH, P., CSABA, G.: (1975) Biogenic amine and amine precursor uptake by thyroidal mast cells. Acta morph. Acad. Sci. hung. In press. — 3. CSABA, G., HODINKA, L.: (1971) Dynamics of the cortisone effect on mast cell formation. Acta morph. Acad. Sci. hung. **19,** 131—139. — 4. CSABA, G., BARÁTH, P.: (1973) Tritiated 5-hydroxytryptamine uptake of the mast cells in the rat thyroid gland. Neuroendocrinology **12,** 67—70. — 5. CSABA, G., BOMBERA, G., MÁNDICS, R.: (1973) Provocation of biogenic amine formation in the foregut endoterm by hormone induction. Z. mikr. anat. Forsch. **87,** 351—356. — 6. CSABA, G., BARÁTH, P.: (1974) Effect of pinealectomy on the ^{3}H-5-HT uptake of mast cells in the thyroid gland of the rat. Acta anat. (Basel) **89,** 442—451. — 7. CSABA, G., NAGY, S. U., BOMBERA, G., MÁNDICS, R.: (1975) Iodine accumulating ability as generalized property of cells of entodermal origin. Endocr. exp. **9,** 121—126. — 8. CSABA, G., BARÁTH, P.: (1975) Evidence of the heterogeneity of mast cell population as emerging from studies with labelled amines and amine precursors. Acta biol. Acad. Sci. hung. In press. — 9. DOUGLAS, W. W.: (1970) Histamine and antihistamines. In: Goodman, L. S., Gilman, A.: The pharmacological Basis of Therapeutics. MacMillan, New York. — 10. GILLIS, C. N.: (1971) Binding of hydroxytryptamine and noradrenaline by rat lung. Experientia, (Basel) **27,** 1317—1319. — 11. HUGHES, J., GILLIS, C. N., BLOOM, F. E.: (1969) The uptake and disposition of dl-norepinephrine in perfused rat lung. J. Pharmacol. exp. Ther. **169,** 237—248. — 12. PEARSE, A. G. E.: (1969) The cytochemistry and ultrastructure of polypeptide hormone producing cells of the APUD series and pathological implications of the concept. J. Histochem. Cytochem. **17,** 303—313. — 13. RAIKHLIN, N. T., KVETNOY, I. M., TOLKACHEV, V. N.: (1975) Melatonin may be synthetized in enterochromaffine cells. Nature (London) **255,** 344—345.

DIE SELEKTIVE 5-HT-AUFNAHME IN DEN EPITHELZELLEN DER RATTENLUNGE

Von

GY. CSABA und F. KOVÁCS

Manche Epithelzellen der Rattenlunge nehmen aus dem Kreislauf Serotonin auf, während sie kein 5-HTP — den Präkursor des Serotonins — und kein Histamin oder Histidin aufnehmen. Aufgrund der Versuchsergebnisse wird die Möglichkeit erwogen, das die Serotoninakkumulation eine Manifestation der allgemeinen Potenzen des Entoderms darstellt.

ИЗБИРАТЕЛЬНОЕ ПОГЛОЩЕНИЕ 5-НТ ЭПИТЕЛИАЛЬНЫМИ КЛЕТКАМИ ЛЕГКИХ КРЫСЫ

Отдельные эпителиальные клетки накапливают из кровообращения серотонин, в то время как они не поглощают ни 5-НТР (прекурсора серотонина), ни система или гистидина. На основе результатов опытов обсуждается возможность, что накапливание серотонина представляет собой проявление общей потенции энтодермы.

Dr. György CSABA
Dr. Péter KOVÁCS
Semmelweis Orvostudományi Egyetem, Biológiai Intézet, 1450 Budapest, Tűzoltó u. 58., Hungary

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Institute of Forensic Medicine, Semmelweis University Medical School, and National Institute of Cardiology, Budapest

ULTRASTRUCTURAL CHANGES IN THE LUNGS INDUCED BY EXTRACORPOREAL PERFUSION

J. Soós, I. Balogh, P. Sótonyi, A. Árvay and E. Somogyi

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The changes induced in the lungs have been studied, in the course of 15 heart operations with extracorporeal circulation. In each case material was obtained from lungs after the thoracotomy, before starting the perfusion and after its termination. The pulmonary structure showed changes against the control material already prior to perfusion, the most conspicuous alternations of the capillary endothelial cells and of the basal membrane. Examination of the surfactant by ruthenium red demonstrated changes in the reaction; from this circumstance the conclusion has been drawn that affection of the surfactant may have a part in the postperfusion syndrome.

Simultaneously with the increase of the number of surgical interventions on the heart the incidence of undesired late effect has increased. It is often difficult to decide whether for the failure the underlying disease some of its consequences or the intervention involving a high number of aphysiological factors was responsible and the stage of the disease cannot be neglected. One of the serious postoperative respiratory complications of operations on the open heart performed with cardiopulmonary bypass is the perfusion lung or postperfusion pulmonary syndrome manifesting with atelectasis haemorrhages and oedema. A damage to the alveolar surfactant during the perfusion has probably an important role in the development of the syndrome.

A study has been made on the effect of extracorporeal perfusion on the alveolar surfactant layer. It was a considerable difficulty in the study that the surfactant had then already suffered changes due to the basic disease. Several methods are known for demonstration of the surfactant; their results concerning the structural characteristics of the layer, its functional significance and basic changes are partly contradictory [2, 3, 6, 7, 9, 10, 11, 15, 16, 17, 25].

Material and methods

Material was obtained from identical parts of the lung of 15 patients subjected to open heart operations under extracorporeal perfusion. The specimens were obtained after thoracotomy before the perfusion and after its termination. Since the results differ the method of fixation the surfactant has been studied also in apparently normal pulmonary specimens from subjects operated upon for tumour.

The specimens were immediately cut into 1 mm pieces and fixed in [18] 2.5% gluteraldehyde with cacodylate buffer containing 0.5 mg/ml ruthenium red. After fixing for 1 hour and

rinsing with cacodylate buffer the material was postfixed in 2% osmium tetroxide with 0.1 M veronal acetate buffer pH 7.2 and 0.5 mg/ml ruthenium red in the dark at +4°C for 3 hours, then dehydrated and embedded in Araldite. The sections were prepared by means of a LKB type ultrotome. Some of them were contrasted with uranyl acetate and lead citrate. HITACHI HU 10A and JEM 100B type electron microscopes were used.

Table I

Duration of perfusion

Minutes	Subjects
30	3
30—60	2
60—120	5
120	5

Table II

Operations

ASD	4
Tetralogy of Fallot	2
Implantation of mitral valve	5
Implantation of aortic valve	2
Implantation of mitral and aortic valve	2
	15

Results

In the alveolar surface of the epithelial cells and of the type II pneumocytes a 150 to 200 Å thick cell-free layer was seen displaying at some sites a granular structure and clearly contouring the microvilli (Plate I, Figs 1, 2, and 3). In the stained layer there was a close resemblance between type I and type II cells and a characteristic ruthenium red positivity on the membranes of the inclusion bodies of type II pneumocytes. The preperfusion pulmonary structure showed changes against the controls, consisting in swelling of the capillary endothelial cells, increased vacuolization, thickening of the basal membranes. This was particularly marked in patients suffering from severe pulmonary congestion. The alveolar surfactant layer before perfusion (Plate II, Figs 1 and 2) formed a continuous layer on the alveolar surface in some cases whereas in others it was narrow discontinuous and sometimes even lacking (Plate II, Figs 3 and 4). After the perfusion, approximately in proportion to its duration, the oedematous swelling of the cells, their vacuolization and the thickening of the basal membrane were more marked. In those cases where before perfusion the surfactant layer showed a characteristic positivity, the positivity decreased in proportion to the duration of perfusion (Plate III, Figs 1 and 2) or even disappeared; initially weak reaction disappeared in almost every case (Plate III, Figs 3 and 4).

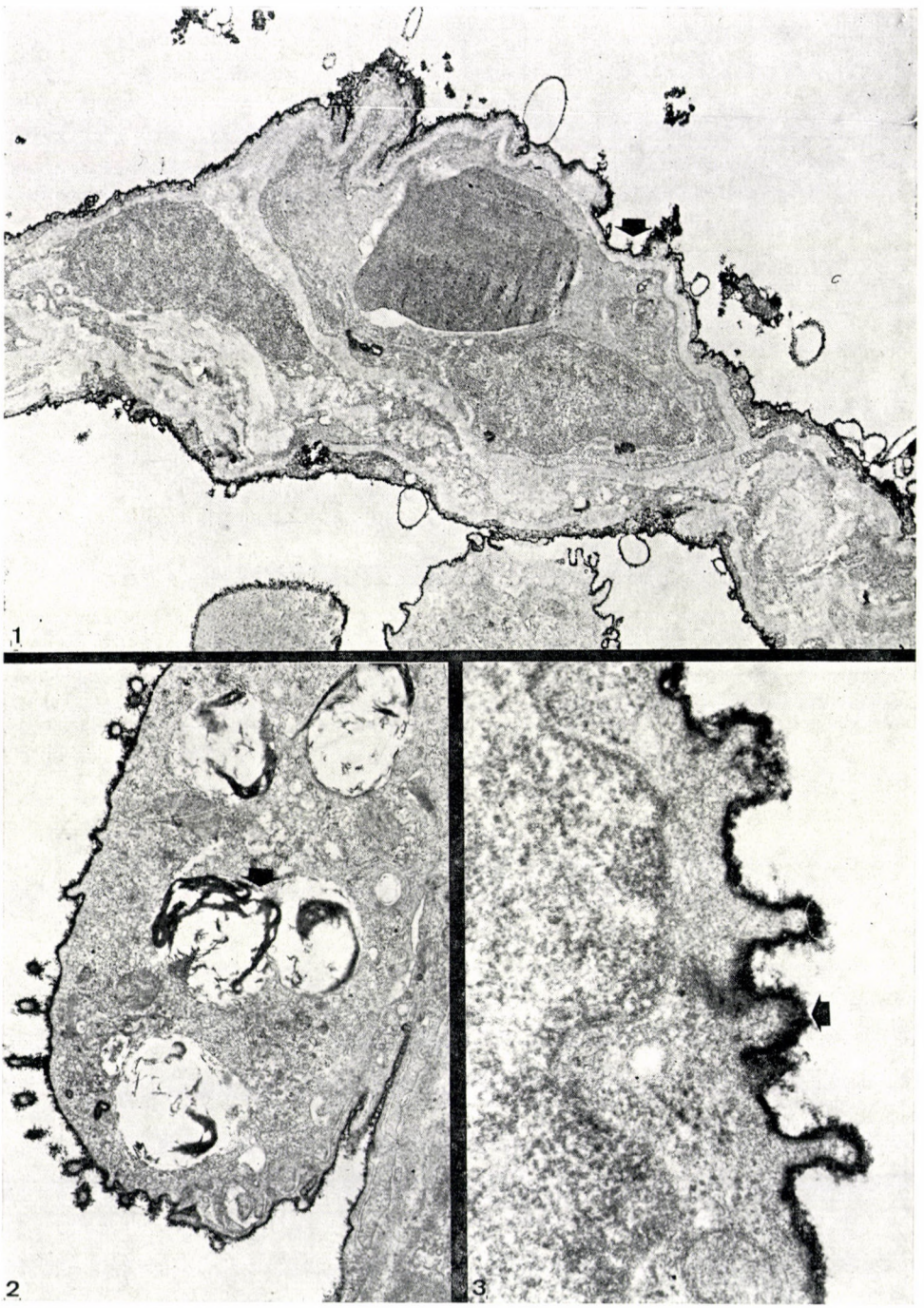

Plate I. Fig. 1: Continuous ruthenium red positive layer on the alveolar surface of epithelial cells. × 8300. *Fig. 2.* Reaction localized to the microvilli and inclusion bodies of type 2 pneumocytes. × 16,600. *Fig. 3.* The reaction is mostly continuous and 150 to 200 Å thick. × 60,560

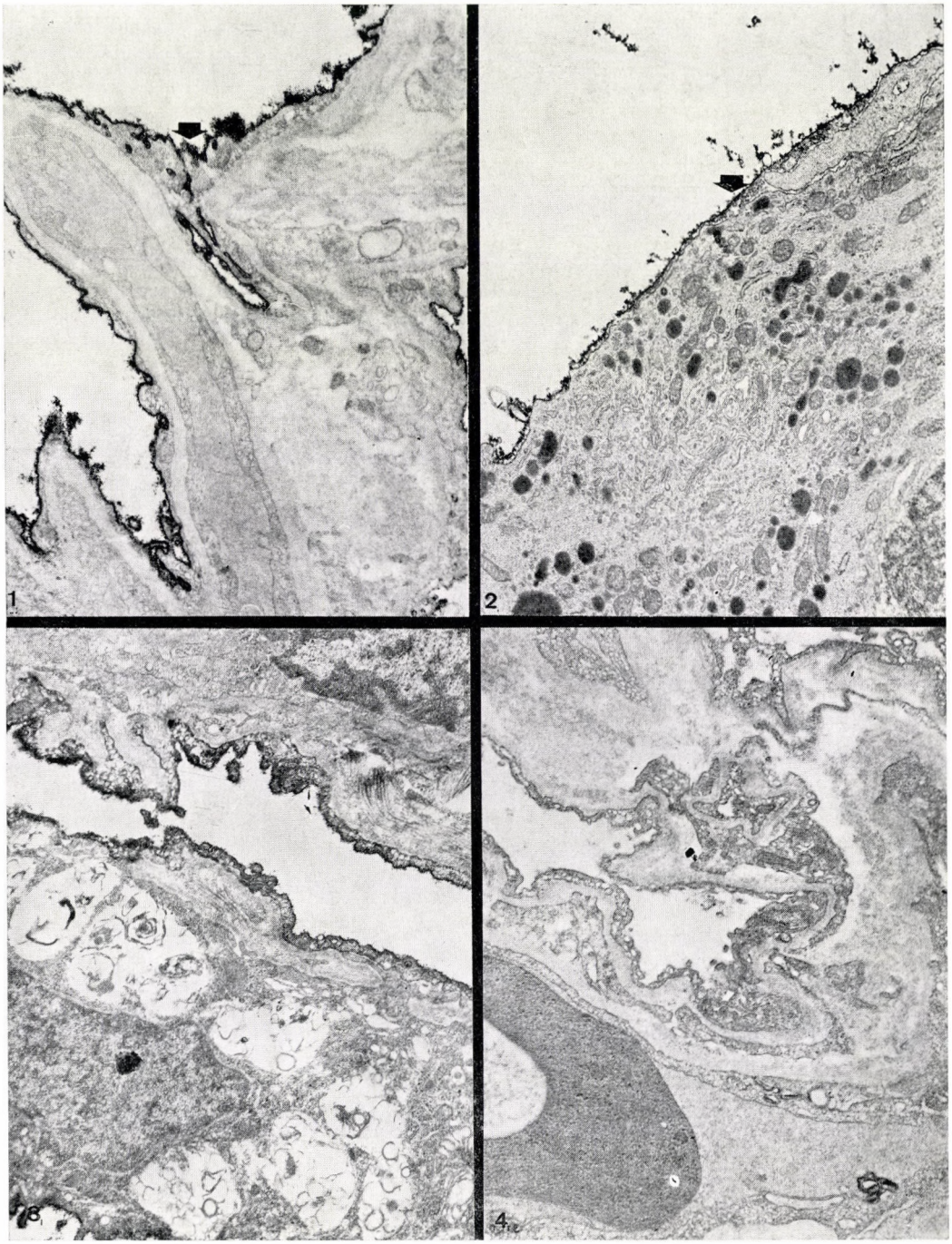

Plate II. Figs 1—2. Layers of various thickness and localization prior to perfusion. × 6600
Figs 3—4. Absence of surfactant on the surface of cells. × 12,400

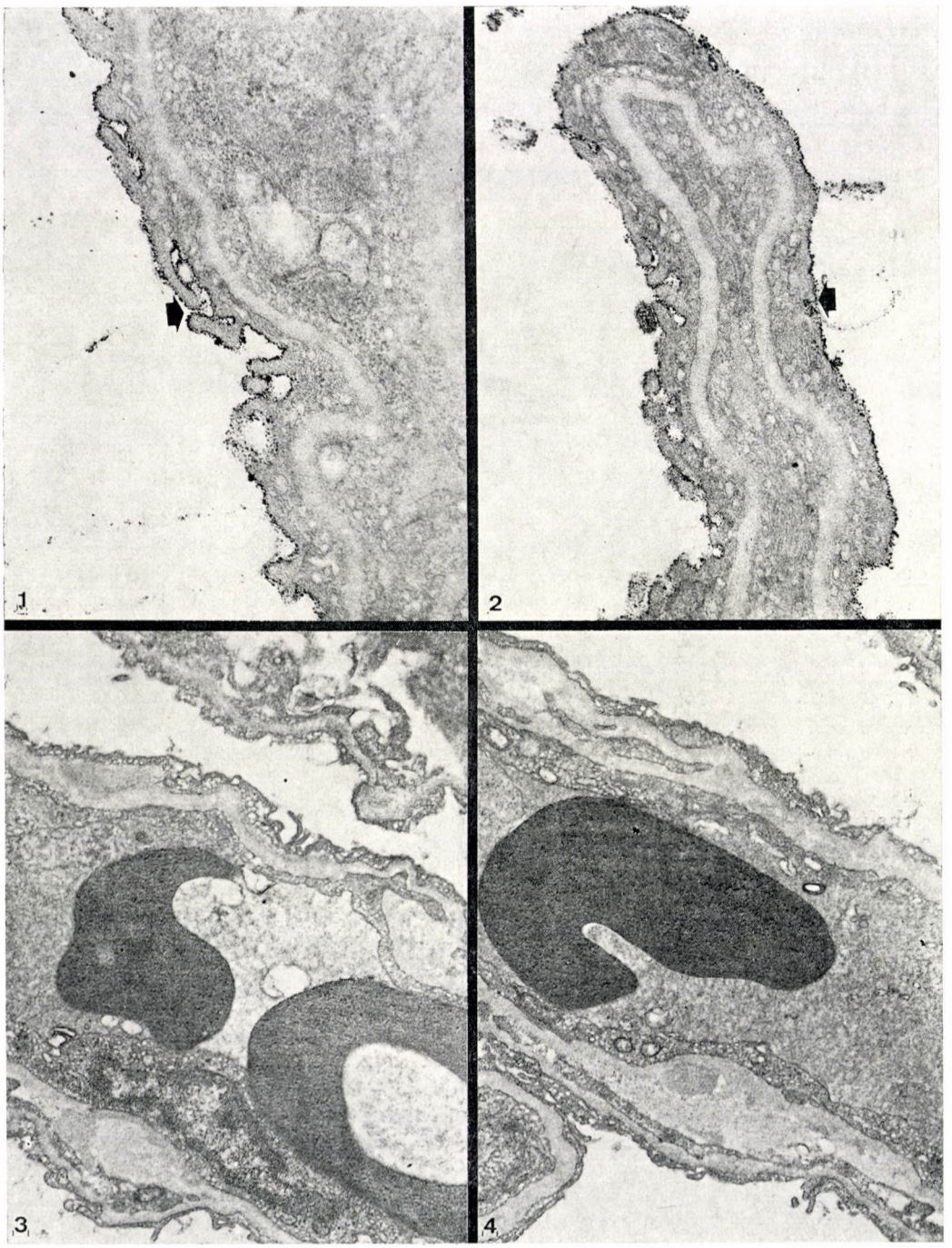

Plate III. Figs 1—2. Decrease of ruthenium red positive layer parallel to the duration perfusion. × 12.400. *Figs 3—4.* Characteristic changes and disappearance of positivity. × 15,800

Discussion

Elasticity of the lung tissue in itself would not suffice maintain the stability required from the organ. The reaction force of the lungs is due mainly to the alveolar surface tension ensured by the surfactant covering the inner surface of the alveoli. In the lack of surfactant, atelectasis disturbances of alveocapillary diffusion inter- and intraalveolar oedema and haemorrhages appear. Formation and function of the surfactant depend on satisfactory blood supply and normal metabolism in the alveoli. The surfactant is affected in a number of pathological processes, e. g. pulmonary arterial occlusion [1] haemorrhagic shock [12], cardiopulmonary bypass [4, 5, 8, 26] oxygen intoxication [13, 22], in operations for mitral stenosis [14] and other open heart operations [23, 27], and owing to irradiation [20]. Following perfusion, vacuolization of the alveolar cells with swelling of their mitochondria and endoplasmic reticulum was found [24, 27]. In shock a swelling of both the granular and the membraneous pneumocytes could be observed with mitochondrial changes restricted to the granular pneumocytes [21]. In oxygen intoxication, a widening of the surfactant layer, its swelling as well as metaplasia of the granular pneumocytes occurred [13]. In the lungs of mitral stenosis patients capillary dilatation and interstitial oedema vacuolization of the membraneous pneumocytes additionally in the capillary endothelial cells abnormal lucens regions, the substitution of the thin membraneous pneumocytes by thick granular pneumocytes as well as oedematous thickening of the surfactant layer was found [14]. Similar changes were observed in mitral insufficiency [23]. Under the effect of irradiation an irregular aggregation of the surface material stained by thorotrast could be observed, at numerous sites the alveolar epithelial layer was torn from the basal membrane [20].

A number of authors reported on cell destruction in the course of extracorporeal perfusion. [19, 23, 27]. The present study revealed the damage of the surfactant layer in the course of the perfusion. Biopsy prior to perfusion already showed an effection of the fine structure of the lung and the surfactant layer, particularly in patients with congestion. Thus the perfusion was only partly responsible for the damage and for the consecutive perfusion lung.

REFERENCES

1. Balis, J. V., Rappaport, E. S., Pifarre, R., Neville, W. E.: (1969) Ultrastructural and surfactant changes of the dog lung following pulmonary artery ligation. Lab. Invest. **20,** 574. — 2. Balogh, I., Sótonyi, P., Szinay, Gy., Soós, J.: (1973) Ultrastrukturális vizsgálatok emberi tüdőbiopsiás anyagon. Morph. Igazságü. Szle. **13,** 196—1966. — 3. Brooks, R. E.: (1971) Ultrastructural evidence for a noncellular lining layer of lung alveoli. A critical review. Arch. intern. Med. **127,** 426—428. — 4. Camishion, C.: (1968) Effect of partial and total cardiopulmonary bypass with whole blood or hemodilution priming on pulmonary surfactant activity. J. surg. Res. **8,** 1. — 5. Connell, R. S., Page, V. S., Bartley, Th. O., Bigelow, J. C., Webb, M. C.: (1973) The effect on pulmonary ultrastructure of dacron-wool

filtration during cardio-pulmonary bypass. Ann. thorac. Surg. **15,** 217. — 6. DERMER, G. B.: (1969) The fixation of pulmonary surfactant for electron microscopy: I. The alveolar surface lining layer. J. Ultrastruct. Res. **27,** 88—104. — 7. FINLAY-JONES, J.-M., PARADIMITRIOU, J. M.: (1972) Demonstration of pulmonary surfactant by tracheal injection of tricomplex salt mixture: electron microscopy. Stain. Technol. **47,** 59—63. — 8. GARDNER, F.: (1962) The effect of cardiopulmon bypass on surface activity of lung extracts. Bull. Soc. int. Chir. **21,** 542. — 9. GILL, J., WEIBEL, E. R.: (1968) The influence of fixation technique and fixative on preservation of the pulmonary surfactant. Fourth European Regional Conference on Electron Microscopy, Rome. — 10. GILL, J.: (1972) Effect of tricomplex fixation on lung tissue J. Ultrastruct. Res. **40,** 122—131. — 11. GRONIOWSKI, J., BICZYSKOVA, W.: (1969) The extraneous coat of lung alveol. Lab. Invest. **5,** 430—436. — 12. HENRY, J. N., MCARDLE, A. H., BOUNOUS, G., HAMPSON, L. G., SCOTT, H. J., GUARD, F. N.: (1967) The effect of experimental hemorrhagic shock on pulmonary alveolar surfactant. J. Trauma. **7,** 691—726. — 13. KAPANCI, Y., WEIBEL, E. R., KAPLAN., ROBINSON, F. R.: (1969) Pathogenesis and reversibility of the pulmonary lesions of oxygen toxicity in monkeys. II. Ultrastructural and morphometric studies. Lab. Invest. **20,** 101—118. — 14. KAY, J. M., EDWARDS, F. R.: (1973) Ultrastructure of the alveolar-capillary wall in mitral stenosis J. Path. **111,** 239—245. — 15. KIKKAWA, Y.: (1970) Morphology of alveolar lining layer Anat. Rec. **167,** 389—400. — 16. KIKKAWA, Y., HAHN, H-S., YANG, S-S., BERNSTEIN, J.: (1970) Mucopolysaccharide in the pulmonary alveolus. II. Electron microscopic observations Lab. Invest. **22,** 272—280. — 17. KUHN, C. H., III., FINKE, E. H.: (1972) The topography of the pulmonary alveolus: scanning electron microscopy using different fixations. J. Ultrastruct. Res. **38,** 161—173. — 18. LUFT, J. H.: (1964) Electron microscopy of cell extraneous coats as revealed by ruthenium red staining. J. Cell Biol. **23,** 54 A. — 19. MANDELBAUM, I., GIAMMONA, S. T.: (1964) Extracorporeal circulation, pulmonary compliance and pulmonary surfactant. J. thorac. cardiovasc. Surg. **48,** 881. — 20. OLEDZKA-SLOTWINSKA, H., MAISIN, S. R.: (1970) Electron microscopic and histochemical observations on the pulmonary alveolar surfactant in normal and irradiated mice. Lab. Invest. **22,** 131—136. — 21. RATLIFF, N. B., WILSON, J. W., HACKEL, D. B., MARTIN, A. M. Jr.: (1970) The lung in hemorrhagic shock. Amer. J. Path. **58,** 353—373. — 22. ROSENBAUM, E. R. M., WITTNER, M., LENGER, M.: (1969) Mitochondrial and other ultrastructural changes in great alveolar cell of oxygen-adapted and poisoned rats. Lab. Invest. **20,** 511—528. — 23. SAKASHITA, I., MIYOSHI, M., HOSHINO, K.: (1968) Pulmonary alveolar lesions following open heart surgery. An electron microscopic study. Jap. Heart. J. **9,** 441—452. — 24. THELMO, W., BECKER, N. M., HANGSTROM, W. C., WEITH, F. J.: (1970) Sequential light and electron microscopy of isolated perfused lungs. Ann. Surg. **171,** 171. — 25. UNTERSEE, P., GIL, J., WEIBEL, E. R.: (1971) Visualisation of extracellular lining layer of lung alveoli by freeze-etching. Respir. Physiol. **13,** 171—185. — 26. WEEDEN, O.: (1970) Effect of oxygen and ventilation on pulmonary mechanics and ultrastructure during cardiopulmonary bypass. Amer. J. Surg. **120,** 584. — 27. YAMAGUCHI, M.: (1970) Fine structural change of the lung following cardiopulmonary bypass. Its effect on the early postoperative course. J. Jap. Ass. thorac. Surg. **18,** 1084—1101.

ULTRASTRUKTURELLE UNTERSUCHUNG DER DURCH EXTRAKORPORALE PERFUSION HERBEIGEFÜHRTEN LUNGENVERÄNDERUNGEN

Von

J. SOÓS, I. BALOGH, P. SÖTÖNYI, A. ÁRVAY und E. SOMOGYI

Im Zusammenhang mit 15 mittels extrakorporaler Perfusion ausgeführten Herzoperationen wurden die unter Perfusionswirkung eintretenden Lungenveränderungen untersucht. In sämtlichen Fällen wurde nach der Thoratokomie vor dem Beginn und nach dem Abstellen der Perfusion aus der Lunge Biopsiematerial entnommen. Im Vergleich zu den Kontrollen zeigte schon die vor Perfusionsbeginn wahrnehmbare Lungenstruktur Veränderungen, von denen die Veränderung der kapillaren Endothelzellen und der Basalmembran besonders auffallend waren. Die Untersuchung der oberflächenaktiven Substanz mit Rutheniumrot zeigte eine Reaktionsveränderung; dies läßt auf die Rolle der oberflächenaktiven Schändigumg in der Entwicklung des Postperfusions-Lungensyndroms folgern.

УЛЬТРАСТРУКТУРНОЕ ИЗУЧЕНИЕ ИЗМЕНЕНИЙ ЛЕГКИХ, ВЫЗВАННЫХ ЭКСТРАКОРПОРАЛЬНОЙ ПЕРФУЗИЕЙ

Й. ШООШ, И. БАЛОГ, П. ШОТОНЬИ, А. АРВАИ и Э. ШОМОДЬИ

В связи с 15 случаями операции на сердце при помощи экстракорпоральной перфузии, авторы изучали изменения легких, возникающие под влиянием перфузии. Во всех наблюдениях после торакотомии до начала и после окончания перфузии авторы продовили биопсию легких для получения материала. По сравнению с контролями в структуре легких изменения наблюдались уже до начала перфузии. Особенно поразительными были изменения эндотелиальных клеток капилляров и основной мембраны. Исследование поверхностноактивного вещества красителем рутеновый кресный показало изменение реакции. Этот факт позволяет заключение, что поражение поверхностноактивным веществом играет роль в развитии постерфузионного легочного синдрома.

Dr. József Soós
Dr. István Balogh
Dr. Péter Sótonyi
Dr. Endre Somogyi
Semmelweis Orvostudományi Egyetem Igazságügyi Orvostani Intézet
1450 Budapest, Pf. 9/41, Hungary

Dr. Attila Árvay
Országos Kardiológiai Intézet Szívsebészeti Osztálya
1450 Budapest, Pf. 88, Hungary

RECENSIONES

J. KUNZ: *Pathologie der Arterienwand* (Pathology of the Arterial Wall). Verlag Volk und Gesundheit, Berlin 1975. Price: M 43.—

The book discusses the role and significance of glycosaminoglycans (formerly mucopolysaccharides) and their participation in the changes of the vascular wall. The basis of the examinations is the definition of the normal structure, but it supplies an excellent and detailed discussion of the significance of glycosaminoglycans in arteriosclerosis research. In addition to their pathogenetic role, these substances are discussed also from other aspects, e. g. as risk factors. The intention is to clarify the aetiological and athogenetical details of the single elements. The most important statements in the study of the glycosaminologlycans concern not so much by the final state than the early changes of the arterial wall. Although no details are supplied concerning the connection of glycosamines with the nervous system or the muscles, their importance and role are also discussed. After treating the importance, role and structure of glycosoaminoglycans in the arterial wall from physiological and biochemical aspects, the changes of other than arteriosclerotic character are reported. The main problems of lipid infiltration and synthesis and vascular changes are dealt with from the aspect of glycosaminoglycans and the author's opinion concerning these basic questions is slated by comparing his own results and the pertinent literary data. For the specialist engaged in arteriosclerosis research many interesting data are supplied concerning the various forms of arteriitis, a field where literary data are scare and have mostly been obtained in studies concerning the various forms of mucopolysaccharidosis. The detailed analysis of the causal factors arteriosclerosis is also of interest; it discusses in addition to lipid infiltration, thrombosis, calcification and hypertension also the changes induced by endocrine factors and vitamins. The significance of smoking and nutrition as well as of genetic factors is also discussed. The book contains an extensive list of references, supplemented until the day of publication. The monograph will be useful for the specialist engaged in the problem of glycosaminoglycans as well as for those who intend to obtain information about their problems.

J. JELLINEK

A. F. ROCHE, H. WIANER, and D. THISSEN: *Predicting adult Stature for Individuals.* Monograph in Pediatrics, Vol 3, with 114 pages 17 figures and 40 tables. Karger, Basel 1975.

The five chapters of the book supply detailed information on the possibility of predicting adult stature in infancy by employing the so-called RWT method termed after the initials of the authors.

The first chapter describes the indication sphere of predicting the adult stature; in the case of abnormally tall or abnormally small children the reliable prediction of adult height may support the indication or contramdication of drug therapy. Knowledge of the correlation between longitudinal growth rate and degree of bone maturation may be of importance for establishing the right time for certain surgical interventions, particularly heart operations or orthopaedic interventions.

Chapter 2 gives a literary survey of the methods used in predicting adult stature, then enumerates and evaluates the pertaining data.

Chapter 3 discusses the methods of examination and of the collection of data. The RWT method has been developed from combination of data of the longitudinal examination of Fels. These studies were carried out in Yellow Springs on children born between 1929 and 1954. In each case body length and body weight were measured, and bone age was estimated on

basis of the Greulich-Pyle atlas. For each child, 80 data were registered; among these 2 were constant and 78 variable. Constant data concern the height of the parents. Among the 78 data varying according to the age of the children, 75 signify bone-specific bone age; the remaining 3 variables are the lying body length, standing height and body weight. The examination were carried out on 100 boys and 100 girls; in the first year of life at the age of 1, 3, 6, 9, and 12 months, afterwards every 6 months until the age of 18 years, and subsequently every second year.

Chapter 4 discusses data processing and analysis. Starting from the study of the linear correlations of the data suited for predicting, eliminated those which contained no essential information and from each group of predictors one was chosen to represent the whole group in respect of sex and age. In the course of final selection four predictors remained:

1. lying body length at the time of study;
2. body weight at the time of study;
3. mean adult height of parents;
4. mean metacarpal bone age of the child.

If the values of these 4 predictors as well as the regression coefficients and constants represented in the RWT tables XXVII and XXVI are substituted in the given equation, the adult stature of the child can be calculated.

The 5th chapter compares the RWT method with the Bayley-Pinneau method used also for predicting adult stature, and evaluates the reliability prediction by the two methods.

The Bayley-Pinneau method allows height prediction from the age of 6 years, whereas the RWT method already from the first year. In contrast to the Bayley-Pinneau method, the RWT method informs concerning adult stature of such children, in whom less than the half of metacarpal bones is of adult maturity. The RWT method can be applied in boys until the age of 16 years, in girls until the age of 14. Except in the period of adolescence, the RWT method supplies more exact prediction than the Bayley-Pinneau method. The possibilities of error with both methods are analysed in three series divided according to age and sex. The comparison proves the higher reliability of the RWT method and its usefulness throughout the entire period of childhood.

BEA KISMARTONI

JUNZO KOIZUMI: *Glycogen in the Central Nervous System* Volume 6, No 4. Gustav Fischer Verlag. Stuttgart 1974. Price: DM 23, —

This monograph, published in the "Progress of Histochemistry and Cytochemistry" series contains on 30 pages 20 electron microscopic pictures of excellent quality. The glycogen metabolism of the central nervous system is reviewed, covering numerous problems, that may offer new information even for the morphologist familiar with the question.

The first chapter deals with the morphology of glycogen structure including the possibilities of reliable differentiation of the single glycogen particles. The second chapter discusses the topography of glycogen in the central nervous system, in the neuronal and glial cells. The third chapter describes the conditions associated with an increase of the glycogen content, e. g. irradiation, injuries of the central nervous system, psychotropic drugs, hibernation, hypoxia, ischemia, cerebral oedema, neuronal degeneration, encephalitis and cerebral tumours. Noteworthy are the examinations demonstrating that the number of beta-particles is higher in the intact tissues, whereas a higher number of alpha-particles may be observed in pathological and experimental material. 4 accumulation of glycogen is not uniform in the different parts of the glia. Chapter four discusses the conditions inducing a decrease of the glycogen content e. g. convulsive processes, insulin, hypoglycaemia, histamine, shock electroshock, experimental catatonia, Cardiozole spasm. A detailed bibliography is added.

The monography supplies valuable information also for the clinicians engaged in experimental pathology.

P. SÓTONYI

L. MÓDIS: *Topo-Optical Investigations of Mucopolysaccharides (Acid Glycosaminoglycans)*. Handbuch der Histochemie (Handbook of Histochemistry). W. Grauman and K. Neumann, Gustav Fischer Verlag, Stuttgart 1975. 134 pages with 98 illustrations. Volume II Polysaccharides. Part 4

Topo-optical polarization microscopic procedures are combined with methods of mucopolysaccharide histochemistry in their study of the pertaining theoretical and methodological problems. New informations were obtained by means of the topo-optical reactions introduced by Romhányi and his school, which support Romhányi's concept of the mucopolysaccharides concerning their oriented microstructural organization. The N,N-diethylpseudocyanine staining process seems well suited for the study, and the critical electrolyte concentration (CEC) procedure for the differentitation, of various mucopolysaccharide structures. Very remarkable are those new examinations where the results obtained with the anisotropic index are utilized for the study of the structure of the ground substance. Detailed studies were performed on the changes and formation of the latter in epiphyseal and articular cartilage. The effect of hypo- and hypervitaminosis in connective tissue as well as the effect of lysine and vasopressin in the course of recovery during callus formation is studied. In the ground substance a marked increase was found. The changes in mucopolysaccharide orientation in various processes affecting the ground substance are also discussed and it is shown that the changes revealed by the topo-optical reaction in the epiphyseal and articular cartilages and in the substantia propria of the cornea manifest themselves in the orientation of the mucopolysaccharides.

The study illustrates excellently the exceptional possibilities of polarization microscopy and mucopolysaccharide histochemistry in morphological studies at the submicroscopical level. The excellent didactic of this book and its classification detailed list of references greatly facilitate understanding of mucopolysaccharide histochemistry. The book will supply new informations for morphologists and also for clinicians.

P. SÓTONYI

INDEX

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Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana Punjab, India

BALBIANI'S VITELLINE BODY IN THE OOCYTES OF VITELLOGENIC AND NONVITELLOGENIC FEMALES OF THE DOMESTIC FOWL: A CORRELATIVE CYTOLOGICAL AND HISTOCHEMICAL STUDY

S. S. Guraya

(Received December 18, 1975)

A cytological and histochemical study has been made of Balbiani's vitelline body in the oocytes of nonvitellogenic and vitellogenic females of the domestic fowl *(Gallus domesticus)*, which consists of yolk nucleus, mitochondria, Golgi bodies and lipid bodies of diverse nature. The yolk nucleus consisting of RNA, protein and lipoprotein shows the greatest development and morphological complexity in the growing oocytes of vitellogenic females. Its possible significance has been discussed in relation, to the variable metabolism and hormonal status of the female during sexual maturation.

Introduction

Guraya [9] reported the presence of a paranuclear basophilic complex of organelles and lipid inclusions in the young oocytes of birds. The structure was termed yolk nucleus complex; it consists of the yolk nucleus, mitochondria, Golgi bodies and lipid bodies of variable size and histochemical composition. Using the electron microscope, Greenfield [7] and Bellairs [4] confirmed the presence of these components in the yolk nucleus complex of fowl oocytes, which they called the Balbiani body, and this term will be used here for the paranuclear complex.

In the present study the changes of Balbiani's vitelline body were examined in the developing oocytes of nonvitellogenic and vitellogenic chicken to reveal differences in the morphology of its yolk nucleus component. The development, structure and function of various components in Balbiani's vitelline body of the animal oocyte are still challenging subjects.

Materials and methods

Ovaries of juvenile (or nonvitellogenic) and adult (or vitellogenic) domestic chicken *(Gallus domesticus)* were used. After decapitation, the ovary was immediately placed in physiological saline, washed free from blood and cut into small pieces. Fixatives used for the histochemistry of lipids included formaldehyde-calcium with and without postchromation [2, 3]. After fixation and subsequent postchromation, the material was embedded in gelatin. Frozen gelatine sections were cut at 10 μ. Sections were washed briefly in water treated with the following histochemical techniques for lipids as cited in [14]: Sudan black B for the demonstra-

tion of lipids in general; Nile blue sulphate technique for neutral and acidic lipids; Sudan III and Sudan IV methods for neutral fats (triglycerides); acid haematin technique together with pyridine extraction control for phospholipids; Schultz's method for cholesterol and/or its esters. Sudanophilic lipids, which stained pink with Nile blue and red with Sudan red, were interpreted as neutral fats (triglycerides). Sudanophilic substances, which stained blue with Nile blue and blue-black with acid haematin, and gave a negative reaction in the material treated with hot pyridine, were interpreted to contain phospholipids. Material fixed in weak Bouin's solution and extracted with hot pyridine [2] and coloured with Sudan black B, was used as a control for lipids in general.

Paraffin sections of material fixed in Zenker's fluid were treated with methyl green-pyronine [14]; controls with ribonuclease and trichloroacetic acid were employed in the study of RNA. Proteins were demonstrated by mercuric bromophenol blue [12].

The following cytological techniques (as cited in [1, 5]) were used for studying the morphology and distribution of cytoplasmic components. The material was fixed according to Lewitsky (Flemming's solution without acetic acid), Champy, Aoyama, and Kolatchev techniques and embedded in paraffin wax. The sections cut at 5 μ were either stained with iron haemotoxylin or mounted unstained for silver nitrate and osmium tetroxide treatment.

Results

As described in previous paper [9] the yolk nucleus and its associated mitochondria, Golgi bodies and lipid inclusion bodies form a subspherical mass in the juxtanuclear ooplasm of oocytes measuring 77 μ in maximum diameter (Fig. 1); most of the lipid bodies that tend to hide the yolk nucleus and its associated organelles are usually lost in the paraffin sections. The early stages in the development, morphology and distribution of yolk nucleus and tis associated components appear to be similar in the corresponding oocytes

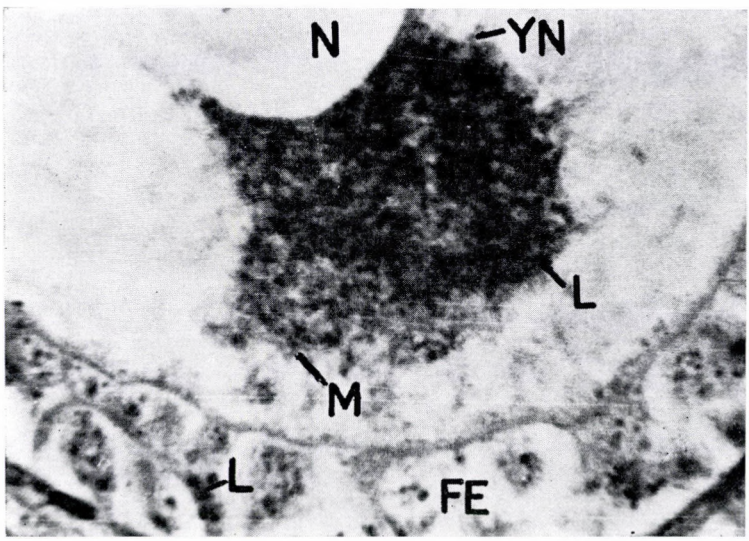

Fig. 1. Portion of oocyte from the ovary of nonvitellogenic female, showing fully developed Balbiani's vitelline body in the juxtanuclear ooplasm. Mitochondria (M) and some lipid bodies (L) are associated with the yolk nucleus (YN) which forms the substrate. Lipid bodies (L) in the follicular epithelium (FE). Portion of nucleus (N) is also seen. Champy/iron haematoxylin. × 1000

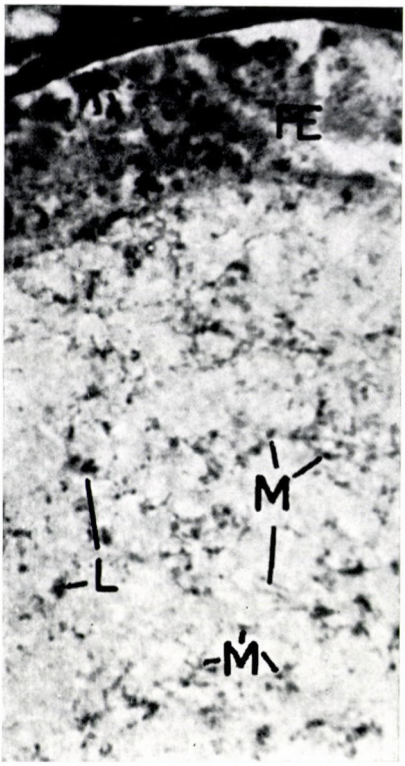

Fig. 2. Portion of oocyte from the ovary of nonvitellogenic female, showing sparsely distributed yolk nucleus substance and mitochondria (M) in the form of granules, rods and filaments. Lipid granules are seen in the follicular epithelium (FE). Champy/iron haematoxylin. ×1000

of nonvitellogenic and vitellogenic ovaries. When the yolk nucleus and its associated organelles and lipid bodies have reached their greatest development in the juxtanuclear ooplasm of oocytes in the nonvitellogenic ovary (Fig. 1), the yolk nucleus itself begins to break up first into irregular masses, then into small elements of the growing oocytes which measure 116 μ in maximum diameter. Simultaneously, the other components of Balbiani's vitelline body are also dispersed in the outer ooplasm. Finally, all the components of Balbiani's vitelline body are sparsely distributed in the ooplasm (Fig. 2). At this stage, the granular, rod-like and filamentous mitochondria, which closely follow the distribution of sparsely distributed yolk nucleus substance in the outer ooplasm, can be studied more precisely. The largest oocytes in the ovaries of juvenile chicken measured 0.6 mm (596 μ) in diameter and yolk formation had not begun in them.

In the vitallogenic ovary, the yolk nucleus still persists in oocytes, which measure 239 μ in diameter, and as a result of its continued growth, it becomes almost as large as the nucleus of the oocyte (Fig. 3). Owing to its dense and

organized nature, the yolk nucleus stands out in sharp contrast to the general ooplasm. Sometimes, two yolk nuclei develop simultaneously in the oocytes of the vitellogenic ovary and continue to persist on oocytes which measure 236 μ in diameter (Fig. 4); the large one lies in the juxtanuclear ooplasm and the small one at the animal pole.

Part of the fully developed yolk nucleus in the oocytes of vitellogenic females takes the form of radiating projections which extend into the outer

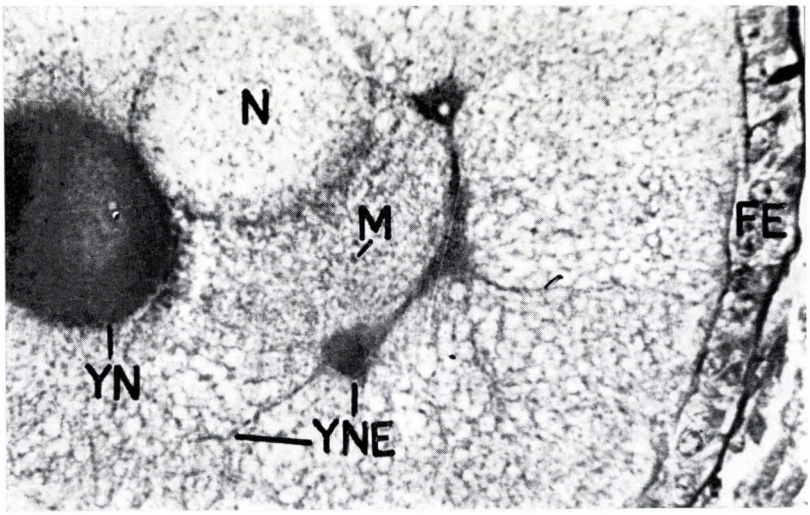

Fig. 3. Portion of oocyte from the ovary of vitellogenic female, illustrating the morphological complexity of the yolk nucleus (N). One extension (YNE) is in the form of arch having several mitochondria (M) and sparsely distributed yolk nucleus substance in its concavity. Portion of follicular epithelium (FE) is also seen. Lewitsky/iron haematoxylin. ×400

ooplasm (Figs 3, 4). The yolk nucleus and its long extensions sometimes develop into a complicated structure (Fig. 3). Its extension may take the form of an arch with its concavity towards the nuclear envelope. The arch consisting of yolk nucleus substance is of variable width and can be distinguished in its thicker and thinner portions (Figs 3, 5). Some secondary projections may also arise from the outer surface, the arch, which reach the cytoplasmic membrane of the oocyte (Fig. 3). The yolk nucleus and its various extensions appear to contribute their substance to the outer ooplasm where it is sparsely distributed. The fully developed yolk nucleus in the oocytes of nonvitellogenic chicken does not develop such extensions of complex nature (Fig. 1).

The fully grown yolk nucleus in some oocytes of vitallogenic females may differentiate into zones of varying density (Figs 3, 6): (1) an inner zone, (2) a middle zone, and (3) an outer zone. Although the fully developed yolk nucleus of the nonvitellogenic ovary (Fig. 1) does not appear to have zones of

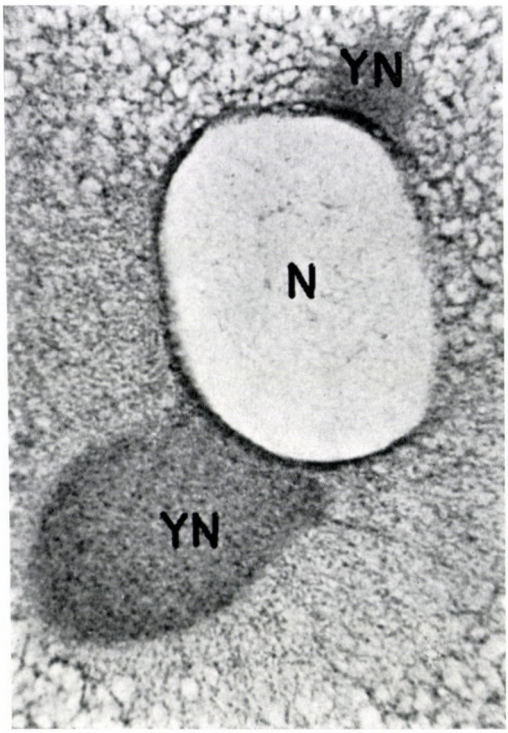

Fig. 4. Portion of oocyte from the ovary of vitellogenic female, showing two yolk nuclei (YN) adjacent to the nucleus (N). Aoyama preparation. ×400

varying density, it does not appear homogeneous since it contains various reticular and granular substructures (Fig. 1).

The substanece of the yolk nucleus and its extensions give a weak positive reaction with Sudan black B, which dissappears in the control material extracted with hot pyridine, revealing the presence of some diffuse lipids in them, in addition to RNA and protein described previously in the yolk nucleus of young oocytes in birds [9]. The diffuse lipid component seems to be due to lipoprotein as judged from its negative reactions in histochemical tests for phospholipids, triglycerides and cholesterol and/or its esters.

Development and differentiation of the yolk nucleus and its extensions in the oocytes of vitellogenic hen are closely accompained by the proliferation and accumulation of various other components in the juxtanuclear ooplasm; they include the mitochondria, Golgi bodies and different types of lipid bodies. The yolk nucleus actually forms the substrate round which the various other organelles continue to multiply and aggregate during oocyte growth. The mitochondria show a higher concentration in the peripheral portions of the yolk nucleus. Their number is also apparently more in the con-

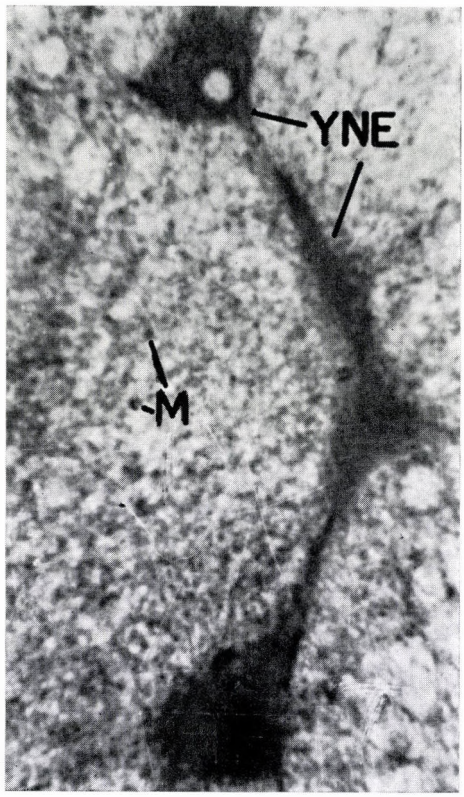

Fig. 5. Higher resolution of yolk nucleus extension (YNE) shown in Figure 3, illustrating mitochondria (M) and sparsely distributed yolk nucleus substance in its vicinity. Lewitsky/iron haematoxylin. ×1000

cavity of the yolk nucleus arch which itself is associated with mitochondria (Figs 3, 5). The distribution and morphology of mitochondria can be best studied in the Champy and Lewitsky iron heamatoxylin preparations where they appear in the form of granules, rods and filaments (Figs 1—3, 5, 6). With histochemical techniques the mitochondria stain mainly as a phospholipid-protein complex [8].

The Golgi bodies form a close morphological association with the outer regions of the fully developed yolk nucleus and could be studied best in material treated by the Aoyama and Kolatchev techniques. In histochemical techniques for lipids, the Golgi bodies appear to stain for lipoprotein as judged from their weak reaction with Sudan black B and negative reactions with other histochemical tests used for the detection of phospholipids, triglycerides and cholesterol and/or its esters. The Golgi bodies like the yolk nucleus resist the action of ethanol and paraffin embedding after various cytological fixations, apparently due to their lipoprotein composition.

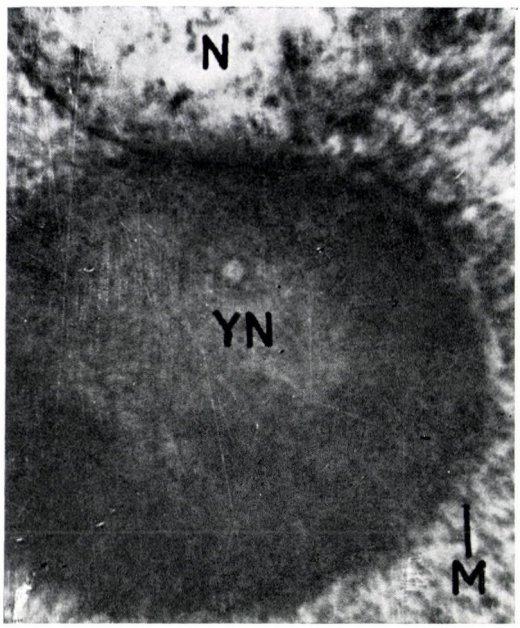

Fig. 6. Higher resolution of yolk nucleus (YN) shown in Figure 3, illustrating its zones. Most of mitochondria (M) are present in the peripheral zone. Portion of nucleus (N) is also seen. Lewitsky/iron haematoxylin. ×1000

Various types of lipid bodies in the form of granules and spheres, that have been described in the young oocytes of birds [8], accumulate along with the growth of yolk nucleus. They usually accumulate in the outer ooplasm. The lipid spheres consisting of triglycerides and cholesterol and/or its esters, are lost in the course of dehydration and paraffin embedding and thus clear vacuoles are forned in the outer ooplasm (Figs 3, 5). The various types of lipid bodies could be studied effectively in the frozen sections of the material fixed in formaldehyde-calcium and postchromed in dichromate calcium and coloured with Sudan black B. They consist mainly of triglycerides, cholesterol and/or its esters and very little phospholipid.

With further growth of the oocyte in the vitellogenic ovary, the persisting yolk nucleus and its extensions finally disappear from view as their substance consisting of RNA, protein and lipoprotein is distributed in the outer ooplasm where it further multiplies and accumulates. The mitochondria, Golgi bodies and lipid bodies are also dispersed in the outer ooplasm and continue to multiply. In some oocytes measuring 255 μ in diameter, the yolk nucleus/nuclei and associated components move away from the juxtanuclear ooplasm and continue to persist as organized structures in the outer ooplasm (Fig. 7) where they appear to proliferate further.

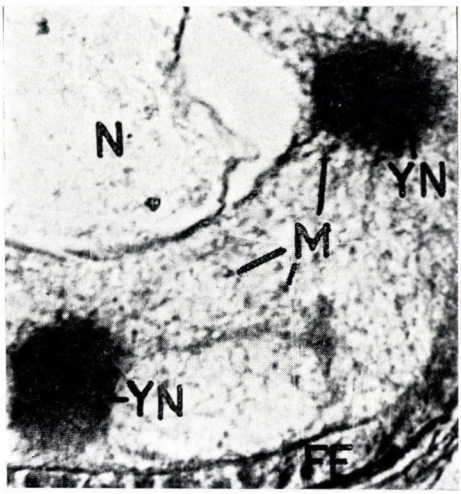

Fig. 7. Portion of oocyte from the ovary of vitellogenic female, showing two yolk nuclei (YN). Mitochondria (M) are also seen. Lewitsky/iron haematoxylin. ×400

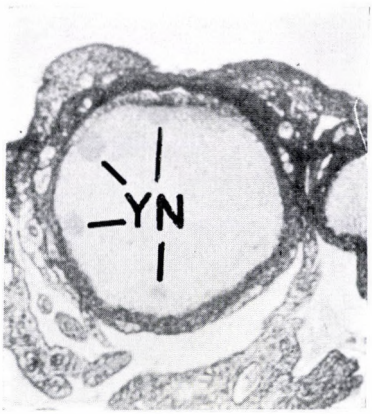

Fig. 8. Oocyte from the ovary of vitellogenic female, showing yolk nuclei (YN) in the cortical ooplasm. Aoyama preparation. ×100

The cortical ooplasm of some growing oocytes (measuring 228 μ in diameter) in vitellogenic females also show 5 to 7 compact spherical bodies which in their morphology and histochemical reactions closely resemble the juxtanuclear yolk nucleus (Fig. 8). They are also associated with the mitochondria, Golgi elments and some lipid granules consisting of phospholipids and triglycerides. These cortical ooplasmic structures are apparently formed from some fragments of original yolk nucleus, which attain a large size by further growth and differentiation. They finally develop into a compact and organized structure in the cortical ooplasm similarly as the juxtanuclear yolk nucleus.

These secondary yolk nuclei of the cortical ooplasm seem to constitute additional sites for the multiplication of yolk nucleus substance, mitochondria, Golgi bodies and possibly of some lipid granules. Finally, they also disappear before the yolk vesicles originate in the central ooplasm. The cortical yolk nuclei are undeveloped in the corresponding oocytes of nonvitellogenic chicken.

Discussion

The present study revealed that Balbiani's body of the fowl oocyte becomes very complex both morphologically and histochemically during maturation of the ovary as described also for the human oocyte [11]. The yolk nucleus component attains its gratest development and morphological complexity in the oocytes of vitellogenic chicken which might in some way be related to yolk deposition on their oocytes and subsequent embryogenesis. Similarly, the persisting yolk nucleus and its associated cell components of vitellogenic females of the wall lizard *(Hemidactylus flaviviridis)* develop a great morphological and histochemical complexity during the breeding season [10].

The paranuclear complex of organelles and inclusions has been described by various terms in previous studies on avian oogenesis [7]. All those terms were used due to the belief that this region of the oocyte has something to do with the formation of yolk. It now appears to be the site of a high metabolic activity concerned with the initial multiplication and differentiation of various organelles and lipid bodies as also supported by its intense acid phosphatase activity.

The greatest development and complexity of the yolk nucleus in the oocytes of vitellogenic females of booth fowl and wall lizard can be attributed to their peculiar metabolism which may be the result of high levels of hormones; the latter are now well known to influence oocyte growth and yolk deposition in non-mammalian vertebrates [6, 13, 15]. Under the effect of those hormones, the metabolic activity of oocytes in vitellogenic females is apparently elevated, and this stimulates the synthesis of abundant complex macromolecules such as RNA, protein, lipoproteins, lipids, etc., used in the construction of the yolk nucleus and its extensions, and of other ooplasmic structures. Simultaneously, their precursor substances might also become available in abundance in the blood plasma of vitellogenic females, and are subsequently transported into the growing oocyte. A similar explanation may be offered for the development of cortical yolk nuclei in the oocytes of vitellogenic hen, which seem to constitute the secondary sites for the multiplication of yolk nucleus substance and other components to be subsequently accumulated in the cortical ooplasm of the vitellogenic ovary. The corresponding oocytes in the nonvitellogenic ovary show a sparse distribution of yolk nucleus substance. Since cytological

(including ultrastructural) and histochemical studies have revealed that the yolk nucleus consists of elements of endoplasmic reticulum (or lipoprotein) and RNP particles [10] the synthesis of such macromolecules is apparently much more stimulated in vitellogenic females than in nonvitellogenic ones. The precise nature of the factors which actually control the developmental processes of the yolk nucleus and other organelles in the growing oocyte, is still a challenging subject for future research. In this regard the study of hormonal effects on the developmental processes of ooplasmic structures, especially of the yolk nucleus, will be rewarding. In general, regulation of the biogenesis of cell organelles and the rules that integrate them to produce cell growth, reproduction, or differentiation are matters about which knowledge is still vague. The results of present study and of a previous one on the lizard oocyte [10] suggest however, that the metabolic status of the female apparently has a great influence on the developmental processes of ooplasmic structures during previtellogenesis.

REFERENCES

1. BAKER, J. R.: Cytological Techniques. Methuen, London 1945. — 2. BAKER, J. R.: (1946) The histochemical recognition of lipide. Quart. J. micr. Sci. **87,** 441—470. — 3. BAKER, J. R.: (1956) Improvements in the Sudan black technique. Quart, J. micr. Sci. **97,** 621—623.— 4. BELLAIRS, R.: (1967) Aspects of the development of yolk spheres in the hen's oocyte studied by electron microscopy. J. Embryol. exp. Morph. **13,** 215—233. — 5. CULLING, C. F. A.: Handbook of Histopathological Techniques. Washington Butterworth, 1963. — 6. GILBERT, A. B.: (1971) The ovary. In: Physiology and Biochemistry of the Domestic Fowl (ed. by D. J. Bell and B. M. Freeman) Vol. 3, pp. 1163—1202 Academic Press, New York. — 7. GREENFIELD, M.: (1966) The oocyte of the domestic chicken shortly after hatching, studied by electron microscopy. J. Embryol. exp. Morph. **15,** 297—316. — 8. GURAYA, S. S.: (1959) Histochemical studies of lipids in oocytes. IV. Lipids in the oogenesis of *Gallus domesticus*, *Streptopelia senigalensis* and *Streptopelia decaocto*. Res. Bull. (N. S.) Punjab Univ. **10,** 119—230. — 9. GURAYA, S. S.: (1962) The structure and function of the so-called yolk nucleus in the oogenesis of birds. Quart. J. micr. Sci. **103,** 411—416. — 10. GURAYA, S. S.: (1968) Further morphological and histochemical studies on the yolk nucleus and associated cell components in the developing oocyte of the Indian wall lizard. J. Morph. **124,** 283—294. — 11. GURAYA, S. S. (1974) Morphology, histochemistry and biochemistry of human oogenesis and ovulation. Int. Rev. Cytol. **37,** 121—151. — 12. MAZIA, A. D., BREWER, P. and ALFERT, M.: (1953) The cytochemical staining and measurements of protein with mercuric bromphenol blue. Biol. Bull. **104,** 57—67. — 13. MCINDOE, W. M.: (1971) Yolk synthesis. In: Physiology and Biochemistry of the Domestic Fowl. (ed. by D. J. Bell and B. M. Freeman), Academic Press, New York, pp. 1209—1220. — 14. PEARSE, A. G. E.: Histochemistry. J. and A. Churchill Ltd., London. 1968. — 15. WALLACE, R. A.: (1972) The role of protein uptake in vertebrate oocyte growth and yolk formation. In: Oogenesis (ed. by J. D. Biggers and A. W. Schuetz), University Park Press, Baltimore, pp. 339—360.

BALBIANISCHE VITELLIN-KÖRPERCHEN IN DEN OOZYTEN VON VITELLOGENEN UND NICHT-VITELLOGENEN HAUSHÜHNERN EINE KORRELATIVE ZYTOLOGISCHE UND HISTOCHEMISCHE STUDIE

S. S. GURAYA

An den in den Oozyten von vitellogenen und nichtvitellogenen Haushühnern *(Gallus domesticus)* befindlichen Balbianischen Vitellin-Körperchen, die aus dem Eikern, aus Mitochondrien, Golgi-Körperchen und Lipiden unterschiedlicher Art bestehen, wurden zytologische und histochemische Untersuchungen ausgeführt. Der aus RNS, Eiweiß und Lipoprotein bestehende Eikern zeigt den höchsten Entwicklungsgrad und die größte morphologische Komplexität in den wachsenden Oozyten der vitellogenen Hühner. Die mögliche Bedeutung dieser Beobachtung in bezug auf den sich im Verlauf der geschlechtlichen Reifung verändernden Stoffwechsel und den hormonalen Status wird besprochen.

ВИТЕЛЛИНОВЫЕ ТЕЛЬЦА БАЛБИАНИ В ООЦИТАХ ВИТЕЛЛОГЕННЫХ И НЕВИТЕЛЛОГЕННЫХ КУР: КОРРЕЛЯТИВНЫЕ ЦИТОЛОГИЧЕСКИЕ И ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ

С. С. ГУРАЙА

Цитологические и гистохимические исследования были проведены на вителлиновых тельцах Балбиани, находящихся в ооцитах вителлогенных и невителлогенных кур (Gallus domesticus). Эти тельца состоят из германитивного ядра, из митохондрий, телец Гольджи и из липидов различной природы. Состоящее из РНК, из белков и липопротеинов герминативное ядро показывает максимальное развитие и наибольшую морфологическую коммплексность в растущих ооцитах вителлогенных кур. Обсуждается возможное значение этого наблюдения в отношении обмена веществ и гормонального статуса в ходе полового созревания самки.

Sardul S. GURAJA: Department of Zoology Punjab Agricultural University, Ludhiana, Punjab India

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Institute of Anatomy, Histology and Embryology, University Medical School, Debrecen

EFFECT OF TRIIODOTHYRONINE (T_3) THYROPARATHYROIDECTOMY AND MERCAPTOIMIDAZOLE TREATMENT ON ENCHONDRAL BONE GROWTH

II. GROWTH DYNAMICS IN THE PROXIMAL GROWTH ORGAN OF THE TIBIA IN YOUNG RATS

G. Lévai, M. Petkó, S. Varga, J. Laczkó* and J. Gyarmati, Jr.

(Received May 29, 1975)

The 24 hour growth rate of the tibial proximal growth organ was investigated in triiodothyronine or mercaptoimidazole-treated, and in thyroidectomized male rats. Longitudinal growth of the tibia of the same period was also studied. After one week treatment, the 24 hour rate of growth in the triiodothyronine-treated rats was retarded as compared with the controls, whereas at the end of the 3rd and 5th weeks the growth rate of the treated animals exceeded that of the controls. The tibia was somewhat longer in the treated rats than in the untreated controls, though the difference was not significant.

The growth rate in the growth organ of mercaptoimidazole-treated and thyroidectomized animals was generally higher than that measured in the controls. The length of the tibia was less than in the controls, but the difference was not significant. The phenomenon is attributed to the altered hormonal conditions.

Introduction

Growth of long bones occurs in their growth organs [11] as a result of enchondral ossification. The growth rate of these bones — apart from a transient acceleration taking place during puberty — gradually decreases until linear bone growth stops altogether [4]. Normal equilibrium of the endocrine system is indispensable for physiological growth. If this equilibrium is upset, profound changes take place in longitudinal growth [15, 21]. Most of growth disturbances having an endocrine origin are caused by the altered function of the thyroid [9, 15, 16, 21]. In a previous work we have analysed by biometrical methods the morphological alterations ensuing in the proximal tibial growth organ of developing rats under the effect of triiodothyronine and mercaptoimidazole treatment and thyroidectomy [10].

In the present work we have studied the changes in longitudinal growth of the tibia, and in that of the proximal growth organ following triiodothyronine and goitrogenic treatment, and surgical thyroidectomy.

* Present address: Central Research Laboratory, University Medical School, Debrecen.

Material and method

Experiments were carried out on the proximal epiphyseal growth organ of the tibia of 28—30-day-old (42—45 g) male rats of our own breed. Fifteen animals were treated with triiodothyronine hydrochloride (Lyothyronin*) in daily doses of 0.01 mg/100 g body weight given orally. Fifteen animals were treated with thiamazole (Metothyrin*) orally, in daily doses of 10 mg/100 g body weight. In 30 rats, thyroidectomy was performed according to GRIESBACH and PURVES [2]. For further processing the material of 15 animals was used in complete extirpation of the thyroid gland was verified after death. The drugs were mixed to the food. The experimental conditions (food, temperature of the room, moisture content of the air, light) were identical with every group. The animals received tap water ad libitum.

After 7, 21 and 35 days of treatment, 5 animals from each group were killed by overdosage of ether. Forty-eight hours before, every animal was given 25 mg/kg oxytetracycline and 24 hours later, 50 mg/kg Fluorexon** (2,4 bis/N,N'-carbomethyl/-fluorescein) intraperitoneally using the method of MODIS et al. [14].

Attention was paid to the precise dosage of fluorochrome and the exact time of killing the animals. Both tibiae of the animals were removed. From the proximal end of the left tibia 18 μ thick cryostat sections were made and after a brief fixation in absolute alcohol the sections were mounted in Fluoromont (Microme) and examined in ultraviolet light with BG 3/4—OG 1/1 filter combination. Tetracycline gave a yellow, and Fluorexon a green fluorescence. The distance between the lower margins of the fluorescent streaks was measured by an oculometer. The results of measurements are shown in Table I, where the data represent the absolute values for growth in μ, taking place in the 24 hours elapsed following tetracycline and Fluorexon administration.

The right tibiae were fixed in acetone and dried at room temperature. Their length was measured with a caliper. The data relating to tibial length and body length (distance between the tip of the nose and base of the tail) are given in Table II.

Results

Table I, in addition to data of measurements, contains also the values calculated for 24 hours of growth of the tibia. The value for 24 hour growth was calculated from tibial length (Table II) by dividing the difference between mean tibial length measured on the 7th and 21st days of treatment by the number of days elapsed (14 days). E.g. the mean length of the tibia of the control animals was 25.17 mm on the 7th, and 27.35 mm on the 21st day of the experiment; the difference (2.18 mm = 2180 μ) divided by 14 gives the value of the average daily growth of the tibia; in the given case it was 155.71 μ.

In control rats the calculated mean daily growth of the tibia reached the highest value in the first week (271 μ) and then declined in the third and fifth week to about the same level (155 μ, and 158 μ, respectively). A similar tendency was noted in the growth rate of the proximal growth organ of the tibia at identical time intervals (129 μ, 110 μ, and 105 μ, respectively).

Following surgical thyroidectomy the calculated values for tibial growth were lower at the end of the first and fifth week as compared with the controls (217 μ, 137 μ), while in the third week they somewhat exceeded the control ones (163 μ). The 24 hour growth rate measured in the proximal growth organ

* = Kőbányai Gyógyszergyár, Budapest
** = Chemopol, Prague

Table I

24-hour growth rate measured in proximal growth organ of the rat tibia, and calculated values for 24-hour tibial growth

Treatment	1 week (37-day-old rat)		3 weeks (51-day-old rat)		5 weeks (65-day-old rat)	
	Growth (μ)					
	tibia	proximal growth organ	tibia	proximal growth organ	tibia	proximal growth organ
Control	271.42	129.6± 6.2	155.71	110.0±10 6	158.57	105.1± 5.6
Triiodothyronine treated	321.42	119.3± 4.3 (1)	150.71	131.6± 7.8 (1)	132.14	109.3± 4.3 (1)
Thiamazole-treated	254.28	150.6± 8.4 (1)	140.00	110.0± 8.2	95.00	144.6±10.1 (1)
Thyroid-ectomized	217.14	183.2±12.1 (1)	163.57	124.4± 7.4 (1)	137.85	109.2± 4.5 (1)

(1) $p < 0.1\%$

Table II

Body length of rats (distance between the tip of nose and base of tail) and length of the tibia

Treatment	Duration (week)	Body length (cm)	Tibial length (mm)
Control	1 (37 day-old rat)	12.94	25.17
	3 (51 day-old rat)	14.20	27.35
	5 (65 day-old rat)	17.80	29.57
Triiodothyronine-treated	1 (37 day-old rat)	13.42 (2)	25.67 (4)
	3 (51 day-old rat)	14.00 (4)	27.78 (4)
	5 (65 day-old rat)	17.88 (4)	29.63 (4)
Thiamazole-treated	1 (37 day-old rat)	12.20 (1)	25.10 (4)
	3 (51 day-old rat)	13.50 (2)	27.06 (4)
	5 (65 day-old rat)	14.92 (1)	28.39 (3)
Thyroidectomized	1 (37 day-old rat)	11.45 (1)	24.67 (4)
	3 (51 day-old rat)	13.56 (1)	26.96 (4)
	5 (65 day-old rat)	16.36 (1)	28.89 (3)

(1) $p < 0.1\%$ (2) $p < 1\%$ (3) $p < 5\%$ (4) $p < 10\%$

of the tibia showed likewise a decreasing tendency, though the values were higher (183 μ, 124 μ and 109 μ respectively) than the control ones.

In thiamazole-treated rats the calculated value for daily tibial growth was lower at all time intervals (254 μ, 140 μ, and 95 μ) than in the controls showing a similar decreasing tendency. The 24-hour growth rate measured in the proximal growth organ of the tibia exceeded the control values in the first and fifth week (150 μ, and 144 μ, respectively), whereas in the third week there was no difference between the treated and control animals (110 μ).

Triiodothyronine treatment enhanced the daily growth rate of the tibia (321 μ) in the first week, and decreased it at the end of the third and fifth weeks (150 μ, and 132 μ, respectively). The growth rate measured in the proximal growth organ of the tibia for the same time interval was lower in the first week (119 μ), whereas at the end of the third and fifth weeks it was higher (131 μ, and 109 μ, respectively) than in the controls.

Thyroidectomy and goitrogenic treatment caused a significant retardation of growth. On the other hand, in rats treated with triiodothyronine, at the end of the first week longitudinal growth significantly exceeded that in the controls. At the end of the third and fifth weeks the difference in body length between the two groups was not significant.

Discussion

The use of markers allows to measure the extent of growth taking place in a given organ in certain time intervals [4]. The first such intravitally given "marker" was the madder, *Rubia tinctoria* KRAPP [3]. Now isotopes [7, 8] and fluorochromes [12, 13] serve as markers. The latter allow to measure longitudinal growth within a short period of time e.g. 24 hours. Despite the scattering of values, the results obtained are acceptable. We could find no data on the application of fluorochromes in distrubances of bone growth of endocrine origin.

In a previous work [10] we have described the histological changes occurring in the proximal growth organ of the tibia in young rats following triiodothyronine or thiamazole treatment and surgical thyroidectomy. In the present study we have measured growth by means of fluorochromes under similar conditions.

The methods and results of our experiments [19] and with a difference of 2—4 days our intervals too correspond to those used by TAPP. The values for the length of the tibia in the 3 intervals were as follows (the first figures are those given by TAPP): 25 mm—25.17 mm; 27.5 mm—27.35 mm; 29 mm—29.57 mm. The 24-hour growth measured on the proximal epiphysis of the tibia was: 180 μ—129 μ; 120 μ—110 μ; 110 μ—105 μ. Thus, our results are in agreement with TAPP's data, but differ from those published by HANSSON et al. [5] obtained on Sprague-Dawley rats. The differences may be due to differences in the rat strains and to the diversity of methods.

In a 24-hour interval SISSONS [17] noted a growth of 180 μ in the distal epiphysis of rat femora in the first five weeks of the experiment. KEMBER [7] measured a growth of 125 μ in the proximal epiphysis of 5—8-week-old rat tibiae. All these data are in close agreement with our results obtained on control rats.

Changes elicited in the endocrine system modify the examined parameters of longitudinal bone growth.

Following triiodothyronine treatment no significant change occurred in tibial length as compared with the controls, and body length of the treated animals was significantly greater only at the end of the first week. The calculated values for daily growth of the tibia differ from the controls by considerably higher values in the first week and lower ones in the third and fifth weeks. Measurements in the proximal growth organ of the tibia showed significantly lower values in the first week, and higher ones at the end of the third and fifth weeks, as compared to the controls. The latter data seem to indicate that triiodothyronine treatment elicits a transient slowing down of growth followed by an acceleration and eventually by a return to the normal level. The changes may be interpreted as resulting from the effect of large amounts of growth hormone released under the effect of the sudden elevation of the blood thyroid hormone level. The slow growth noted on the 7th day was probably the result of a transient growth hormone deficit due to the mobilization of reserves. For the production of growth hormone the presence in the blood of thyroid hormones is required [6, 18]. The proximal growth organ of the tibia is the most sensitive indicator of the blood growth hormone level [1]. In the changes elicited in the initial period of treatment the primary triiodothyronine effect exerted directly on the growth organ may have been involved [10]. In the later period, the growth rate approached that of the controls; this may be interpreted as a sign of adaptation of the endocrine system to the changed conditions.

Thiamazole treatment and thyroidectomy elicited a significant decrease in growth. A decrease was noted in the growth of the tibia. The 24-hour growth measured in the proximal growth organ of the tibia likewise differed remarkably from the control values. In fact, these values in two of the three intervals examined were higher in the treated animals. Owing to the different endocrine conditions, the results obtained in the two groups will be discussed separately despite the finding that the serum thyroid hormone level decreased in both cases. In thiamazole-treated animals the production of thyroid hormone showed a gradual decrease [6], while the thyrotropic hormone (TSH) level was raised. These animals, unlike thyroidectomized ones, possess complete C-cells and parathyroid glands.

When goitrogenic treatment is applied we have to reckon with the direct peripheral effect of the drug. In the case of thyroparathyroidectomy the major part of C-cells and of the parathyroid substance is removed. Consequently, a degranulation of the acidophilic cells of the adenophypophysis [6, 9, 16], decrease of growth and of the blood thyroid hormone levels occurs. Nevertheless, a minimum amount of thyroid hormone always persists in the circulation [6]. These observations may well explain the differences in growth rate of the two groups of animals. After surgical thyroidectomy the endocrine equilibrium is

suddenly upset. We suppose that this change in the endocrine system, with the simultaneous elevation of TSH production, may also have a share in the increased mobilization of growth hormone. The latter process may result in a degranulation of acidophilic cells. The growth observed at the end of the fifth week approaches the control values; this seems to be due to the adaptational efforts of the organism and, in addition, to the persistence of a slight amount of thyroid hormone in the blood [6]. The minimum hormone level may be sufficient for the production of an amount of growth hormone ensuring an almost normal growth of the proximal tibial growth organ [1]. Thiamazole treatment elicited a considerable increase in growth at the end of the first and fifth weeks. The changes noted in the first week seem to be likewise explained by the increased mobilization of growth hormone. Increased growth at the end of the fifth week may be interpreted as the result of higher TCT secretion of the C-cells of the thyroid. WUTTKE et al. [22] observed increased activity in the thyroid C-cells after goitrogenic treatment, and this increased activitiy might be associated with TCT secretion. According to TARSOLY and BUCHER [20], prolonged administration of TCT leads in rats to a proliferation of chondrocytes in the proximal tibial epiphyseal cartilage which is the morphological sign of accelerated growth. The alterations become conspicuous only after several weeks treatment.

Our control measurements are in agreement with the data in the literature, which is a proof of the usefulness of the method employed. The results of measurements obtained under pathological endocrine conditions may serve as basis for similar investigations of growth dynamics. For the clarification of the exact background of the changes observed, further studies are required.

REFERENCES

1. EVANS, H. M., SIMPSON, M. E., MARX, W., KIRBICK, E.: (1943) Bioassay of the pituitary growth hormone. Width of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats. Endocrinology **32,** 13—25. — 2. GRIESBACH, W. E., PURVES, H. D.: (1945) The significance of the basophil changes in the pituitary accompanying various forms of thyroxine deficiency. Brit. J. exp. Path. **26,** 13—17. — 3. HALES, S.: (1727) Statical essays: containing vegetable statics: organ account of statical experiments on the sap in vegetables. Innys, Woodward and Peele, London. Vol. I. — 4. HANSSON, L. I.: (1967) Daily growth in length of diaphysis measured by oxytetracycline in rabbit normally and after medullary plugging. Acta Orthop. scand. Suppl. No. 101. — 5. HANSSON, L. I., MERANDER-SELLMAN, K., STENSTRÖM, A., THORNGREN, K-G.: (1972) Rate of normal longitudinal bone growth in the rat. Calc. Tissue Res. **10,** 238—251. — 6. HERLANT, M.: (1964) The cells of the adenohypophysis and their functional significance. Int. Rev. Cytol. **17,** 299—382. — 7. KEMBER, N. F.: (1960) Cell division in enchondral ossification. A study of cell proliferation in rat bones by the method of tritiated thymidine autoradiography. J. Bone Jt. Surg. **42—B** 824—839. — 8. KEMBER, N. F., WALKER, K. W. R.: (1971) Control of bone growth in rat. Nature (Lond.) **229,** 428—429. — 9. KONEFF, A. A., SCOW, R. O., SIMPSON, M. E., LI, C. H., EVANS, H. M.: (1949) Response by the rat thyro-parathyroidectomized at birth to growth hormone and thyroxine given separately or in combination. II. Histological changes in the pituitary. Anat. Rec. **104,** 465—476. — 10. LÉVAI, G., VARGA, S., GYARMATI, J., LACZKÓ, J.: (1975) Effect of triiodothyronine treatment, thyroparathyroidectomy and mercaptoimidazole treatment on enchondral bone

growth. I. Changes in the histological structure of the growth organ. Acta morph. Acad. Sci. hung. **23,** 9—19. — 11. McLean, F. C., Urist, M. R.: (1968) Bone. Fundamentals of the Physiology of Skeletal Tissue. University of Chicago Press, Chicago.— 12. Milch, R. A., Rall D. P., Tobie, J. E. : (1957) Bone localization of the tetracyclines. J. nat. Cancer Inst. **19,** 87—93. — 13. Milch, R. A., Rall, D. P., Tobie, J. E.: (1958): Fluorescence of tetracycline antibiotics in bone. J. Bone Jt. Surg. **40—A,** 897—910. — 14. Módis, L., Petkó, M., Földes, I.: (1969): Histochemical examination of supporting tissues by means of fluorescence. II. Fluorochromes as an indicator of bone mineralization. Acta morph. Acad. Sci. hung. **17,** 157—166. — 15. Riekstnice, Emilija, Asling, C. W.: (1966) Thyroxine augmentation of growth hormone induced endochondral osteogenesis. Proc. Soc. exp. Biol. (N. Y.) **123,** 258—263. — 16. Scow, R. O., Simpson, M. E., Asling, C. W., Li, C. H., Evans, H. M.: (1949) Response by the rat thyro-parathyroidectomized at birth to growth hormone and thyroxine given separately or in combination. I. General growth and organ changes. Anat. Rec. **104,** 445—463. — 17. Sissons, H. A.: (1971) The growth of bone. In: The Biochemistry and Physiology of Bone. Vol. III. Ed: Bourne, G. H. Academic Press, New-York — 18. Suzuki, M., Shibasaki, K.: (1970) Early effect of thyroxine on synthesis of growth hormone and prolactin in the adenohypophysis of thyroidectomized rat. Endocr. exp. **4,** 187—199. — 19. Tapp, E.: (1966) Tetracycline labeling methods of measuring the growth of bones in the rat. J. Bone Jt. Surg. **48—B,** 517—525. — 20. Tarsoly, E., Bucher, O.: (1973) Histologische und enzymhistochemische Untersuchung des Epiphysenknorpels von Ratten nach langdauernder Calcitonin-Verabreichung. Acta histochem. (Jena) **45,** 10—20. — 21. Thorongen, K-G., Hansson, L. I.: (1972) Effect of thyroxine and growth hormone on longitudinal bone in the hypophysectomized rat. Acta endocr. (Kbh) **74,** 24—40. — 22. Wuttke, H., Kessler, F. J., Junger, A.: (1971) Mikrokinetischer Einfluss des thyrotropen Hormons auf die C-Zellen der Schilddrüsse. Z. ges. exp. Med. **155,** 20—31.

DIE WIRKUNG DER TRIJODTHYRONIN-(T_3) BEHANDLUNG, DER THYREOPARATHYREOIDEKTOMIE UND DER MERKAPTOIMIDASOL-BEHANDLUNG AUF DAS ENCHONDRALE KNOCHENWACHSTUM

II. Untersuchung der Wachstumsdynamik am proximalen Wachstumsorgan der Tibia junger Ratten

G. LÉVAI, M. PETKÓ, S. VARGA, J. LACZKÓ und J. GYARMATI jr.

Am proximalen Wachstumsorgan der Tibia junger männlicher Ratten wurde nach Trijodthyronin- und Merkaptoimidason-Behandlung sowie nach Thyreoidektomie das 24-Stunden-Wachstum und die Längenveränderungen der Tibia untersucht. Unter Trijodthyroninwirkung sind die am proximalen Wachstumsorgan der Tibia am Ende der ersten Behandlungswoche im 24-Stundenwachstum ermittelten Werte geringer als die gleichen Werte der Kontrolltiere; demgegenüber sind am Ende der 3. und 5. Behandlungswoche die Werte der Versuchstiere größer als diejenigen der Kontrollratten. Die Tibialänge der behandelten Tiere übersteigt zwar etwas die der Kontrollen, doch ist der Unterschied nicht signifikant. Unter Merkaptoimidasol-Wirkung bzw. nach Thyreoidektomie übersteigt der Wert des im proximalen Wachstumsorgan der Tibia gemessenen 24-Stunden-Wachstums in der Regel die Kontrollwerte. Zugleich ist die Tibialänge im allgemeinen geringer als die Kontrollwerte, indessen nicht in signifikantem Ausmaß. Als Erklärung für diese Erscheinung werden die im Laufe der Eingriffe entstandenen hormonellen Veränderungen angeführt.

ДЕЙСТВИЕ ТРИЙОДТИРОНИНА (T_3), МЕРКАПТОИМИДАЗОЛА И ТИРЕОПАРАТИРЕРОИДЕКТОМИИ НА ЭНХОНДРАЛЬНЫЙ РОСТ КОСТЕЙ

II. Изучение динамики роста на проксимальном органе роста большеберцовой кости молодых крыс

Г. ЛЕВАИ, М. ПЕТКО, Ш. ВАРГА, Й. ЛАЦКО и Й. ДЯРМАТИ МЛ.

На молодых крысах-самцах после дачи трийодтиронина или меркаптоимидазола и после проведения тиреоидектомии, авторы изучали в проксимальном органе роста большеберцовой кости размер роста, происходящего в течение сутки, и изменения длины большеберцовой кости. Было установлено, что под влиянием дачи трийодтиронина суточный

рост проксимального органа роста большеберцовой кости к концу первой недели опыта отстает от такового у контрольных животных, в то время как к концу третьей и пятой недели дачи препарата, величины подопытных животных превышают величины измеренные у контролей. Длина большеберцовой кости подопытных животных в незначительной мере превышает длину большеберцовой кости контролей, причем разница не явичины достоверной. Под влиянием дачи меркаптоимидазола или после тиреоидектомии величины суточного роста проксимального органа роста большеберцовой кости в общем превышают величины контрольных крыс. В то же время длина большеберцовой кости, как правило, отстает — хотя только в незначительной мере — от величин контрольных животных. Для объяснения этого явления авторы приводят гормональные условия, развивающиеся в ходе вмешательств.

Dr. Géza Lévai
Dr. Mihály Petkó
Dr. Sándor Varga
Dr. Jenő Laczkó
Dr. János Gyarmati

DOTE Anatómiai, Szövet- és Fejlődéstani Intézet
4012 Debrecen
Pf. 14, Hungary

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Institute of Forensic Medicine, Semmelweis University Medical School, Budapest

SCANNING ELECTRON MICROSCOPIC INVESTIGATION OF THERMAL DAMAGE OF THE TEETH

L. HARSÁNYI

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Sound teeth were heated to 200 °C and 1300 °C and the gradually developing morphological changes have been studied. The cementum structure was destroyed at about 500 °C, the enamel structure between 700 and 900 °C, whereas dentine preserved its canalicular structure even after the inorganic salts had melted at 900° to 1000 °C. At 1300 °C the mineral substances of the tooth were melting into atypical, globular formations. Scanning electron microscopic examination of dental residues damaged by high temperature seems valuable from forensic, criminological as well as anthropological aspects, since the origin of the finding can be determined, from a small fragment, the material can be identified with a tooth and conclusions can be drawn concerning the temperature inducing the damage.

Teeth are well-known to resist high temperatures. Examination of dental residues may be important in cases where on the basis of parts of corpses, human remains found after a fire, blast or combustion expert opinion has to given concerning the origin of the finding, wether the material would be part of a tooth, and to clarify the circumstances of death. Thermal damage to the teeth has been investigated, experimentally by a number of authors and the ensuing morphological changes have been described in detail in the pertaining literature [1, 14, 15, 18, 19, 22, 24, 25, 28, 29, 32, 35, 43—46, 48, 51, 56]. In the course of anthropological studies, expert examination and evaluation of skeletal and dental findings originating from urn burials provided considerable help [2, 11, 12, 16, 17, 38].

Electron microscopy offered new possibilities for the recognition of teeth by transmission, replica, as well as scanning (SEM) techniques [7—10, 13, 20, 23, 31, 36, 42, 50, 52].

SEM was soon included among the examination methods of forensic medicine, by its application, observations were carried out on electric current marks, fibrin network developing in haemorrhages, wounds, bone injuries [3—6], the marks of instruments on bones [21, 39, 40] and bite-marks [53]. In the criminological mark-reading, trassology, particular attention was paid to micro-marks: textile and plastic fibres, dye granules, lacquer fragments, metal and wood particles, mineral granules, hair, etc. [55].

The present paper reports on a study of the thermal damage to teeth by SEM, a technique not yet applied for such porposes.

Material and method

From the fresh corpses of 35 to 45 years old men and women sound incisor and premolar teeth were removed and weighed together with adhering shreds of soft tissue and the dental pulp. Preserving agents were not applied. Four teeth each from every corpse formed one experimental group. Thermal treatment was carried out in an electric incinerator. The temperature was elevated to 1300 °C in steps of 100 °C. Each group reached the experimental temperature in 5 minutes; thermal treatment lasted for further 55 minutes.

The teeth removed from the furnace were cooled at room temperature and weighed again. The loss in weight agreed well with data in the literature, amounting to 25 to 28% after heating to between 200 °C and 1300 °C [49]. About 66% of the weight loss the major occurred between 200 °C and 1300 °C, owing to dehydration [35]. The lost water originated from the about 10 to 12% water content of dentine and of the higher content of the dental pulp. Further weight loss amounting to 5 to 8% of the initial weight occurred between 300 °C and 1000 °C.

The teeth were first observed by a magnifying glass. After registering the gross phenomena, SEM of smaller fragments was performed. Corresponding to the technical possibilities of our electron microscope, fragments about $6 \times 6 \times 4$ mm in size were examined. The material was fixed to the holding plate with Elektrodag 416 Acheson, Canada adhesive. Steaming was performed in 4×10^{-5} vacuum by a JEOL JEE 4.B rotating device. Na carbon layer has been applied; over the surface of the material an about 30 nm (300 Å) gold layer was spread. A scanning appliance mounted on a JEM 100B apparatus was used. From the enamel, dentine and cementum substance of each tooth several specimens were prepared; the external surface of the enamel. The bony substance and the fracture surface of dentine were studied. The structural changes of the teeth treated at identical temperatures were in full agreement. The Figures demonstrate specimens originating from the same tooth.

Results

At 200 °C only changes in colour were induced.

300 °C, 1 hr

Gross changes: Crown and root are dark greyish brown: on the crown small crevices can be observed. The fraction surface of dentine is light greyish brown in colour. The enamel cap starts to peel off in some places in the form of small crevices. The cavities are not narrowed.

SEM (Plate I)

A. Enamel. Small crevices can be seen, which form no network. In the areas between the crevices the enamel surface is intact.

B. Dentine. The structure is preserved. The tubules have opened up horizontally or longitudinally; their morphology is unaffected.

C. D. Cementum. The evaporating water lifts the cementum from dentine, "vesicles" with disrupted walls are formed. On the denuded surface the tubular orifices can be seen.

500 °C, 1 hr

Gross changes. The enamel is grey, the root light bronwish-gray, the fraction surface of dentine dark greyish-black. The crown displays deep longitudinal furrows, almost dividing it into several pieces. The pulp chamber and the root canal are preserved and not narrowed.

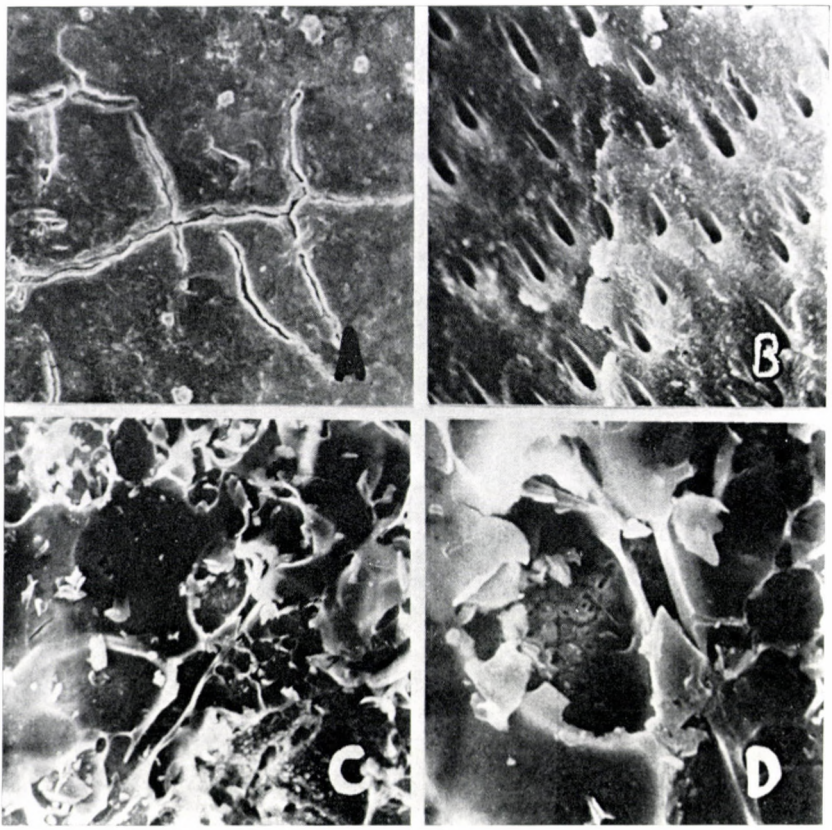

Plate I. 300 °C A. Enamel ×430 B. Dentine ×3300 C. Cementum ×430 D. Cementum ×1100

SEM (Plate II)

A. B. Enamel. The enamel substance is divided by a deep network of crevices into irregular, multiangular plates.

C. Dentine. The structure is preserved. The open dental canalicules are not narrowed, their anatomical properties can excellently be studied. According to KOMORI [32] as well as FURUHATA and YAMAMOTO [14] the dentine structure would be rendered indistinguishable at 500 °C. This is an erroneous statement, due obviously to the HIRANO [24] method applied by these authors: they embedded the fragment into gelatin and stained it with fuchsin to obtain light microscopic preparation. This could not succeed with the calcinated, brittle material. The SEM method saves the brittle substance, induces no changes on its surface and this will result in blameless persistence of dentine.

D. Cementum. The long substance is aggregated into large plates divided by deep furrows. The single portions are irregularly multiangular and measure about 30 to 60 μ in diameter.

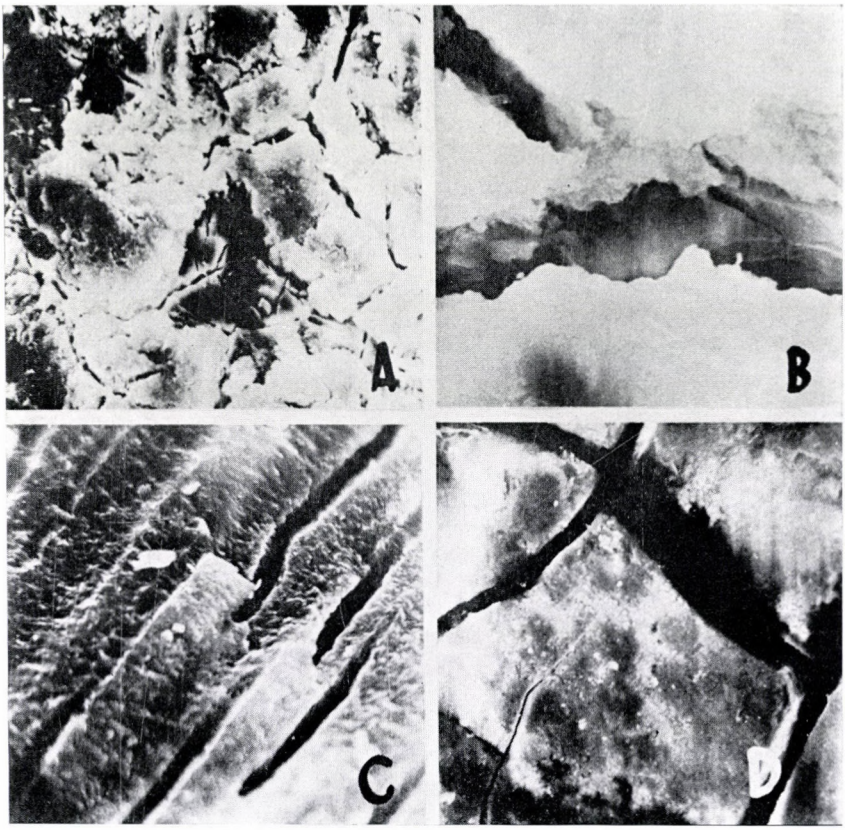

Plate II. 500 °C A. Enamel ×430 B. Enamel ×1100 C. Dentine ×3300 D. Cementum ×430

700 °C, 1 hr

Gross changes. The crown and root are light greyish-white, the dentine is pale grey in colour. The crown is broken into fragments, in some parts the pulp chamber and the root canal can be recognized but narrow.

SEM (Plate III)

A. Enamel. It consists of fine grained granules; the original surface cannot be recognized.

B. C. Dentine. The dentine tubules are somewhat narrowed but can be studied well. The hypermineralized peritubular zone described by JOHANSEN and PARKS [27] is about 0.5 μ thick; it can be against the environment and the intertubular dentine substance as a slightly prominent, differentiated layer. Owing to its high salt content the peritubular zone is more heat-resistant than the intertubular dentine, which contains more organic material and water.

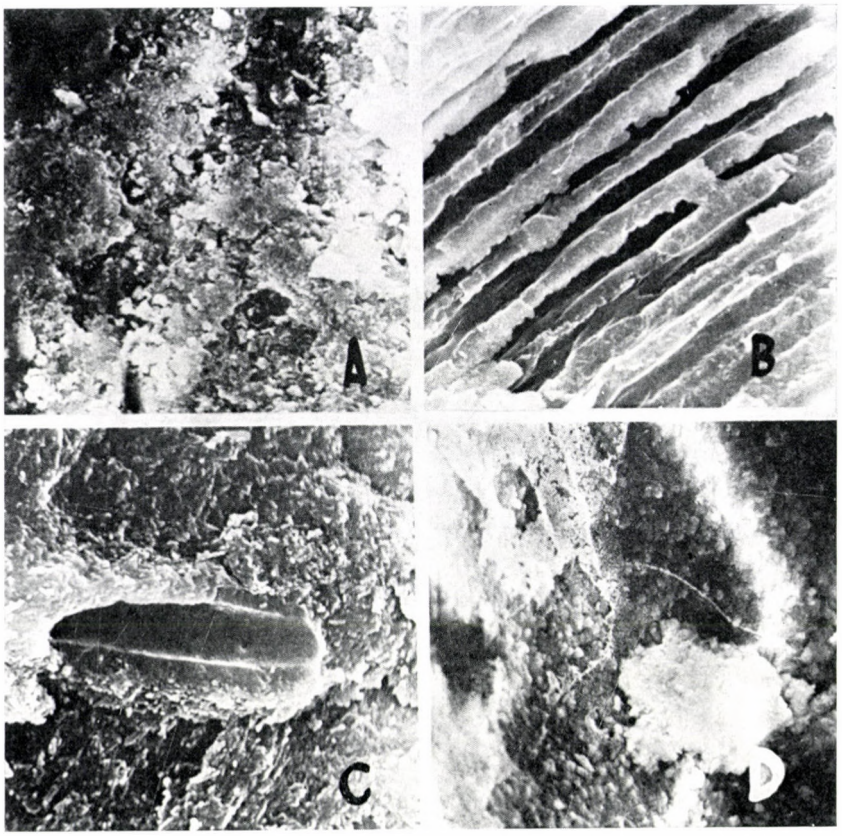

Plate III. 700 °C A. Enamel ×1100 B. Dentine ×3300 C. Dentine ×11,000 D. Cementum ×1100

D. Cementum. The surface finely granular, the original structure cannot be seen.

900 °C, 1 hr

Gross changes. Crown, root and dentine are equally light, almost white. The crown is broken into smaller, the root into larger pieces; on the fragments the narrowed cavities of the tooth can be recognized.

SEM (Plate IV)

A. Enamel. The enamel grains start to fuse; the structure cannot be recognized.

B. C. Dentine. The structure can be studied well. The narrowed dentine tubules measure 1.5 to 1.7 μ in diameter; the orifices of the anastomoses between tubules cannot be seen, since the salts have fused into smooth flat plates irregular in size and rounded off at their margins. The melting point of apatite (hydroxy-apatite, francolite, etc.), which forms the bulk of the anorganic

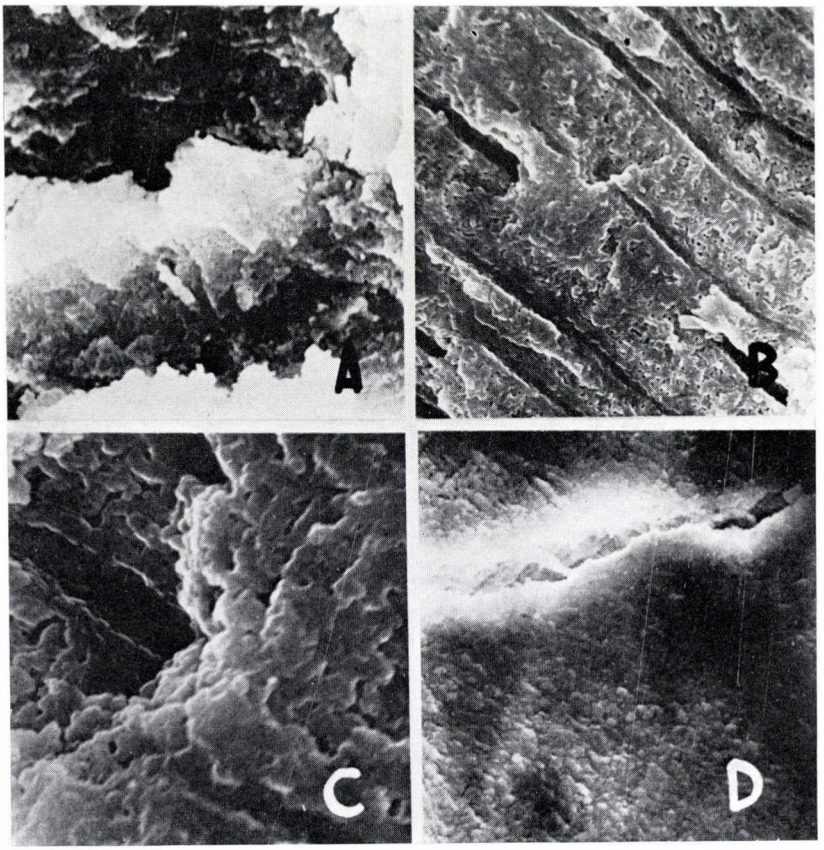

Plate IV. 900 °C A. Enamel ×3300 B. Dentine ×3300 C. Dentine ×11000 D. Cementum ×1100

substances of the tooth is, considerably higher than 900 °C; e.g. KOCH and SZTRÓKAY [30] estimated it at 1550 to 1650 °C. However, in addition to calcium and phosphorus the tooth contains a number of other elements e.g. sodium, potassium, chlorine, magnesium, copper, zinc, fluorine and iron originating from organic substances. These decrease the melting temperature of apatite.

D. Cementum. The surface is granular penetrated by deep and wide crevices; the original structure is decomposed.

1000 °C, 1 hr

Gross changes. Crown root and dentine are equally porcelain-white; the tooth has broken up into pieces; in the fragments the narrow pulp chamber and the root canal can slightly be distinguished.

SEM (Plate V)

A. Enamel has liquefied into smooth plates which have lost their structure.

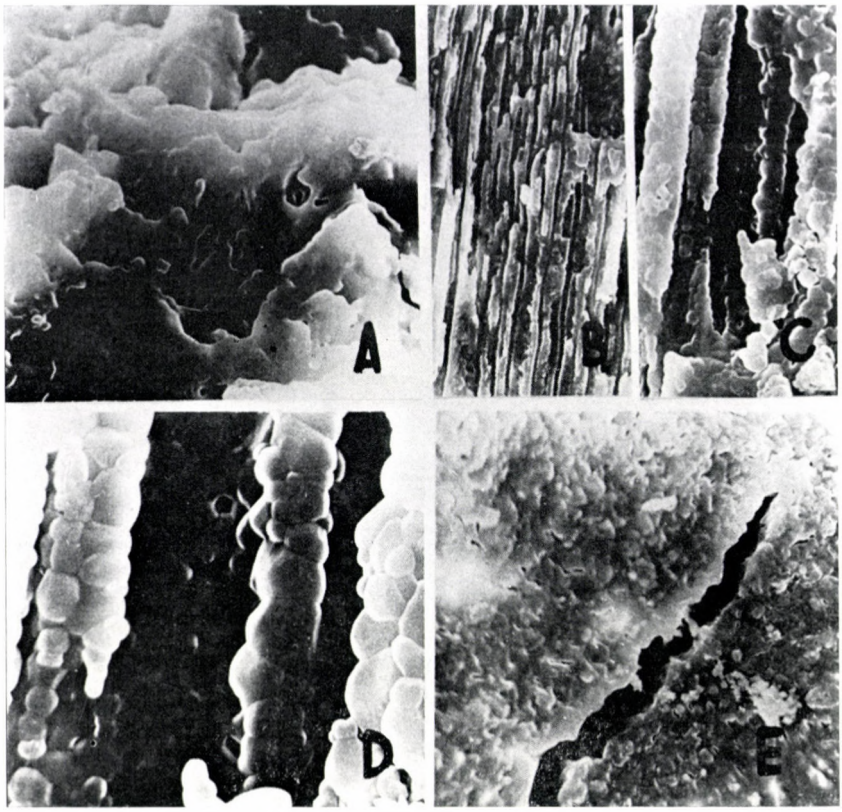

Plate V. 1000 °C A. Enamel ×3300 B. Dentine ×1100 C. Dentine ×3300 D. Dentine ×11,000 E. Cementum ×1100

B. C. D. Dentine. The characteristic tubular structure is still preserved, but under higher power the substance is seen to have melted into minute pearls. The structure consists of small globules 0.1 of 0.2 μ in diameter connected like a string of pearls. Such micro-pearls have not been reported in the literature. They differ from the "bone-pearls" of macroscopic size, formed on the bones of the skull and the extremities under the effect of high voltage current at about 3000 °C to 4000 °C [26].

E. Cementum is seen to cover the dentine as a homogeneous, melted unconnected layer; in some places the open orificies of the dentine tubules can be seen.

1100 °C 1 hr

Gross changes. The tooth is broken down into small fragments porcelain white in colour; on single pieces it can be still recognized, whether they were parts of the crown or of the root. On the surface deep large crevices are visible;

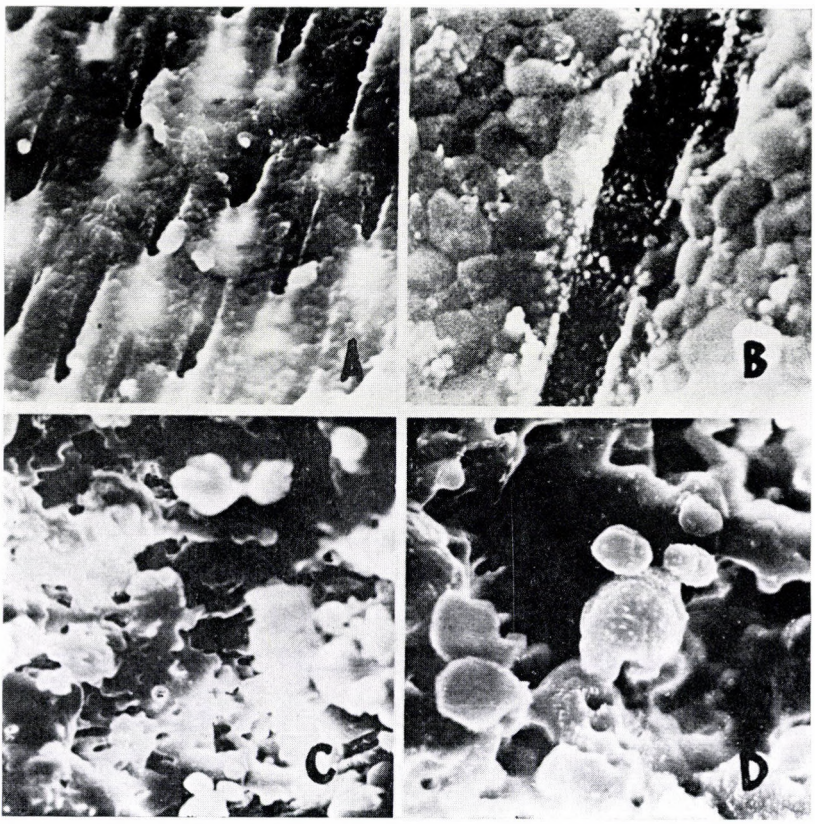

Plate VI. 1100 °C; 1300 °C A. Dentine. 1100 °C ×3300 B. Dentine. 1100 °C ×11,000 C. Dentine. 1300 °C ×3300 D. Dentine. 1300 °C ×11,000

the narrow pulp chamber and root canal can be observed on the corresponding pieces.

SEM (Plate VI)

The original structure of enamel or cementum cannot be recognized; the picture is identical with that seen after heating at 1000 °C.

A. B. Dentine. The tubular structure is still recognized; the anastomoses and the parts of the narrow tubules, cannot be distinguished since the substance has liquefied and formed round plates and granules of uneven size.

1300 °C, 1 hr

Gross changes. The whole tooth is transformed into minute smooth porcelain-white fragments. Some pieces can be recognized as parts of the crown or the root. Their surface is like glass; crevices cannot be seen on it. On larger pieces the remains of the narrowed pulp chamber and the root canal may be suspected in form of small identations or openings.

C. D. The original structure of the tissues is decomposed, the inorganic salts have fused into round formations of various sizes.

Conclusions

1. SEM is a method excellently suited for the study of heat-damaged teeth. Their preparation presents no difficulty and the small, brittle pieces are not damaged during preparation.

2. The hard tissues of the tooth are resistant to heat. Dentine will preserve for the longest time its original architecture which circumstance depends on its peculiar structure determined by the tubules. The hypermineralized peritubular zone has a considerable role in the resistance of dentine.

3. Melting of inorganic crystals starts at 900 ° to 1100 °C and not at that higher melting point of apatite. The decrease of the melting point is one to the presence beside apatite of other elements.

4. Despite melting, dentine preserves its characteristic tubular structure up to about 1100 °C. When the tooth has been damaged by such a high temperature it can be still determined whether the fragment is of dental origin.

5. When the tooth has been exposed to a temperature of 1300 °C or higher, it cannot be estabilished, either macroscopically, or by SEM whether the fragment was part of a tooth.

6. The gross characteristics and the SEM picture allow exact conclusions concerning the temperature inducing the damage.

7. It seems advisable to apply the SEM technique in forensic and anthropological examinations after the detection of burned findings.

REFERENCES

1. Amoedo, O.: (1897) The role of dentists in the identification of the victims of the catastrophe "Bazar de la Charité", 4th of May, 1897. Dent. Cosmos **39,** 905—912. — 2. Arendt, N.: Studien an Zahn und Kieferresten aus Leichenbränder vom säptkaiserlichen Urnenfeld Burg bei Magdeburg. Dissertation, Humboldt Universität, Berlin 1958. — 3. Boehm, E.: (1970) Untersuchungen an experimentellen Stromverletzungen mit Elektronenstrahlen. Z. Rechtsmed. **67,** 293—308. — 4. Boehm, E.: (1974) Zur Ultrastruktur von Blutungen nach wiederholter Traumatisierung. Z. Rechtsmed. **74,** 197—206. — 5. Boehm, E.: (1974) Zur Faser- und Netzstruktur des Fibrins bei intravasculärer Gerinnung. Z. Rechtsmed. **74,** 217—223. — 6. Boehm, E., Tschomakov, M.: (1973) Ein Sekundenphänomen der vitalen Reaktion. Beitr. ger. Med. No. **31,** 221—229. — 7. Boyde, A., Lester, K. S.: (1967) An electron microscope study of fractured dentinal surfaces. Calc. Tiss. Res. **1,** 122—136. — 8. Boyde, A., Lester, K. S.: (1967) Electron microscopy of resorbing surfaces of dental hard tissue. Z. Zellforsch. **83,** 538—548. — 9. Boyde, A., Stewart, A. D. G.: (1962) A study of the etching of dental tissues with argon ion beams. J. Ultrastruc. Res. **7,** 159—172. — 10. Boyde, A., Stewart, A. D. G.: (1963) Scanning electron microscopy of the surface of developing mammalian dental enamel. Nature (Lond.) **198,** 1102—1103. — 11. Breitinger, E.: (1959) Das Brandknochenmenge von Dauborn. Nassau. Ann. **65,** 48—51. — 12. Chochol, J.: (1958) Bisherige Ergebnisse einer anthropoligischen Analyse der Lausitzer Brandgräber in den Böhmischen Ländern. Pamatky Archeol. **49,** 559—582. — 13. Fromme, G. H., Riedel, H.,

VAHL, J.: (1967) Beobachtungen zum Gefügeaufbau der Zahnhartsubstanzen mit Hilfe des Rasterelektronen-Auflichtmikroskopes. Dtsch. zahnärtztl. Z. **22,** 395—400. — 14. FURUHATA, T., YAMAMOTO, K.: Forensic Odontology, Thomas, Springfield 1957. — 15. GEBHARDT, H.: (1923) Verbrennungserscheinungen an Zähnen und Zahnersatz und ihre gerichtsärztliche Bedeutung für die Identifizierung verbrannter Leichen. Dtsch. ges. gerichtl. Med. **2,** 191—209. — 16. GEJVALL, N. G.: Westerhaus. Medival Population and Church in the Light of Skeletal Remains. H. Ohlssons Boktryckeri, Lund 1960. — 17. GRIMM, H., THEIS, G.: (1952/53) Anthropologische Untersuchungen am Leichenbrandinhalt von Urnen der frühen Eisenzeit aus Berlin-Britz. Wiss. Humboldt-Univ. Berlin, Math.-Naturwiss. Reihe **2,** 85—87. — 18. GUSTAFSON, G.: Forensic Odontology. Staples Press, London 1966. — 19. GUSTAFSON, G.: Forensic oral Pathology. In: Gorlin, R. J., Goldman, H. M. (Eds): Oral Pathology. Mosby, St. Louis 1970, pp. 1112—1139. — 20. HAJÓSSI, GY., KOHÁRI, S., BÓNA, K.: (1956) The enamel and dentine of sound teeth under the electron microscope. Acta morph. Acad. Sci. hung. **7,** 107—117. — 21. HANTSCHE, H., SCHWARZ, W.: (1971) Das Rasterelektronen-mikroskop als Hilfsmittel zur Identifizierung von Werkzeugspuren. Arch. Kriminol. **148,** 24. — 22. HARSÁNYI, L., SZUHOVSZKY, GY.: Igazságügyi Fogorvostan. Egyetemi jegyzet. Fővr. Nyomdaipari Váll., Budapest 1971. — 23. HELMCKE, J. G.: Ultrastructure of enamel. In: Miles, A. E. W. (Ed.): Structural and Chemical Organisation of Teeth. Academic Press, New York 1967. Vol **2,** pp. 135—163. — 24. HIRANO, K.: (1935) Studies on effect of heat on the tooth. I. Heating of human teeth. Kokubyo Gakkai Zasshi **9,** 375—395. — 25. HOFMANN, E.: (1882) Die gerichtsärztliche Aufgabe bei der Sicherstellung der Identität von Leichen. Wien, med. Wschr. **32,** 57—62. — 26. IRÁNYI, J., IRÁNYI, J., SOMOGYI, E.: Az Elektromos Baleset és a Villámcsapás Klinikuma és Pathológiája. Akadémiai Kiadó, Budapest 1967. — 27. JOHANSEN, E., PARKS, H. F.: (1962) Electron microscopic observations on sound human dentine. Arch. oral. Biol. **7,** 185—193. — 28. KEISER-NIELSEN, S.: (1951) Odontologisk identification of forbraendte dödsorfe efter en fly-katastrofe. Odont. T. **59,** 57—69. — 29. KENYERES, B.: Törvényszéki Orvostan. MOKT, Budapest, 1909. — 30. KOCH, S., SZTRÓKAY, K. I.: Ásványtan. Tankönyvkiadó, Budapest 1967. — 31. KOHÁRI, S., BÓNA, K.: (1960) Vergleichende elektronenmikroskopische Untersuchungen von Zahnschmelz und Dentin bei Individuen verschiedenen Alters. Dtsch. Zahnärztl. Z. **15,** 1009—1020. — 32. KOMORI, H.: (1960) On the changes of the hard tissues of extracted human teeth under high temperature. Jap. J. leg. Med. **14,** 5. — 33. LEPKOWSKY, V. V., WACHHOLZ, L.: (1903) Über Veränderung natürlicher und künstlicher Gebisse durch extreme Temperatur und Faulnis. Ärztl. Sachverst. Z. **9,** 119—142. — 34. MACHLITT, J.: Folgen Thermischen Einflusses auf Zahn und Zahnreihe Thesis, Leipzig 1973. — 35. MANNERBERG, F.: (1951) Undersökning av kariesfria tänders utseende efter kremerting. Odont. Revy **2,** 67—72. — 36. MILES, A. E.W. (Ed.): Structural and Chemical Organization of Teeth. Academic Press, New York 1967. — 37. MORARU, I.: Medicina Legálá. Ed. Medicalá, Bucuresti 1967. — 38. NEMESKÉRI, J., HARSÁNYI, L.: (1968) A hamvasztott csontvázleletek vizsgálatának kérdései. Anthrop. Közl. **12,** 99—116. — 39. OHNSORGE, J.: (1970) Festkörperoberflechenuntersuchungen mit Hilfe des Rasterelektronenmikroskops. Z. Orthop. **107,** 213—220. — 40. OHNSORGE, J.: (1972) Möglichkeiten und Grenzen der Rasterelektronenmikroskopie. Beitr. gerichtl. Med. **24,** 259—265. — 41. OHNSORGE, J., HOLM, R.: (1970) Die Spongiosafeinstruktur des Hüftkopfes im Rasterelektronenmikroskop. Arch. orthop. Unfall-Chir. **68,** 15—27. — 42. POOLE, D. F. G., JOHNSON, N. W.: (1967) The effects of different demineralizing agents on human enamel surfaces studied by scanning electron microscopy. Arch. oral Biol. **12,** 1621—1634. — 43. PRINZ, H.: (1915) A contribution to the tooth in its relation to forensic medicine. Brit. dent. J. **36,** 683—686. — 44. RENNER, K.: Zähne. In: Neureiter, F., Pietrusky, F., Schütt, E. (Eds): Handwörterbuch d. ger. Medizin und naturwissenschaftlichen Kriminalistik. Springer, Berlin 1940, pp. 940—952. — 45. RÖTZSCHER, K., REIMANN, N.: Die forensische Stomatologie. In: Prokop. O., Göhler, W. (Eds): Forensische Medizin. Volk. u. Gesundheit, Berlin 1975, pp. 545—564. — 46. SCHIRNDING, Hi.: (1934) The teeth and their significance in forensic medicine, with special regard to the identification of corpses. Dent. Cosmos **76,** 853—859. — 47. SCHNEIDER, V., HANTSCHE, H.: (1973) Über Untersuchungen an Strommarken mit Rasterelektronenmikroskop und Mikrosonde. Beitr. ger. Med. **31,** 192. — 48. SCHRANZ, D.: Törvényszéki Stomatológia. Budapest 1944. — 49. SCHRANZ, D.: Igazságügyi Orvostani és Stomatológiai vizsgálatok a személyazonosság meghatározására. D. Sc. Thesis, Budapest 1968. — 50. SCHUMACHER, G. H., SCHMIDT, H.: Anatomie und Biochemie der Zähne. Volk und Gesundheit, Berlin 1972. — 51. SCOTT, D. B.: Dental evidence in identification and criminology. In: Gradwohl, R. B. H. (Ed.): Legal Medicine. Mosby, St. Louis 1954, pp. 451—478. — 52. SCOTT, D. B., WYCKOFF, R. W. C.: (1952) The study of enamel by electron microscopy. J. Amer. dent. Ass. **44,** 377—385. — 53. SOGNNAES, R. F.: (1975) Forensic identifications aided by scanning electron microscopy of silicone-epoxy microreplicas of calcified and cornified structures. 33rd Abb. Proc. Electron

Micr. Soc. Amer. 678—679. — 54. SPEETER, D., OHNSORGE, J.: (1973) Rasterelektronenmikroskopisch erfassbare Schusszeichen am Knochen. Z. Rechtsmed. **73,** 137—143. — 55. TAYLOR, M. E.: (1973) Scanning electron microscopy in forensic science. J. forens. Sci. Soc. **13,** 269—280. — 56. ZILLNER, E.: (1882) Beitrag zur Lehre von der Verbrennung nach Befunden an Leichen beim Ringtheaterbrande Verunglückter. Vjschr. gerichtl. Med. **37,** 65—72.

RASTERELEKTRONENMIKROSKOPISCHE UNTERSUCHUNG DER THERMISCHEN SCHÄDIGUNG VON ZÄHNEN

L. HARSÁNYI

Im elektrischen Äscherofen wurden intakt Zähne erhitzt, und die zwischen 200 °C und 1300 °C allmählich entstehenden Veränderungen untersucht. Die charakteristische Struktur des Zahnkitts löst sich bei etwa 500 °C auf, im Zahnschmelz bei etwa 700—900 °C, während das Dentin seine durch die Zahnbeinkanälchen geprägte, äußerst charakteristische Struktur, selbst nach dem Schmelzen der anorganischen Salze (bei etwa 900—1000 °C), beibehielt. Die mineralischen Stoffe der Zähne schmelzen bei 1300 °C zu uncharakteristischen, rundlichen Gebinden ein. Die rasterelektronenmikroskopische Untersuchung von hitzegeschädigten Gebißresten ist von gerichtsmedizinischen, kriminalistischen und anthropologischen Gesichtspunkten gleicherweise wichtig, denn sogar aus kleinen Fragmenten läßt sich der Ursprung des Fundes ermitteln und feststellen, ob das untersuchte Material ein Zahn ist; ferner kann man aus der Analyse des erhaltenen Bildes auf die die Schädigung herbeiführende Temperatur folgern.

ЭЛЕКТРОННОМИКРОСКОПИЧЕСКОЕ СКЕННИРОВАНИЕ ТЕРМИЧЕСКОГО ПОВРЕЖДЕНИЯ ЗУБОВ

Л. ХАРШАНЬИ

В электрической зольной печи автор нагревал недовредженные зубы и изучал морфологические изменения, постепенно возникающие между 200° и 1300°С. В зубном цементе разложение характерной структуры происходит под влиянием температуры примерно в 500°С, в зубной эмали — после применения примерно 700°—900°С, в то время как дентин сохраняет свою очень характерную структуру, определенную дентинными канальцами, даже после применения 900°—1000°С, т. е. после расплавления неорганических солей. При температуре в 1300°С минеральные вещества зубов расплавляются в нехарактерные, кругловатые образования. Электронномикроскопическое сканнирование остатков зубного аппарата является важным как с судебномедицинской, так и с криминалистической и антропологической точек зрения, так как даже из небольших фрагментов можно выявить происхождение находки, определить, что исследованное вещество представляет собой зуб, а из анализа полученной картины можно сделать заключения о температуре, вызвавшей повреждение.

Dr. László HARSÁNYI: Semmelweis Orvostudományi Egyetem, Igazságügyi Orvostani Intézete, 1450 Budapest, Pf. 9. Hungary

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First Institute of Anatomy, Semmelweis University Medical School, Budapest

A QUANTITATIVE HISTOLOGICAL METHOD FOR THE IDENTIFICATION OF A NUCLEUS IN THE BRAIN. THE PERIFORNICAL NUCLEUS

M. PALKOVITS

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Cytoarchitectonic characterization of the hypothalamic perifornical nucleus in the rat, has been attempted by quantitative histological analysis. Data are given on the volume, cell numbers and cell densities of the nucleus. The applied method proved suitable for the identification and clear separation from their backround of individual brain nuclei and for describing in exact terms the cellular heterogeneity.

Introduction

A group of cells above and partly around the column of the fornix at about the coronal level of the dorsomedial hypothalamic nucleus has been described as nucleus perifornicalis (NPF) [6]. High concentrations of neurotransmitters and their related enzymes [2–4, 10, 14—16] and intense staining with acetylcholinesterase [8] and thiaminepyrophosphatase [1] were found in this nucleus.

Cytoarchitectonics is largely based on subjective judgments such as variations of staining, or neuronal density in different cell groups. Systematic quantitative analysis is however, be required if the nucleus does not clearly stand out from its enviroment, and such data are essential to gain exact numerical information on cell numbers, sizes and densities in any region. For the knowledge of a nucleus it is also important to analyse the homogeneity of its cell population. If the nucleus proves to be heterogeneous, further analysis is needed in order to clarifity, whether the different kinds of cells are mixed at random or whether they form separate subdivisions. The method described in this study has been applied to the perifornical nucleus but it is easily adaptable to any other region of the brain.

Materials and methods

Adult rat (200 ± 10 g) brains were fixed by perfusion with 4% formalin, and embedded in paraffin. Serial sections of 10 μm thickness were stained with luxol fast blue and cresyl violet [7].

General technical requirements of quantitative studies

Quantitative histological analyses should comply with two basic requirements: a) determination of shrinkage caused by the histotechnical procedures; and, b) exact and reproducible orientation of plane of sectioning.

Ad a) Shrinkage is an improtant factor even in linear measurements (10—30%) and increases in quadratic or cubic rate in case of calculating for surfaces or volumes, and thus highly influences the results obtained [12]. To establish the rate of histotechnical shrinkage two parallel incisions of previously determined distance were cut with a fine knife and this distance was re-measured on the mounted sections.

Ad b) The head of the anesthetized rats had to be fixed by a stereotaxic instrument and the frontal plane of the sections was adjusted with the aid of the stereotaxic instrument. The brains were inclined to a 5° nose-down position of the upper incisor bar-binaural plane. A coronal plane of "origin" was cut through the posterior hypothalamus by a transversely oriented knife manipulated by the vertical and transversal movements of the electrode carrier of the stereotaxic instrument.

Topographic determination of the brain nuclei

Ten μm thick serial sections were cut and samples were taken at 70 μm-distances (calculated for the living state). Photomicrographs of the corresponding sections and containing the whole hypothalamus were superimposed by a rectangular grid of lines running parallel with and perpendicular to the midline at distances of 70 μm (Figs 1 and 3). The sides containing the same areas were projected by the aid of a Visopan (Reichert) miscroscope with a square window of the opaque screen corresponding to the 70×70 μm object distance for all magnifications used. Starting with the lowest square bordering the midline, all neurones having visible and focussable nucleoli were counted. The number of cells per square were classified into an arbitrary scale subdividing the span between the two extremes into four categories marked by different symbols. In the resulting diagram the nuclei of higher density were thus sharply outlined (Fig. 1).

Absolute number and density of cells in the brain nuclei

From the cell numbers on the sections and the volume of any nucleus, the absolute number of cells in this nucleus can be determined. This requires a) the determination of the volume; and b) calculating the cell number per section of the nucleus investigated.

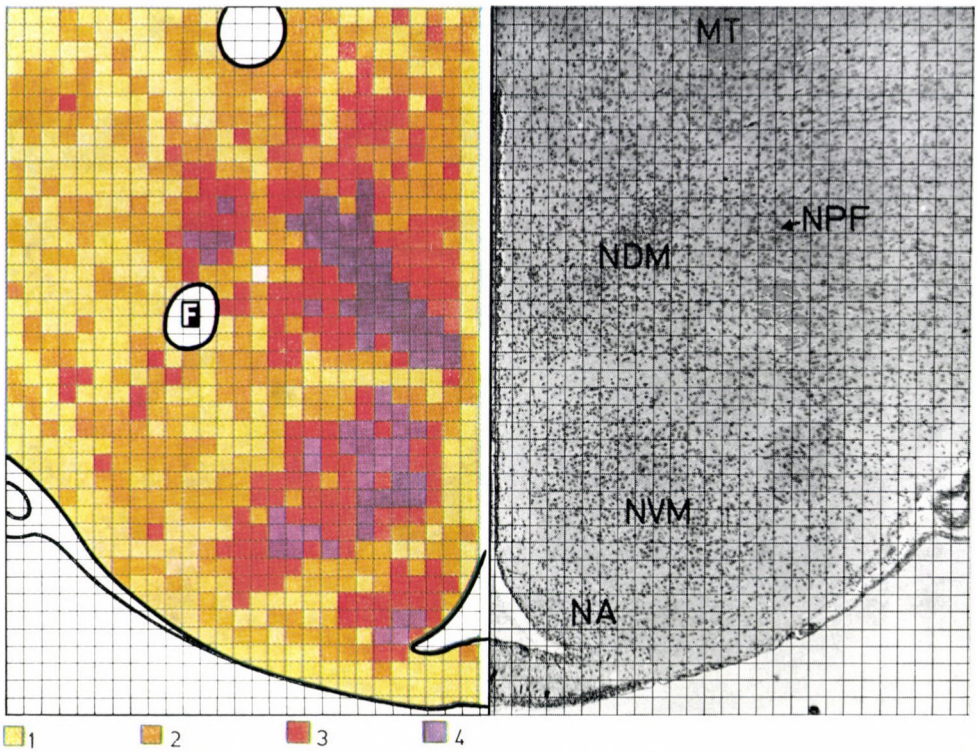

Fig. 1. Neurone density in the hypothalamus at the perifornical nucleus level. 1. 0—4 cells/square (70 × 70 μ); 2. 5—8 cells/square; 3. 9—12 cells/square; 4. more than 12 cells/square

Ad a) The section surface areas multiplied by the distances between sections give the volume of the nucleus. The section surfaces were measured with a planimeter, taking into consideration the quadratic shrinkage.

Ad b) The cell density of the nucleus can be calculated from counting of cells in the section surfaces and measuring the section surface areas. It is important to express cell density in section volume instead of density in section surface. The volume of the sections is calculated by multiplying the section surface area with the section thickness using a correction factor. The FLODERUS [5] correction seems to be most suitable for the purpose [12, 13]. For this knowledge of the average diameter of the nuclei (usually 100 measurements are sufficient for its determination) and the diameter of a nuclear cap that would be still recognized as a nucleus, is indispensable. On the basis of section volume, cell number per sections, and total nucleus volume, the total cell number is determined by employing rule of three.

Dot diagram method for separation of different brain nuclei and their subdivisions

The principle of the dot diagram [11, 13] method is as follows. Each cell nucleus corresponds to one dot in a coordinate; different types of cell show differences in size and shape of their nuclei. Longest and bisecting perpendicular short diameters of cell nuclei were measured using a projection microscope with × 3000 magnification. Choice of the coordinate depends on

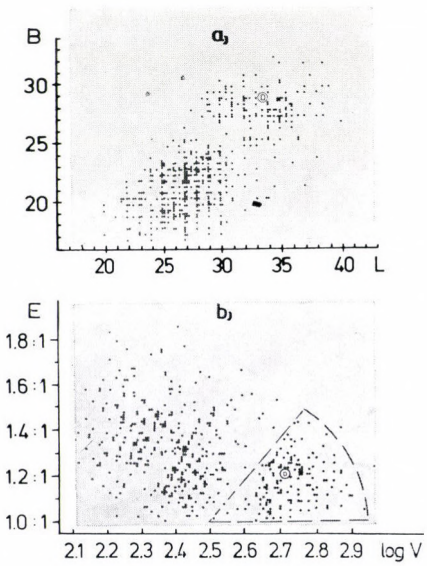

Fig. 2. Dot diagrams of the cell nuclei of the perifornical nucleus (500 measurements each). *A)* Longitudinal (L) and short, peripendicular (B) bisectors of cell nuclei of the two coordinates; *B)* elongation (E) of cell nuclei (quotient of long and short diameter) and the logarithmic classes of the nuclear volume (log. V) on the two coordinates. D indicates neurones having large spherical nuclei which appear as densely packed clouds, well separated from the dots of the other cell nuclei

the object under investigation. The simplest way is to plot diameters on the two coordinate axes (Fig. 2A). Direct indication of the size (nuclear volume = v) and shape (elongation (E) of the nuclei = a quotient of the long (L) and short (B) diameter) of cell nuclei on the coordinates (Fig. 2B) proved to be a better index than the diameters. (Nuclear volume is obtained from a table based on the formula: $v = \pi/6 \cdot LB^2$.) Dots representing 300 – 500 cell nuclei are either distributed homogeneously or in various groups of different packing densities depending on the cell types in the area investigated.

The cell nuclei were again measured by scanning 70 × 70 μm squares and recording in a dot diagram. The small and the large, the round and the elongated cell nuclei became separated by indicating them with four different symbols on the diagram of the hypothalamus (Fig. 3). Thus we can ascertain whether

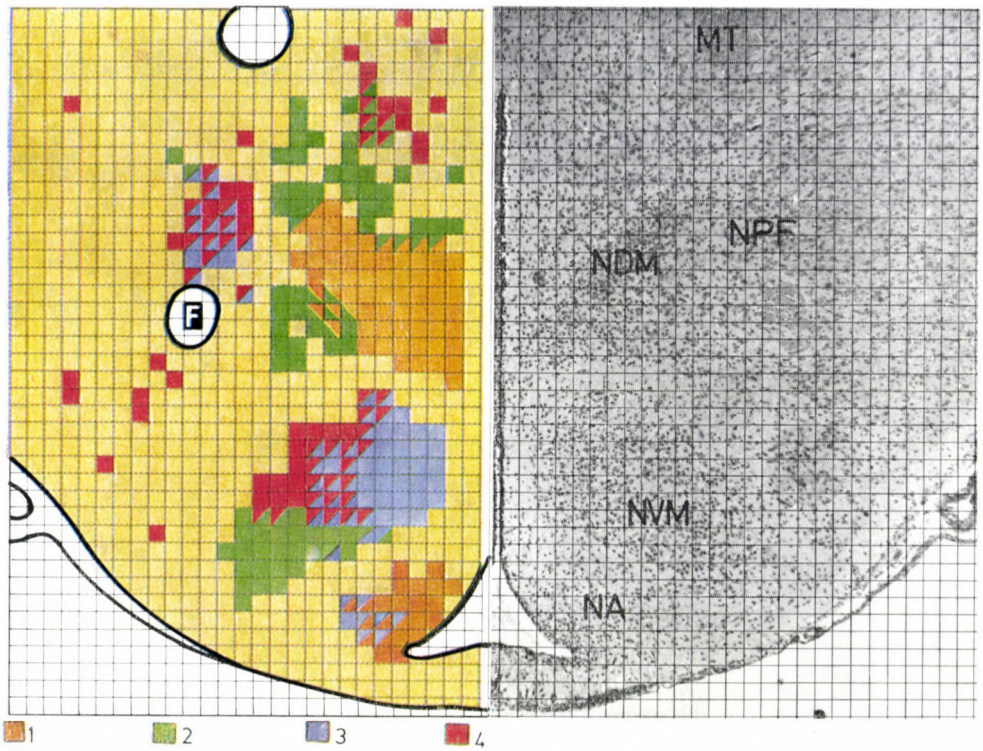

Fig. 3. Different types of cell in the hypothalamus at the perifornical nucleus level. 1. Cells having small, round nuclei; 2. cells having small, elongated nuclei; 3. cells having large, elongated nuclei; 4. cells having large, round nuclei

any given nucleus is homogeneous or is made up of various subdivisions. If the cell population is heterogeneous, 300—500 cell nuclei have to be used for a summarized dot diagram. In this case even the percentual proportion of the different cell types may be calculated.

Data concerning the perifornical nucleus (volume, density, cell numbers) have been calculated from data of 8 animals (mean $\pm$ S. E. M.).

Results

The perifornical nucleus is located in the middle third of the hypothalamus mainly dorsally and slightly medially to the fornix (Fig. 4). The nucleus extends for 1000—1100 μm length in the rostro-caudal direction; usually between the distance of 2400 μm and 3300 μm caudally from the bregma of a 200 g rat. The size of the nucleus (both sides) calculated for the living state was found to be 0.315 ± 0.0148 mm^3. Neuronal density was 21684.9/mm^3, the total number of neurones 6830.5 ± 95.6.

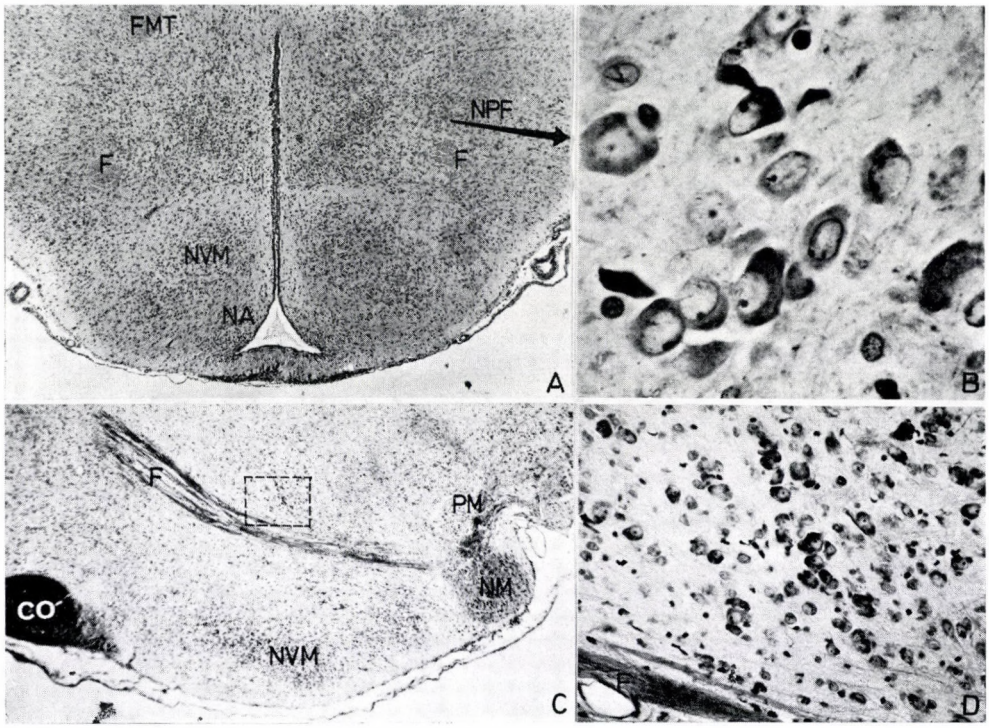

Fig. 4. Topography of the perifornical nucleus (NPF). Abbr.: F — fornix; FMT — fasciculus mammillothalamicus; PM — pedunculus mammillaris; NVM — nucleus ventromedialis; NA — nucleus arcuatus; NM — mammillary body, CO — optic chiasma. *A)* Frontal plane section, haematoxylin-eosin, ×34; *B)* luxol fast blue and cresylviolet staining. ×680; *C)* sagittal plane section, luxol fast blue and cresylviolet. ×20; *D)* NPF from Fig. 4C, under high power (×200)

With the aid of the dot diagrams two types of cell could be separated (Fig. 2). The distribution of small and medium-size cell nuclei on the elongation-volume diagram (Fig. 2B) hints at the possibility of a third type of cell, which would be smaller and more elongated than the two other types.

Partial distribution of the cell having large, spheric nuclei (D-cells on the dot diagram) reached 29.2% of the total number of NPF cells; their average number was 1994.5; and their density, 6332.6/mm^3. The perikarya of these cells are large and stain intensely with cresyl violet (Figs 4A and 4B). Although the perifornical nucleus contains different types of cells, it cannot be separated into subdivisions because the various cell types are arranged diffusely (Fig. 3). Distribution of the cells is not absolutely homogeneous, as the D cells are located mainly in the oral part of the nucleus and their participation in the nucleus declines in the caudal direction (Fig. 5). The numerical distribution of D-cells in a 150 μm thick section in oro-caudal direction was 40.0%, 32.7%, 33.0%, 32.7%, 24.7%, and 12.0%.

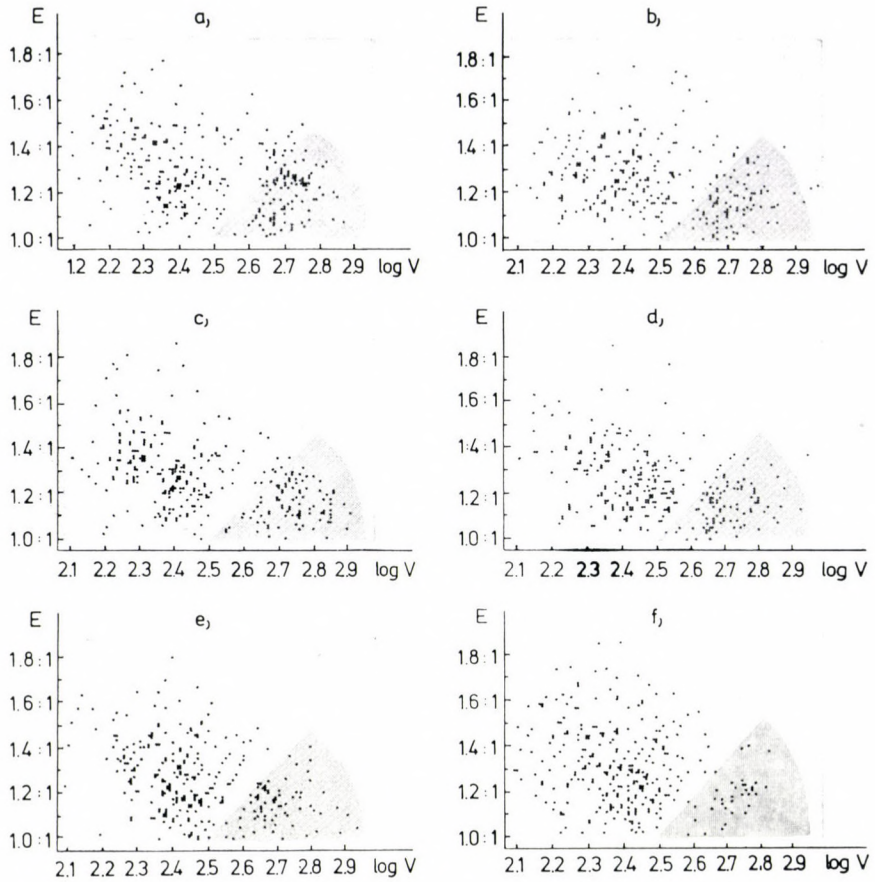

Fig. 5. Dot diagrams (elongation = E nuclear volume in logarithmic classes — Log. V) of NPF from various rostro-cadual portions of the nucleus (300 measurements each. D-cells are shaded. *A)* Dots from NPF cells located rostral to the 2500 μ (bregma level = 0); *B)* samples from the NPF between 2500 and 2650 μ; *C)* 2650—2800 μ; *D)* 2800—2950 μ; *E)* 2950—3100 μ; *F)* caudal to the 3100 μ

Discussion

Nuclei are circumscribed areas of the brain where the density of cells is generally higher than that is in the surrounding areas. Identification of brain nuclei is based traditionally on subjective impression. Numerical determination of the cell density proved to be a suitable method for the delineation of specific regions of the brain on the basis of more exact criteria. The brain nuclei and areas could be separated according to differences in density of their cells.

Nuclei are either homogeneous or heterogeneous. If they are heterogeneous the cell types are arranged diffusely like in the perifornical nucleus,

or can be separated into subdivisions (ventromedial or dorsomedial nuclei, see Fig. 1B). Different types of cells display differences in the size and shape of their nuclei.

Although the nucleus perifornicalis was described in the rat nearly 50 years ago [6], its correct topography and borders as well as its quantitative parameters (size, cell density, cell number, cell types) have not been determined previously. The dot diagrams and mainly the elongation-volume diagram have proved suitable for the demonstration of the heterogeneity of the nucleus. About one third of the neurones, represent a type of perifornical cells, roughly 2000 cells per animal, that differ significantly from the other cell types without, however, making a separate subnucleus.

The physiological importance of the nucleus perifornicalis is unknown. Recent studies (PALKOVITS, deJONG, SZALAY and deWIED, in preparation) point to its role in salt and water balance; it may even correspond to the so-called "drinking center" [9, 17].

Acknowledgement

The author is indebted to Prof. J. SZENTÁGOTHAI for valuable advice and critical comments.

REFERENCES

1. BARA, D., SKALICZKI, J., BŐTI, Z.: (1971) Die Thiaminpyrophosphatase-aktivität der Kernregionen des Hypothalamus. Endokrinologie **58,** 103—110. — 2. BROWNSTEIN, M., KOBAYASHI, R. M., PALKOVITS, M., SAAVEDRA, J. M.: (1975) Choline acetyltransferase levels in diencephalic nuclei of the rat. J. Neurochem., **24,** 35—38. — 3. BROWNSTEIN, M. J., PALKOVITS, M., SAAVEDRA, J. M., KIZER, J. S.: (1975) Tryptophan hydroxylase in the rat brain. Brain Res., **97,** 163—166. — 4. BROWNSTEIN, M. J., SAAVEDRA, J. M., PALKOVITS, M., AXELROD, J.: (1974) Histamine content of hypothalamic nuclei of the rat. Brain Res., **77,** 151—156. — 5. FLODERUS, S., (1974) Untersuchungen über den Bau der menschlichen Hypophyse mit besonderer Berücksichtigung der quantitativen mikromorphologischen Verhältnisse. Acta path. microbiol. scand. **53** (Suppl.), 1—276. — 6. GURDJIAN, E. S.: (1927) The diencephalon of the albino rat. J. comp. Neurol. **43,** 1—114. — 7. KLÜVER, H., BARRERA, E.: (1953) A method for the combined staining of cells and fibers in the nervous system. J. Neuropath. exp. Neurol. **12,** 400—403. — 8. MÉSZÁROS, T., CSURI, J., HÁZAS, J., PALKOVITS, M.: (1969) Esterase activity in the hypothalamus. Acta morph. Acad. Sci. hung. **17,** 210—215. — 9. MONTEMURRO, D. G., STEVENSON, J. A. F.: (1955) The localization of hypothalamic structures in the rat influencing water consumption. Yale J. Biol. Med. **28,** 394—403. — 10. PALKOVITS, M., BROWNSTEIN, M. J., SAAVEDRA, J. M., ALEXROD, J.: (1974) Norepinephrine and dopamine content of hypothalamic nuclei. Brain Res. **77,** 137—149. — 11. PALKOVITS, M., FISCHER, J.: (1968) Karyometric Investigations, Akadémiai Kiadó, Budapest. — 12. PALKOVITS, M., MAGYAR, P., SZENTÁGOTHAI, J.: (1971) Quantitative histological analysis of the cerebellar cortex in the cat. I. Number and arrangement in space of the Purkinje cells. Brain Res. **32,** 1—13. — 13. PALKOVITS, M., MAGYAR, P., SZENTÁGOTHAI, J.: (1971) Quantitative histological analysis of the cerebellar cortex in the cat. III. Structural organization of the molecular layer. Brain Res. **43,** 1—18. — 14. SAAVEDRA, J. M., BROWNSTEIN, M. J. PALKOVITS, M., KIZER, S., AXELROD, J.: (1974) Tyrosine hydroxalase and dopamine-β-hydroxylase: distribution in the individual rat hypothalamic nuclei. J. Neurochem. **23,** 869—871. — 15. SAAVEDRA, J. M., PALKOVITS, M., BROWNSTEIN, M. J., AXELROD, J.: (1974) Localization of phenylethanolamine N-methyl transferase in the rat brain nuclei. Nature (Lond.) **248,** 695—696. — 16. SAAVEDRA, J. M.,

PALKOVITS, M., BROWNSTEIN, M. J., AXELROD, J.: (1974) Serotonin distribution in the nuclei of the rat hypothalamus and preoptic region. Brain Res. **77,** 157—165. — 17. STEVENSON, J. A. F. (1969) Neural control of food and water intake. In: HAYMAKER, W., ANDERSON, E., NAUTA, W. J. H. (Eds): The Hypothalamus. C. C. Thomas, Springfield pp. 524—621.

QUANTITATIVES HISTOLOGISCHES VERFAHREN ZUR IDENTIFIKATION DER ZELLGRUPPEN DES ZENTRALNERVENSYSTEMS. NUCLEUS PERIFORNICALIS

M. PALKOVITS

Die genaue Zytoarchitektur des Nucleus perifornicalis wurde mittels quantitativer histologischer Analyse ermittelt und über das Volumen des Zellkerns, die Zellzahl und die Zelldichte wurden Angaben mitgeteilt. Die Methode läßt sich zur Identifizierung der Kerne des Zentralnervensystems sowie zu ihrer Isolierung von der Umgebung allgemein verwenden, und sie ist auch zur Charakterisierung gemischter Zellpopulationen geeignet.

КОЛИЧЕСТВЕННЫЙ ГИСТОЛОГИЧЕСКИЙ МЕТОД ДЛЯ ИДЕНТИФИКАЦИИ КЛЕТОЧНЫХ ГРУПП ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ. *НУКЛЕУС ПЕРИФОРНИКАЛИС*

М. ПАЛКОВИЧ

Методом количественного гистологического анализа была установлена точная цитоархитектоника **Nucleus perifornicalis.** Сообщаются данные объема клеточного ядра, а также числа и густоты клеток. Метод пригоден для идентификации ядер центральной нервной системы, как и для их точного обособления от окружности, и применим также для характеристики смешанных клеточных популяций.

Dr. Miklós PALKOVITS; Semmelweis Orvostudományi Egyetem,
I. Anatómiai Intézete
1450 Budapest, Pf. 95, Hungary

Acta morphologica Acad. Sci. hung. 23 (4), pp. 293—302, 1975

Institute of Anatomy, University of Medicine Tirgu-Mures, Rumania

SPONTANEOUS REVERSIBILITY OF ADVANCED TOXIC LIVER CIRRHOSIS

T. Maros, L. Seres-Sturm, O. Lakatos, Magda T. Seres-Sturm and V. Blazsek

(Received December 23, 1974)

The spontaneous restoration of liver cirrhosis induced by 6 and 9 month CCl_4 treatment has been studied. The OH-proline content of the liver stroma, the DNA content of the parenchyma, and the Co/DNA ratio were determined. Observations lasted for 4 months after completion of treatment.

Cirrhosis developed after 6 month, CCl_4 administration was reversible in 3—4 months after the discontinuation of tratment; the normal stroma parenchyma ration had gradually normalized.

Nine month treatment exhausted the capacity of the stroma for spontaneous recovery and the parenchyma regenerated to a lesser extent. Fibrosis remained practically irreversible 4 months after CCl_4 administration.

Under experimental conditions the spontaneous reversibility of liver cirrhosis may be estimated by different criteria. Structural and functional recovery of the liver may take some weeks to some months.

Materials and methods

In the experiments rats of either sex, weighing 150 g were used, liver cirrhosis was induced by the subcutaneous administration of 0.1—0.2 ml per 100 g of CCl_4 in sunflower oil. The surviving animals were divided into two groups.

1. The first group comprising 40 animals received 45 doses of CCl_4 during 6 months. Ten of these animals (group M 1) served as controls and were killed on the last day of treatment. The rest was sacrificed 1.5 months (subgroup I/a), 3 months (subgroup II/a) and 4 months (subgroup III/a) later.

2. The second group consisted of 39 animals. They received 75 doses of CCl_4 in the course of 9 months. 15 animals were killed on the last day of treatment and served as controls (group M 2). The remaining animals were killed in groups of 12 three months (subgroup IIb) and 4 months (subgroup III/b) after treatment.

Specimens were fixed in neutral formalin (1 : 9), fixed in cooled 80% alcohol and Carnoy's solution, embedded in paraffin and sectioned with a freezing microtome. Sections were stained with haematoxylin–eosin, Sudan III. van Gieson, and Székely Goldner's trichrome dye. The liver stroma and parenchyma were examined by scanning electron microscopy. Quantitation of collagen was made on the basis of OH-proline content [9, 2]. DNA was estimated spectrophotometrically using diphenylamine. Calculations were made by means of Agrell's formula.

Results

Morphology of the liver

Group M 1 (control). Typical macronodular cirrhosis, the lobular architecture is disintegrated, severe dystrophic lesions, multiple necrotic foci, new bile canaculi with marked diffuse septal and perihepatocellular fibrosis with several pseudolobuli are noted. The three dimensional model demonstrates a blurred network of rough fibrous septa breaking up the parenchyma into irregular fragments (Figs 1/a and 1/b).

Subgroup 1/a. Six weeks after the end of CCl_4 treatment the liver regains its original smooth surface. The lobular architecture is moderately distorted, dystrophic signs and disseminated necroses are detectable only in the centre of lobuli. The number of new bile canaculi is decreased. The pericellular fibrosis has disappeared, the interlobular septa are fragmented. The three dimensional picture reveals a general decrease of fibrosis with an overall thinning and reorganization of connective tissue septa toward the interlobular area.

Subgroup II/a. Regeneration is complete 3 months after the end of CCl_4 treatment. In the portal regions some residual fibrosis is still detectable.

Subgroup III/a. The liver presents a normal microscopic picture the periportal connective tissue does not exceed the usual limit.

Group M 2 (control). Typical macronodular cirrhosis; in 11 of 15 rats it is associated with ascites. Type and gravity of the hepatocellular lesions are more distinct but correspond to those described for group M 1. Septal fibrosis divides the pseudolobuli into further fragments, in the septa poor in cells several vessels can be seen. The three dimensional model demonstrates the many connective tissue labyrinths (Figs 3/a and 3/b).

Subgroup II/b. The picture of macronodular cirrhosis is unchanged 3 months after ceasing the CCl_4 treatment. The lobular architecture is disorganized, there are many regenerated hepatocytes though dystrophic ones also occur. The septal and pericellular fibrosis shows the same characteristic as in group M 2, but the septal fragmentation is absent. The scanning electron microscopic picture of the stroma is similar to that noted in group M 2.

Subgroup III/b. The cirrhosis is unchanged 4 month after stopping the treatment. No ascites is seen. The picture of the parenchyma corresponds to that found after 3 month, but there are less newly formed bile canaculi. Fibrosis surrounding the hepatocytes is less distinct than in subgroup II/b. The intralobular septa are sporadically fragmented and tortuous. The ratio of fibrosis and of pseudolobuli is similar as in the previous phase. The threedimensional model clearly visualizes the sinus-like, occasionally interrupted course of the connective tissue septa.

Table I comprises the results of biochemical measurements.

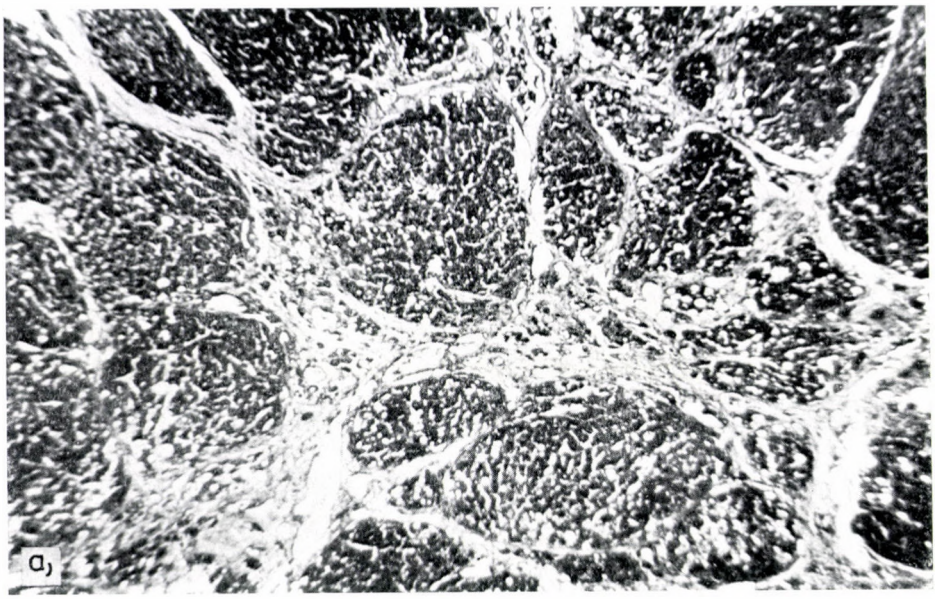

Fig. 1/a. Liver cirrhosis after 6 months CCl_4 treatment Székely-Goldner's trichrome ×87

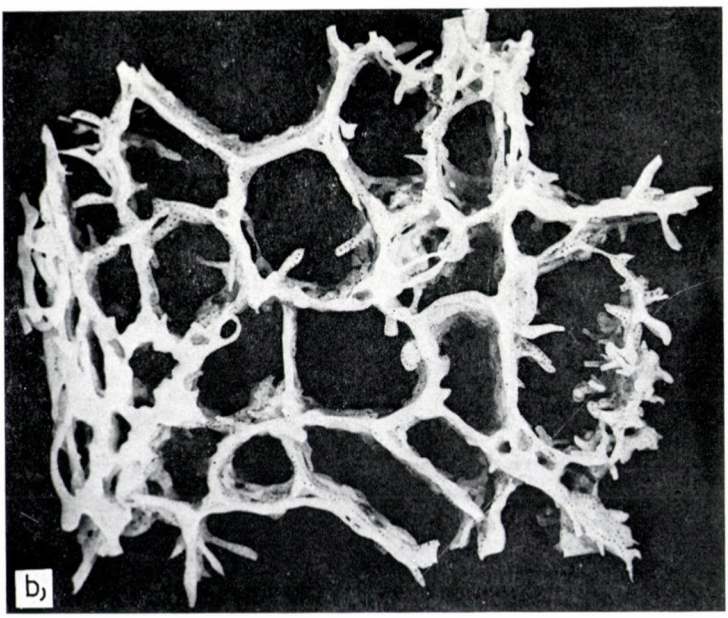

Fig. 1/b. Three-dimensional reconstruction of hyperplastic connective tissue in 6 months old cirrhosis

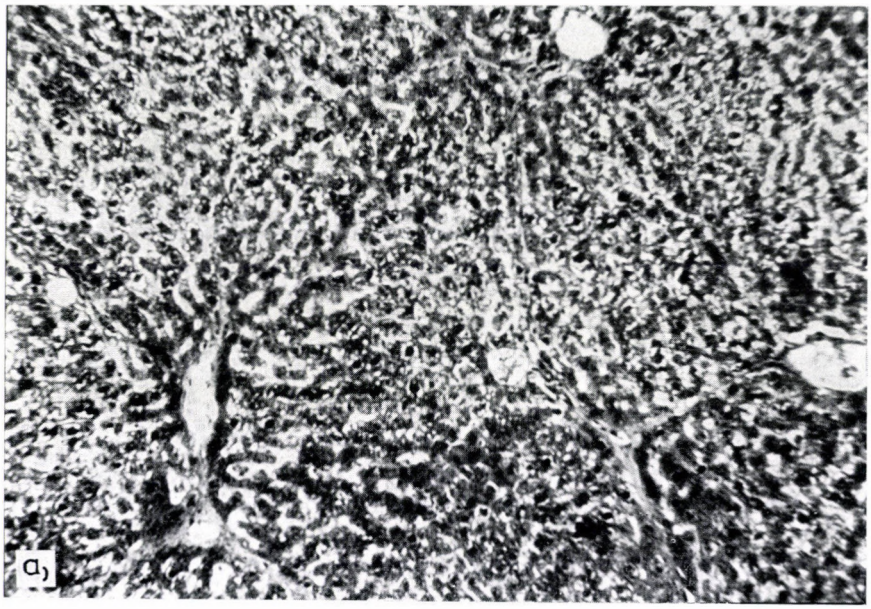

Fig. 2/a. Rat liver after 6 months of CCl_4 treatment at 1.5 months following interruption of the treatment. Székely-Goldner's trichrome. ×87

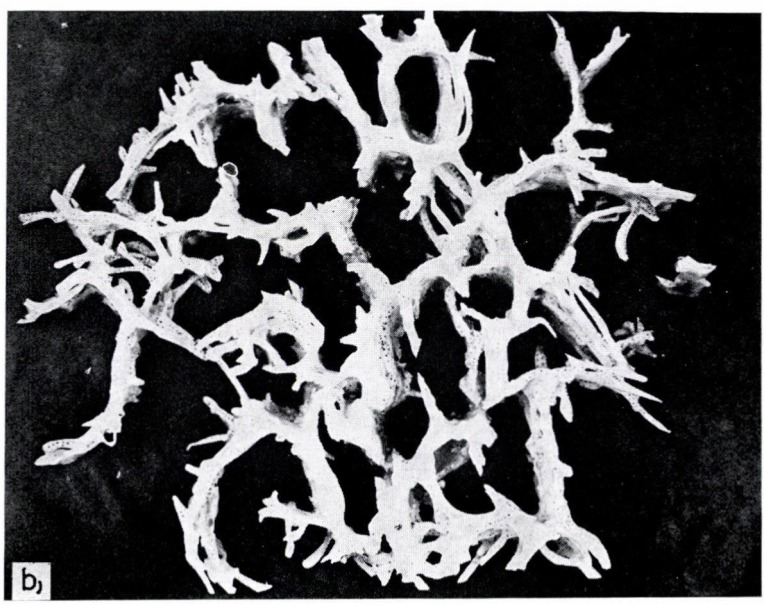

Fig. 2/b. Three-dimensional reconstruction of connective tissue of cirrhotic animal treated with CCl_4 for 6 months; at 1.5 months after the end of treatment

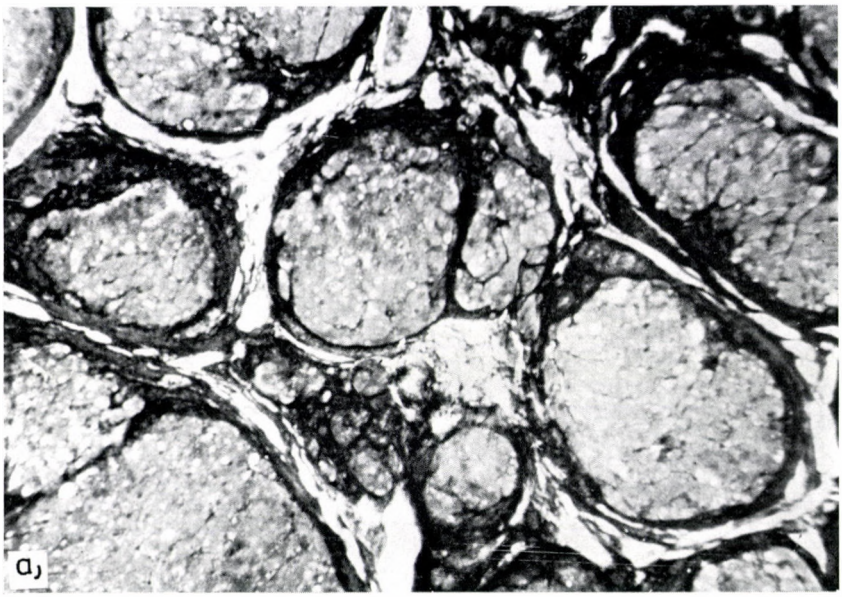

Fig. 3/a. Advanced liver cirrhosis after 9 months CCl_4 treatment. Székely-Goldner's trichrome. ×87

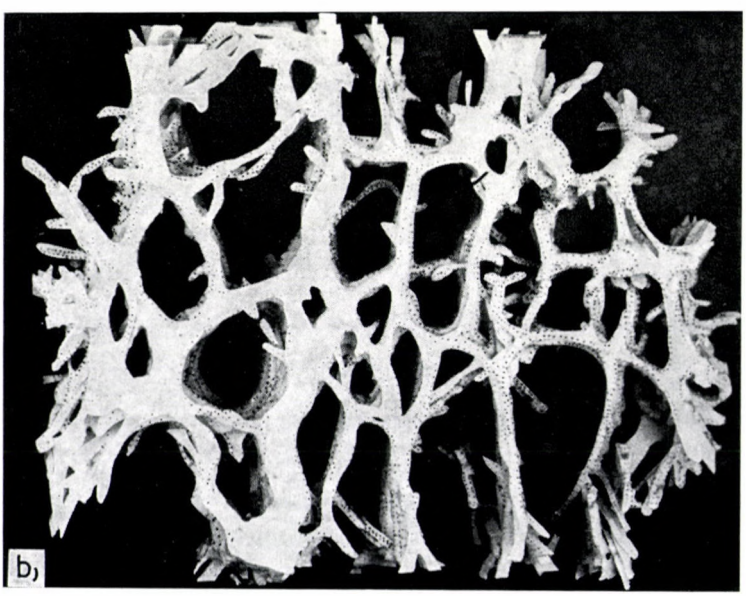

Fig. 3/b. Three-dimensional reconstruction of connective tissue in 9 months liver cirrhosis

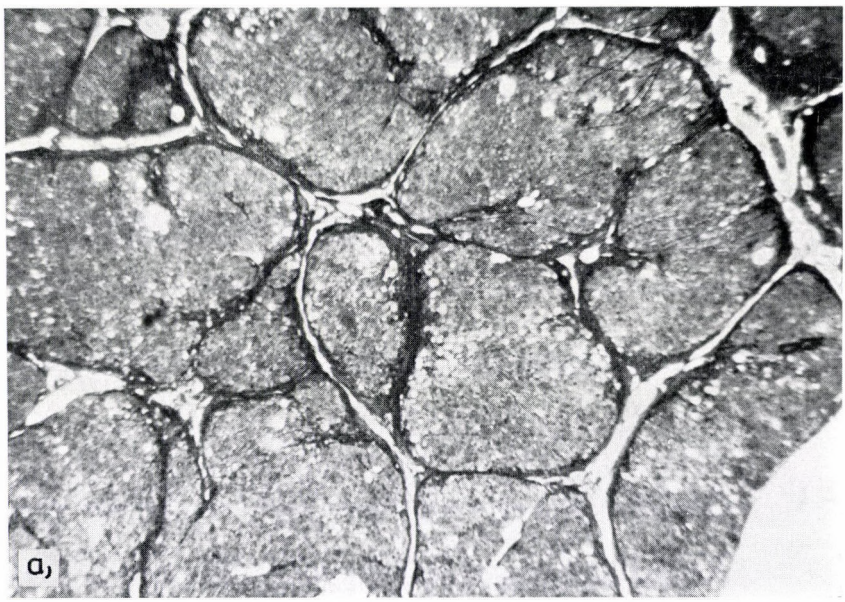

Fig. 4/a. Rat liver treated for 9 months with CCl_4; 4 months after the end of treatment. Székely-Goldner's trichrome staining ×87

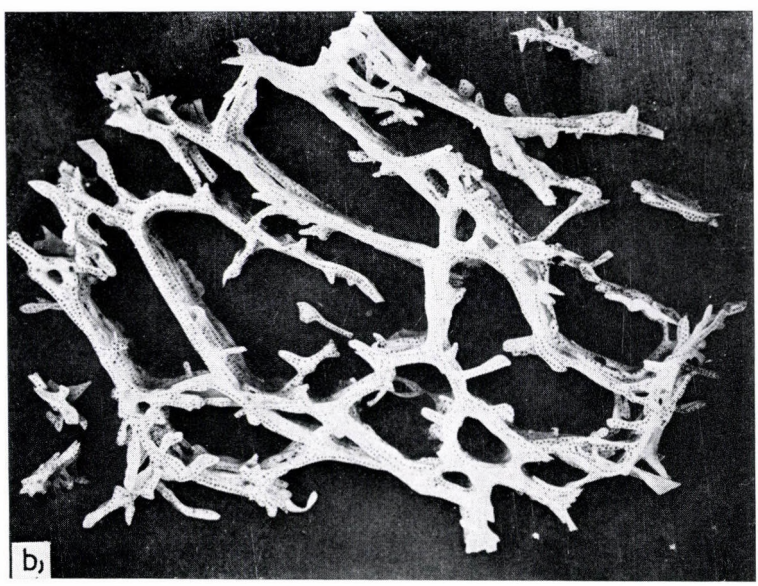

Fig. 4/b. Three-dimensional reconstruction of hepatic connective tissue of cirrhotic animal, treated with CCl_4 for 9 months; 4 months after the end of treatment

Table I

Figures in brackets show minimum and maximum values in the respective groups; P_1 means significance versus healthy controls and P_2 versus groups M_1 and M_2

			Collagen (mg/100 g liver)		DNA (mg/100 g liver)		Co
			$\bar{x} \pm S\bar{x}$	P	$\bar{x} \pm S\bar{x}$	P	DNA
Healthy		12	211.3± 8.5	—	344.5±13.8	—	0.6
Series of 6 months	Group M_1	10	449.0±12	—	377.0± 7.8	—	1.2
	Subgroup I/a	10	359.6±11	P_1 0.001 P_2 0.05	359.0±10.7	P_1 0.90	1.2
	Subgroup II/a	10	262.0±14.2	P_1 0.40 P_2 0.01	Not determined	—	—
	Subgroup III/a	10	198.4± 8.0	P_1 0.90 P_2 0.001	331.0± 7.5	P_1 0.90 P_2 0.40	0.6
Series of 9 months	Group M_2	15	713.6±22.1 (640—876)	—	288.5± 8.4	—	2.4
	Subgroup II/b	12	652.8±52.2 (408—820)	P_2 0.30	345.0±14.1	P_2 0.01	1.8
	Subgroup III/b	12	627.2±31.7 (480—770)	P_2 0.10	321.0± 9.7	P_2 0.01	1.8

Discussion

The time needed for the induction of irreversible cirrhosis by CCl_4 treatment ranges from 6 to 9 weeks according to some authors [13—15, 17] while others put it at 3 to 5 months [4, 6].

These data are not reliable not only for the few experiments made but for the short period (2—3 weeks) that elapsed from the end of the treatment to the time of examination. Olsson [10] and Bengmark and Olsson [3] considerably contributed to the better understanding of the problem; they found that even an 8 month chronic CCl_4 administration does not exceed the threshold of spontaneous reversibility.

In our experiments the treatment lasted for 24 to 36 weeks, with 45 and 75 doses respectively. These values for both the duration of treatment and the number of doses applied are much higher than the 140 days limit of reversibility considered by several authors.

Six months after the onset of CCl_4 treatment (group M 1) the histological picture and the biochemical tests reflected an impaired liver function in agreement with the advanced cirrhosis. Parenchymal lesions and fibrosis then gradually disappeared 1.5 to 4 months after the end of CCl_4 administration and the normal stroma/parenchyma relation was restored spontaneously (Co/DNA + + 0.6).

Group M 2, exposed to 9-month treatment revealed the classical macronodular picture of cirrhosis associated with ascites. A striking increase in the

collagen content, 3.5 fold higher than the normal and the upper limit of group M 1, was characteristic.

RUBIN and POPPER [16] found the collagen content of the liver to increase in parallel with the degree of CCl_4 poisoning to a certain point and to stabilize beyond that. Their observation seem to support that we have achieved the upper limit of collagen accumulation.

The DNA content was much lower as compared to the comparatively stable values for the 6-month group, while the Co/DNA ratio was about 4 fold of that observed in rats and twofold that in group M 1.

The parallelism between the high biochemical values and the amount of proliferated connective tissue has been pointed out by other authors, too [11, 12]. Three to four months after the discontinuation of treatment ascites and the parenchymal lesions disappeared in contrast to the fibrosis that retained its extent and topographic characteristics which had become stable after 9 months.

The decrease of collagen content observed in group M 2 was not significant. Results obtained in the 3rd and 4th month were 3 fold higher than the normal ones and they were comparable to the upper values for group M 1. The wide scattering of the data for collagen with a predominance of maximum values can be attributed to individual reactions. The occasional interruption of interlobular septa and disintegration of the connective tissue surrounding the hepatocytes might be ascribed to a spontaneous catabolism of collagen, particularly 4 months after the end of treatment.

The biochemical findings however failed to support this hypothesis.

In spite of the decrease in the Co/DNA ratio from 2.4 to 1.8 the level still remained high, about threefold of the limit regarded as normal. The phenomenon can mainly be attributed to DNA production (parenchymal regeneration) and not to a decreased collagen content.

CCl_4 treatment for nine months did not change the amount of collagen in the interlobular and intralobular areas of the cirrhotic livers and in spite of a spontaneous regeneration of the hepatocytes the fibrosis retained its characteristic.

Transformation of connective tissue into homogeneous and thick septa dividing the parenchyma into pseudolobules, as well as the sporadic discontinuity of interlobular fibrosis recognizable 4 months after treatment, reflect a tendency to macromolecular reorganization in the physicochemical structure of the accumulated collagen.

Six to 9 months after the beginning of CCl_4 treatment the accumulation of "long lived collagen" of low turnover, refractory to spontaneous degradation was noted. This may have been one to the decreased activity of lysosomal enzymes necessary for collagen disintegration or to an increase of chondroitin B sulphate which is resistant to these enzymes. [5]

REFERENCES

1. AGRELLI, I. P. S.: (1969) Nucleohistone relations in model experiments. Biochem. biophys. Acta (Amst.) **186,** 226. — 2. ASATIANI, V. S.: (1965) Novije metodi biochemicseszkoj fotomotori, Moszkva, Medgiz. pp. 133. — 3. BENGMARK, S., OLSSON, R.: (1966) Biochemical studies on carbon tetrachloride induced liver fibrosis after partial hepatectomy. Acta chir. scand. Suppl. **366,** 44. — 4. CAMERON, G. R., KARUNARATNE, W. A. E.: (1936) Carbon tetrachloride cirrhosis in relation to liver regeneration. J. Path. Bact., **42,** 1. — 5. HUTTERER, F., EISENSTADT, M., RUBIN, E.: (1969) Turnover of hepatic collagen in reversible and irreversible fibrosis. Experientia (Basel) **26,** 244. — 6. ISLAMI, A. H., PACK, G., HUBBARD, D. J.: (1958) Regenerative hyperplasia of the cirrhotic liver following partial hepatectomy. Cancer **11,** 663. — 7. MAROS, T., LÁZÁR, L., LAKTOS, O., BÁLINT, E.: (1971) Appraisal of the degree of reversibility of experimental cirrhotic lesions by the three-dimensional reconstruction. Morfol. norm. Sci. pat. **16** 337. — 8. MAROS, T., SERES-STURM, L., LAKATOS, O., SERES-STURM, M. T. BLAZSEK, V.: (1973) Investigations regarding the possibility of resorption of hepatic collagen in experimental toxic cirrhosis, under the effect of "essential" phospholipids. Arzneim.-Forsch. **23,** 1538. — 9. NEUMAN, R. E.: (1956) Determination of hydroxiproline. In: Aminoacid Handbook (R. J. Block) ed. Thomas, Springfield pp. 48. — 10. OLSSON, R.: (1966) Partial hepatectomy in experimental carbon tetrachloride-induced liver fibrosis. Acta chir. Suppl. 366, 1. — 11. PÉREZ-TAMAYO, R.: (1965) Some aspects of connective tissue of the liver. In: Progress in Liver Diseases (H. Popper, F. Schaffner. (eds) Vol. 2. Chapt. 14., pp. 192. Grune a. Stratton New York. — 12. POPPER, H., HUTTERER, F.: (1970) Hepatic fibrogenesis and disturbance of hepatic circulation. Ann. N. Y. Acad. Sci. **170,** 88. — 13. RABINOVICI, N., WIENER, E.: (1961a) Liver regeneration after partial hepatectomy in carbon tetrachloride-induced cirrhosis in the rat. Gastroenterology **40,** 416. — 14. I d e m : (1961b) Collagen and desoxyribonucleic acid concentrations during regeneration in the hepatectomized cirrhotic liver. Gastroenterology **41,** 251. — 15. I d e m: (1963) Nucleic acid and protein nitrogen in the regenerated liver of the cirrhotic rat. Surg. Gynec. Obstet. **116,** 533. — 16. RUBIN, E., POPPER, H.: (1967) The evolution of human cirrhosis reduced from observations in experimental animals. Medicine **46,** 163. — 17. WAHI, P. N., TANDON, H. D., BHARADWAY, T. P.: (1956) Adrenal cortex and hepatic cirrhosis. I Role of adrenal cortex in evolution of carbon tetrachloride-induced cirrhosis. Arch. Path. **62,** 200.

DAS PROBLEM DER SPONTANEN REVERSIBILITÄT DER FORTGESCHRITTENEN LEBERZIRRHOSE

T. MAROS, L. SERES-STURM, O. LAKATOS, M. T. SERES-STURM und V. BLAZSEK

Die Verfasser untersuchten das makro- und mikroskopische sowie das dreidimensionale Bild der Leber, den OH-Prolin- und den DNA-Gehalt des Leberstromas bzw. des Leberparenchyms, ferner das Co/DNA-Verhältnis im Hinblick auf die spontane Reversibilität der mit Tetrachlorkohlenstoff induzierten Zirrhose 6 und 9 Monate nach Behandlungsbeginn sowie 4 Monate nach Abschluß der Behandlung.

6 Monate nach Behandlungsbeginn entsprach der morphologische und biochemische Status dem bei fortgeschrittener Zirrhose ermittelten Bild. Nach dem Abschluß der Tetrachlorkohlenstoff-Behandlung hatte sich das normale Stroma/Parenchym-Verhältnis stufenweise wiedereingestellt.

Wurde die Behandlung 9 Monate fortgesetzt, so führte dies zur Erschöpfung der potentiellen spontanen Wiederherstellung des Stromas, während die Leberzellen vollkommen, jedoch mit weit geringerer Intensität regenerierten.

Obwohl 4 Monate nach Absetzen der CCl_4-Gaben die Wirkung auf die für die Kollagenmobilisation verantwortlichen Mechanismen nachweisbar ist, bleibt die Fibrose selbst praktisch irreversibel.

Unter experimentellen Bedingungen ist die potentielle spontane Reversibilität der Leberzirrhose aufgrund verschiedener Kriterien feststellbar. Diesbezügliche, generell mit Tetrachlorkohlenstoff durchgeführte Untersuchungen zeigen, daß zur Wiederherstellung des strukturellen und funktionellen Zustands der Leber einige Wochen bis Monate erforderlich sind.

Ausgehend von diesen Erwägungen untersuchten die Verfasser das Verhältnis zwischen Stroma und Parenchym, die Möglichkeiten einer spontanen Ausheilung im fortgeschrittenen Stadium der Leberzirrhose, 6 und 9 Monate nach dem Beginn einer CCl_4-Behandlung.

ИЗУЧЕНИЕ ПРОБЛЕМЫ САМОПРОИЗВОЛЬНОЙ ОБРАТИМОСТИ ДАЛЕКО ЗАШЕДШЕГО ЦИРРОЗА ПЕЧЕНИ

Т. МАРОШ, Л. ШЕРЕШ-ШТУРМ, О. ЛАКАТОШ, М. Т. ШЕРЕШ-ШТУРМ и В. БЛАЖЕК

Авторы изучали макро- и микроскопическую, также как и трехмерную картину печени, содержание OH-пролина и DNA в печеночной строме и паренхиме и соотношение Co/DNA с точки зрения спонтанной обратимости цирроза, вызванного четыреххлористым углеродом, спустя 6 и 9 месяцев после начала и 4 месяца после окончания дачи соединения.

Спустя 6 месяцев после начала дачи CCl_4 морфологическое и биохимическое состояние печени соответствовало картине, выявляемой при далеко зашедшей стадии цирроза. После окончания дачи соединения постепенно восстанавливается нормальное соотношение строма: паренхима.

Продолжение дачи CCl_4 в течение 9 месяцев привело к истощению потенциального спонтанного восстановления стромы, в то время как печеночные клетки полностью восстановились, правда с значительно меньшей интенсивностью.

Несмотря на то, что спустя 4 месяца после окончания дачи CCl_4 можно установить действие, оказанное на механизмы, ответственные за мобилизацию коллагена, сам фиброз практически остается необратимым.

В экспериментальных условиях для оценки возможной самопроизвольной обратимости цирроза печени имеются различные критерии. Исследования такого направления, проводимые — как правило — четиреххлористым углеродом, показывают, что для восстановления структурного и функционального состояния печени необходимо от нескольких недель до нескольких месяцев.

Исходя из этих размышлений авторы изучали соотношение паренхимы и стромы печени, возможность спонтанного излечения в далеко зашедшей стадии цирроза, спустя 6 и 9 месяцев после начала дачи четыреххлористого углерода.

Dr. T. Maros
Dr. L. Seres-Sturm
Dr. O. Lakatos
Dr. Magda T. Seres-Sturm
Dr. V. Blazsek

Department of Anatomy of the University of Medicine and Pharmacology, Tirgu-Mures, str. Gh. Marinescu, Romania

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Institute of Pathology, Maulana Azad Medical College, New Delhi, India

SPLENIC LYMPHOCYTIC RESPONSE TO ACUTE HAEMOLYSIS

B. IYENEGAR

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Adult albino rats, weighing 150—250 g, were administered a sublethal dose of copper sulphate, phenylhydrazine and anti-rat erythrocyta l serum by the intracardiac route to produce equivalent haemolysis of 3 mg se-bilirubin in 24 hr. The animals were sacrificed at intervals of 1, 2, 3, 5 and 7 days and the spleen was studied with particular reference to the lymphatic sheath. Copper sulphate treatment was followed by an initial increase in the number of lymphocytes within the perivascular sheath, indicating a rapid mobilization of thymic lymphocytes. Later these lymphocytes displayed blastic transformation and increased mitotic activity. After phenylhydrazine treatment activity was slight. Antiserum caused a complete loss of lymphocytes which were then replaced and germinal centers were formed.

Erythrophagocytosis did not play a part in the conversion seen after phenylhydrazine treatment. The response to antiserum was slow.

As copper increases the glycolytic activity of the cell on raising the copper level the quiescent small lymphocytes are converted into metabolically active blast cells.

Earlier studies on the effect of 3 different types of acute haemolysis on the lymphoid cells of the spleen revealed a cell proliferation in the marginal zone [5]. The present study was carried out to observe the relationship between the lymphoid reaction and that of RE cells.

Material and methods

Adult albino rats of either sex weighing from 150 to 250 g were used. Copper sulphate (AR Grade) was administered in a sublethal dose of 5000 μg/200 g body weight by the intracardiac route. Phenylhydrazine (20 mg/200 g body weight) and anti rat erythrocyte serum (1 ml of tenfold dilution) were given to produce 3 mg of serum-bilirubin in 24 hr. Batches of 9 rats each were sacrified at intervals of 24, 48, 72 hours 5 days and 7 days in each group. The spleen was removed and fixed in 10% buffered formalin. Paraffin sections 5 μ in thickness were stained with Harris' haematoxylin and eosin and the changes produced in the white pulp were studied. The width of the lymphoid sheath as compared to the number of germinal follicles per 10 low power fields (LPF) were calculated in each group, in ten control rat the normal morphology of the splenic white pulp was studied.

Results

The lymphatic sheath in normal animals is composed mainly of small lymphocytes. No definite area of blast transformation or germinal centres can be seen. The width of the sheath is approximately equal to the diameter of the arteriole (Fig. 1).

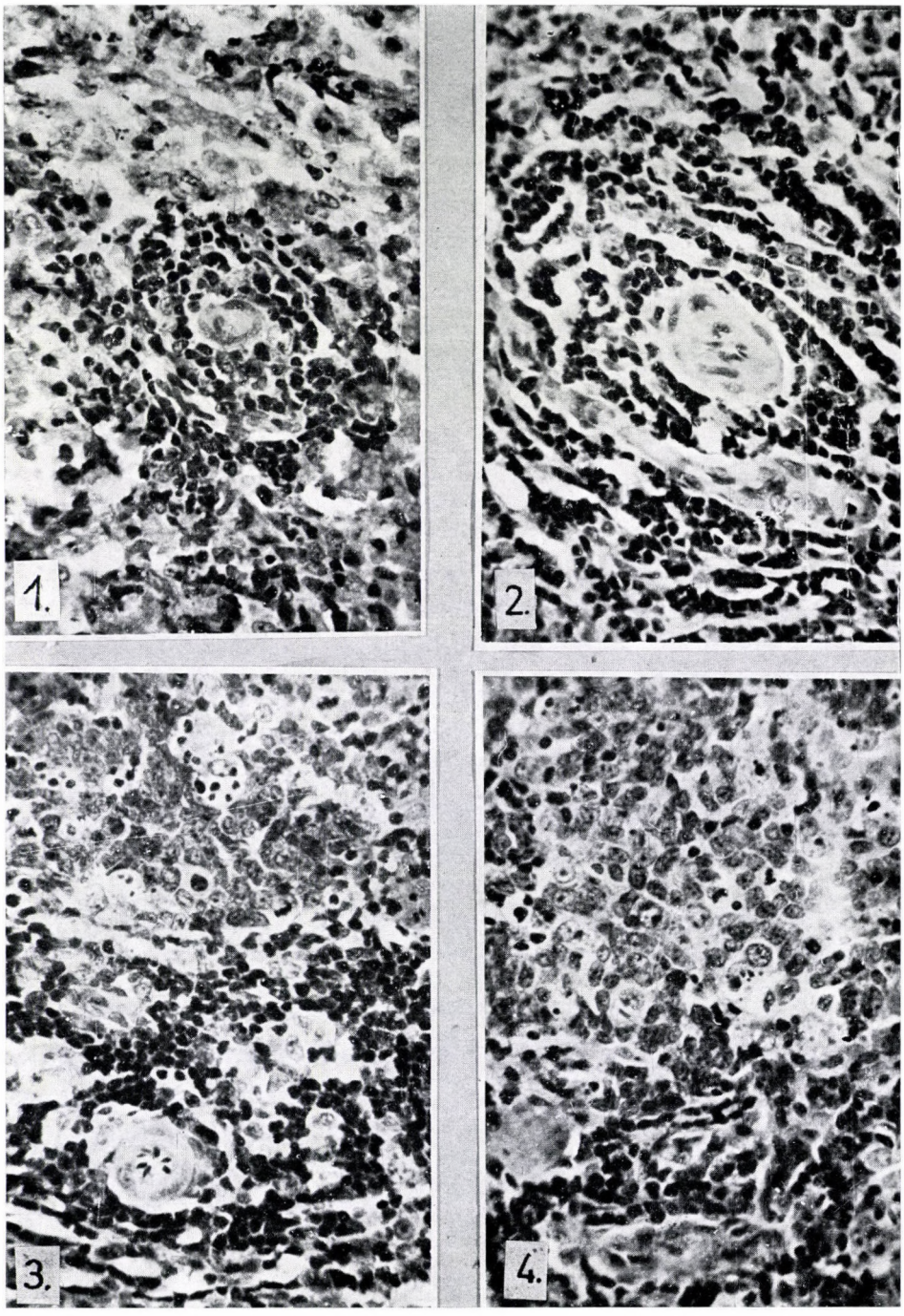

Fig. 1. Normal perivascular lymphatic sheath in the spleen of a control rat, composed of small lymphocytes (HE × 400) *Fig. 2.* Marked increase of the perivascular lymphatic sheath 2 days after copper sulphate. (HE × 400)

Fig. 3. Clones of blast cells appear at the periphery of the lymphatic sheath, protruding into the surrounding red pulp on the 3rd day following copper sulphate. (HE × 100)

Fig. 4. Blast cells showing vesicular nuclei with prominent nucleoli and mitosis. (HE × 400)

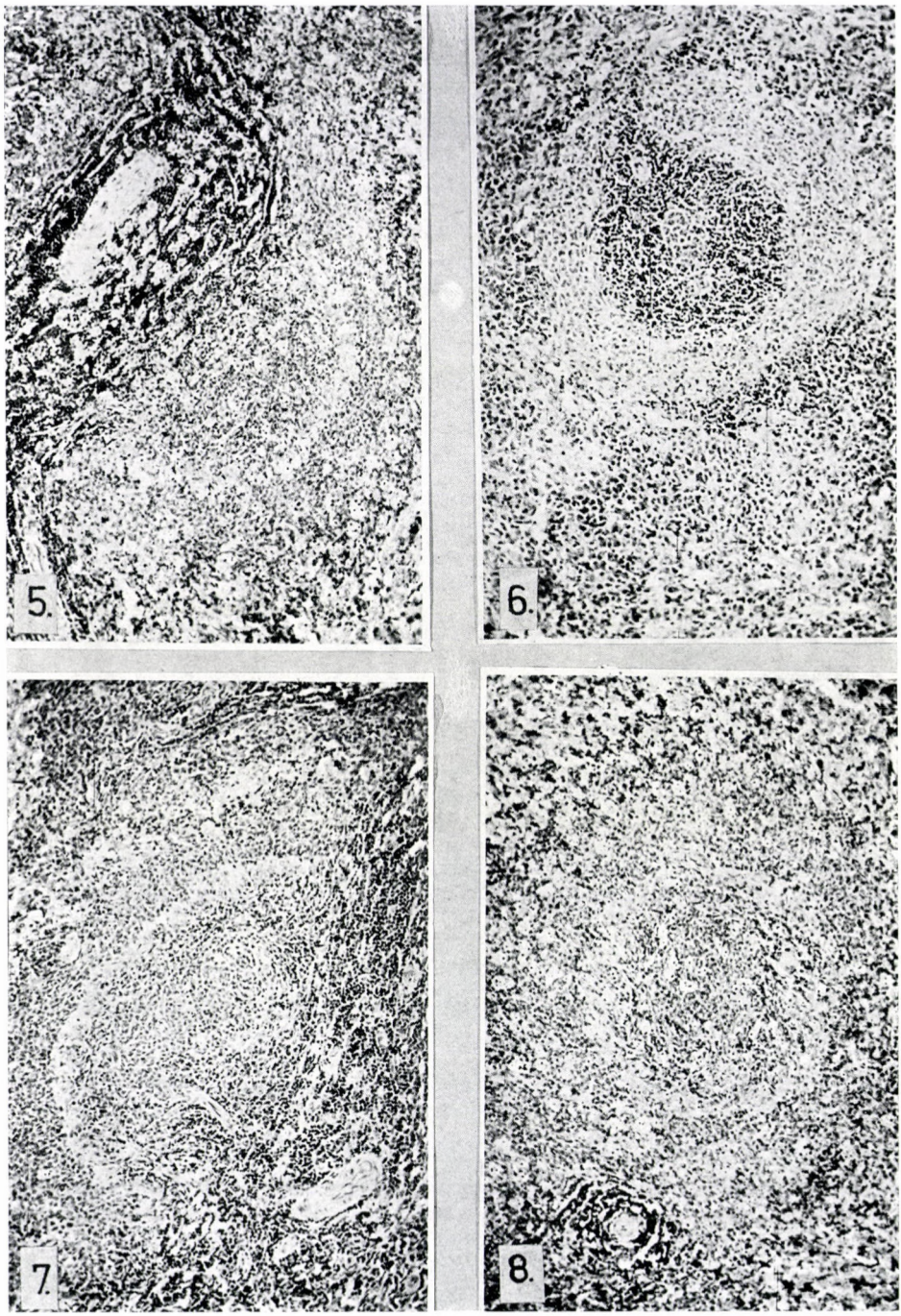

Fig. 5. Graph showing (a) the lymph sheath thickness and (b) germinal follicle formation. (———: copper sulphate, — — —: phenylhydrazine, —.—.—: antiserum) *Fig. 6.* Blast cells showing mitosis (HE × 100) *Fig. 7.* A follicle which has separated from the lymphsheath. The sheath is seen in the upper right corner (HE × 100) *Fig. 8.* Vascular channel arising from the central arteriole to run between the peripheral sheath and the blast cells (HE × 100)

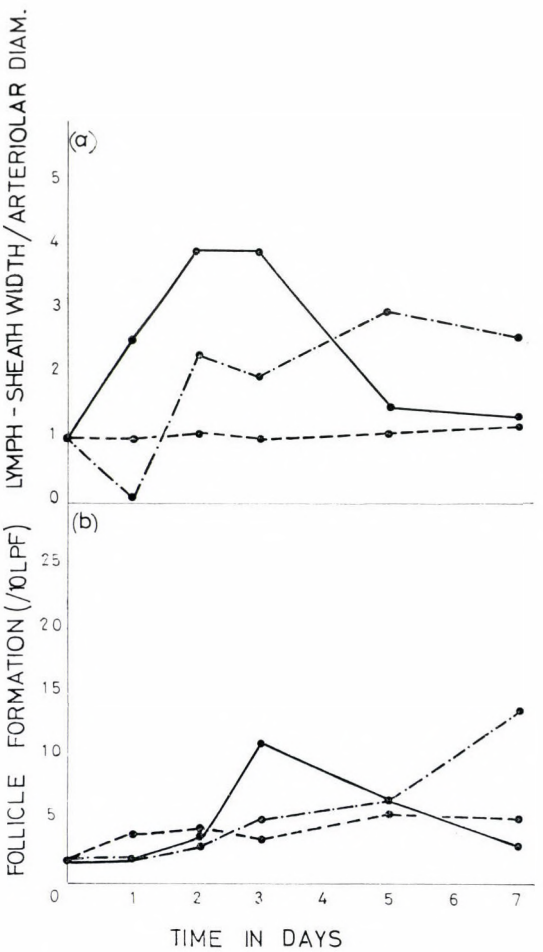

Fig. 9. The germinal centre gradually being replaced by small lymphocytes on the 5th day following copper sulphate. (HE × 100)

Copper Sulphate. In the animals treated with copper sulphate the lymphatic sheath widens to reach almost 4 times the width of the arteriole by the 2nd day (Fig. 2). After the 3rd day the sheath becomes again narrow and is almost completely replaced by RE cells of the marginal zone by the 7th day.

On the 2nd day, at different points at the periphery of the lymphatic sheath, the small lymphocytes grow in size, the nuclei become vesicular with nucleoli and show mitoses (Figs 3, 4). These groups of cells which gradually take on a blastoid appearance start protruding into the surrounding red pulp (Fig. 5). These areas are later covered by a mantle of small lymphocytes. (Fig. 6).

As the blast cell groups are formed, thin vascular channels are seen growing out from the central arteriole to surround the group of blast cells (Fig. 7). These channels lie between the peripherial sheath of small lymphocytes and the blast cells. The cells lining these channels are actively phagocytic and take up the damaged red cells[6].

As the germinal follicles separate out from the perivascular sheath (Fig. 8) the vascular channels become less prominent.

Maximum activity is seen on the 3rd day, after which there is a gradual fall in blastic transformation, the mitoses wane within the blast cell groups and the blast cells are replaced by mature small lymphocytes. There is a corresponding fall in the number of separate germinal follicles within the red pulp.

Phenylhydrazine. After the administration of phenylhydrazine there is a slight increase in the width of the lymphoid sheath. The germinal follicles show a similar curve (Fig. 9).

Anti-rat erythrocyte serum. After the administration of antiserum there is a gradual increase in the lymphatic sheath after an initial destruction which is midway between that of copper sulphate and phenylhydrazine. The germinal centres become active by the 7th day, 4 days later than in the copper sulphate treated animals. Peak activity is similar to that seen after copper sulphate (Fig. 9).

Discussion

On comparing the results after the different haemolytic agents, it is seen that lymphoid hyperplasia and blast transformation are not causally related to the haemolysis. Thus, phenyl hydrazine, which causes a progressive extravascular haemolysis, hardly produces any lymphoid reaction. Antiserum, which is antigenically active, produces an early destruction of the lymph sheath cells followed by a gradual increase of the lymphocytes. This increase is much less marked than that produced by copper sulphate which reaches the peak in 2 days and is followed by a quick fall.

The lymph-sheath cells are related to the antigenic stimulus but the reaction being much more prominent with copper, it appears that copper has a direct effect on these cells.

The conversion of lymphoid cells into blast cells with the formation of germinal centres appears to be correlated with the increase of the lymphoid sheath cells. Phenylhydrazine causes a slight activity while after antiserum the increase in blast cells is gradual, attaining the peak on the 7th day.

Blast transformation and lymph sheath activity are not connected with RE cell activity, particularly erythrophagocytosis. Erythrophagocytosis is highest under the effect of phenylhydrazine but then hardly any activity is seen in the lymphoid elements. Moreover, erythrophagocytic activity caused

by antiserum was much lower than that released by sulphate or phenylhydrazine and yet the lymphoid activity increases gradually to a peak on the 7th day. Thus the effect of antiserum is due to an immunologic stimulation while copper is not antigenic.

In the response the formation of metalloprotein complexes might be implicated [9]. As copper produces a proliferative response in haemopoietic tissue [5] as well as in the RE cells [5], the effect appears to be a generalized phenomenon not confined to immune activity.

Copper is normaly present in all the cells of the body as a constituent of the enzymes of redox systems [3] and plays an important role in the glycolytic activity of cells. When the copper level is raised, as in Wilson's disease, the metal is deposited in many organs, mainly in the neurones of the basal ganglia [12], the hepatocytes and the renal tubular cells [11, 2], but every cell may contain deposits of copper in variable amounts.

It is seen that excess copper causes a rapid raise in glycolytic activity, with an increase in the cellular metabolic rate [13]. The lysosomal membranes are broken down by peroxidation [8] and hydrolytic enzymes are released. The neurones and red cells [8] and renal tubular cells are destroyed due to autooxidation and auto-digestion by the released lysosomal enzymes [13]. The cell phospholipid membranes are also affected [1].

In the present experiments, the initial broadening of the perivascular lymphatic sheath indicates that the number of small lymphocytes has increased. As there is no mitosis or blast transformation at this stage, these cells appear to be transported into the spleen by the circulation. As the perivascular sheath becomes populated by thymic lymphocytes [4], copper seems to stimulate the release of thymocytes.

These lymphocytes are gradually transformed into blast cells which multiply to give rise to clones of cells which then form germinal follicles. Thus the lymphocytes are not destroyed like the neurones and red cells but show an increased activity and proliferate.

Normally the small lymphocyte is an inert cell with a low metabolic rate. With a rise in the copper level, the increased glycolytic activity and metabolic rate converts it into a metabolically active blast cell. BOWLER and DUNCAN [1] have shown that several aspects of cellular activity, particularly the activity of the membrane enzymes, are altered by copper. Peroxidation of the lysosomes with selective release of hydrolytic enzymes may be involved in this process. Activation of lysosomal enzymes has also been implicated in blast transformation produced by antigenic stimulation and by PHA [12]. These changes, which are lethal to the non-mitotic and metabolically active neurones, enchance the activity of lymphocytes. In the red cells there is a quicker utilization of the metabolites with early death of the cells.

Gradually, the stimulatory effect on the cell wanes and the blasts are re-

placed by lymphocytes, so that there is a fall in the number of follicles and blast cells.

Thus, copper gives rise to a blastic transformation of splenic lymphocytes. This is in contrast to the cell death produced in red cells and neurones. The rise in metabolic activity produced by copper destroys the non-mitotic cells by auto-oxidation, whereas the excessive energy released is utilized by the lymphocytes for blastic transformation and mitosis.

REFERENCES

1. Bowler, K., Duncan, D. J.: (1970) The effect of copper on membrane enzymes. Biochem. biophys. Acta Amst. **196,** 116—119. — 2. Goldfischer, S., Sternlieb, I.: (1968) Changes in the distribution of hepatic copper in relation to the progression of Wilson's disease (hepatolenticular degeneration). Amer. J. Path. **53,** 883—901. — (1963) 3. Harper, H. A.: Review of Physiological Chemistry. (9th ed.) K. Lange Medical Publications, San Francisco (1963) — 4. Humphrey, J. H., White, R. G.: Immunology for Students of Medicine. (2nd ed.) Blackwell Scientific Publications, Oxford 1964. — 5. Iyenegar, B.: Influence of haemolysis in the marginal zone reticuloendothelial cells of the spleenc (In press). — 6. Iyenegar, B., Chandra, K.: (1973) Sites of iron deposition in the spleen after haemolysis. Acta morph. Acad. Sci. hung. **21,** 343—350. — 7. Iyenegar, B., Chandra, K.: (1974) Splenic feedback in red cell regeneration. Acta haemat. **51,** 290—296. — 8. Iyenegar, B., Gupta, D. N.: (1973) Haemolysis produced by copper sulphate. I. Extravascular haemolysis produced by heavy metal poisoning. Ind. J. med. Res. **61,** 195—198. — 9. Jandl, J. H., Simmon, R. L.: (1957) Agglutination and sensitisation of red cells by metallic cations. Brit. J. Heamat. **3,** 19. — 10. Lindquist, R. R.: (1967) Acid phosphatase activity in copper loaded rat livers: Studies on the pathogenesis of hepatolenticular degeneration. Amer. J. Path. **51,** 471—481. — 11. Vogel, F. S.: (1959) The deposition of exogenous copper under experimental conditions with observations on its neurotoxic properties in relation to Wilson's disease. J. exp. Med. **110,** 810.— 12. Vogel, F. S., Evans, J. W.: (1961) Morphological alterations produced by copper in neural tissues with consideration of the role of the metal in the pathogenesis of Wilson's disease. J. exp. Med. **113,** 997. — 13. Vogel, F. S., Kemper, L.: Biochemical reactions of copper within neural mitochondria, with consideration of the role of the metal in the pathogenesis of Wilson's disease.

DIE REAKTION DER MILZ-LYMPHOZYTEN AUF DIE AKUTE HÄMOLYSE

B. IYENEGAR

An vollentwickelten Albinoratten von 150—250 g Gewicht wurden subletale Dosen von Kupfersulfat, Phenylhydrazin und Rattenerythrozyten-Antikörper-1 enthaltendes Serum in einer Menge intrakardial eingeführt, die innerhalb von 24 Stunden eine der Wirkung von 3 mg Serumbilirubin äquivalente Hämolyse herbeiführte. Die Tiere wurden in 1-, 2-, 3-, 5- und 7tägigen Intervallen getötet und ihre Milz, besonders im Hinblick auf die lymphatische Kapsel, untersucht.

Unter der Wirkung von Kupfersulfat wurde zunächst in der perivaskulären Kapsel ein Anstieg der Lymphozytenzahl registriert, was auf die rasche Mobilisierung der Thymuslymphozyten hinweist. Im weiteren Verlauf zeigten diese Lymphozyten eine blastische Transformation und eine gesteigerte mitotische Aktivität. Nach Phenylhydrazin-Verabreichung war diese Aktivität gering. Unter der Wirkung von Immunserum verschwanden die Lymphozyten völlig. Ihre Neubildung ging mit dem Auftreten von Germinationszentren einher.

In den mit Phenylhydrazin herbeigeführten Veränderungen spielte die Erythrophagozytose keine Rolle. Die durch Antiserum ausgelöste Reaktion entwickelte sich langsam.

РЕАКЦИЯ ЛИМФОЦИТОВ СЕЛЕЗЕНКИ НА ОСТРЫЙ ГЕМОЛИЗ

Б. ИЕНЕГАР

Взрослым белым крысам, весом в 150–250 г, вводили сублетальные дозы сернокислой меди, фенильгидразина и сыворотку с содержанием противотел против эритроцитов крысы, в количестве, достаточном для вызывания в пределах сутки гемолиза, эквивалентного гемолизу от 3 мг сывороточного билирубина. Животных убили с 1, 2, 3, 5, и 7 дневными интервалами и изучали их селезенку с особым вниманием на лимфатическую капсулу.

После введения сернокислой меди в околососудистой капсуле вначале повысилось число лимфоцитов, что указывает на быструю мобилизацию лимфоцитов зобной железы. В дальнейшем эти лимфоциты показали бластозную трансформацию и повышенную лимфатическую активность. В случае введения фенильгидразина активность оказалась незначительной. Под влиянием иммунной сыворотки лимфоциты совершенно исчезли. Из новообразования сопровождалось появлением зародышевых центров.

В изменениях, вызванных введением фенильгидразина, эритрофагоцитоз не играет роли. Реакция на введение иммунной сыворотки развилась только медленно.

Bhanu IYENEGAR: B-72, Pandra Road New Delhi, 110003, India

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Department of Infectious Diseases, Institute of Internal Medicine, and Department of Histology and Embryology, Institute of Biostructure, Medical School, Bialystok, Poland

HISTOPATHOLOGICAL CHANGES OF SOME ORGANS IN THE COURSE OF EXPERIMENTAL SALMONELLA AGONA INFECTION OF RABBITS

DANUTA, PROKOPOWICZ and L. REJNIAK

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Organ changes evoked by infection with *Salmonella agona* were evaluated in 30 rabbits. Pathological changes were found not only in the intestines but also in the liver, lungs, spleen, kidney and suprarenal glands. The changes were different from those induced by other serotypes of Salmonella.

In the last decade, diseases evoked by *Salmonella* (S.) *agona* serotypes have appeared in many countries of the world. [6] *S. agona* has an antigenic structure of 4, 12: f, g, s and is included in group B of the Kauffmann White schema.

The sources of infection are animals [8] fed on infected fish meal [6].

The aim of the present investigation was to study the effect of *S. agona* infection on the organs of rabbits.

Material and methods

The experiments were carried out on 30 male rabbits weighing 2—4 kg. Twenty-seven were infected, and 3 shame-infected served as controls. The material for infection was obtained from patients with salmonellosis due to agona.

The rabbits were orally infected with 0.25×10^{10} per kg body weight of cultured *S. agona*. Opium tincture in a dose of 3 ml/kg was injected interperitoneally to facilitate infection by inhibiting peristalsis [9]. Before and after the infection 30 ml/kg of milk was introduced into the stomach through a tube to neutralize the gastric juice [9].

Weight and condition of the animals were evaluted every day.

The animals were killed by injection of air into the ear vein. Materials from the following organs were examined: liver, spleen, kidney, suprarenal gland, lungs and intestine with lymph sac and vermiform appendix. Routine histological methods were used to prepare tissues for microscopic examinations.

The disease was confirmed by the identification and isolation of the organisms from blood, faeces, liver, spleen, kidney, suprarenal gland and lungs. Isolation and identification of bacteria was performed in Wojewodship Epidemiologic Station.

Results

Among 27 infected rabbits a decrease of body weight was observed in 18 animals. Diarrhaea was noted in a single case only. Between the 2nd and 7th days of the disease, 22% of the animals died. In these animals gross exami-

nation revealed distension and congestion of intestines, especially of the lymph sac and vermiform appendix.

Diagnosis of the disease was confirmed by isolation of *S. agona* from small and large intestines and lymph sacs.

In 30% of the animals bacteria were found in the liver, kidney, suprarenal gland, spleen and lungs. Blood cultures were negative.

A. Animals died between the 2nd and 7th days after infection

In the rabbits which died in the first week of the disease, there was a hypertrophy of the lymphatics of the intestine and lymph sac (Fig. 1), with inflammatory infiltrations of the intestinal and lymph sac epithelium. (Fig. 2). Necrotic changes were noted in the intestinal mucosa and submucosa.

Fig. 3 shows vacuolated degeneration and single necrotic foci in the liver; numerous infiltrations consisting of mononuclear cells and single macrophages were visible in the lobules (Fig. 4).

In the spleen phagocyting cells filled with pigment, and enlarged lymph nodes were observed.

In the lungs, inflammatory changes (Fig. 5), in the alveoles and bronchi exudate and inflammatory cells were observed (Figs 5, 6).

The suprarenal glands displayed congestion of the medulla the cells had a spumy cytoplasm in the zona fasciculata.

B. Animals killed after 4 weeks of infection

After four weeks, chronic inflammatory changes in the intestines and lymph sac (Fig. 7) and the spleen were noted.

Fig. 8 shows degenerated hepatocytes and numerous inflammatory infiltrations in the liver lobules.

In the kidney parenchymatous degeneration of the convoluted tubules (Fig. 9), eosinphilic fluid under the Bowman's capsule, erythrocytes outside the blood vessels and necrosed convoluted tubules were seen (Fig. 10).

Fig. 11 shows inflammatory changes in the lung tissue and bronchi. The pulmonar vessels exhibited distinct endothelial lesions (Fig. 12).

In the control group no changes in the tested organs were observed.

Discussion

Since *S. agona* has been known to clinicians only for the last five years, no information is available concerning experimental disease evoked by these bacteria. According to some authors, changes of the lymphatic system are typical of salmonellosis [4], while other authors found changes in many other organs [10].

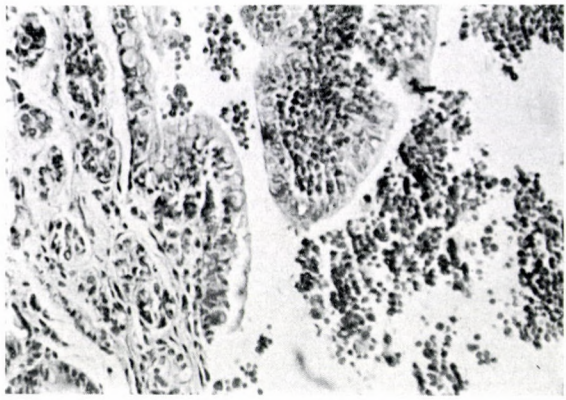

Fig. 1. Hypertrophy pf lymphatic system in lymph sac. H and E ×240

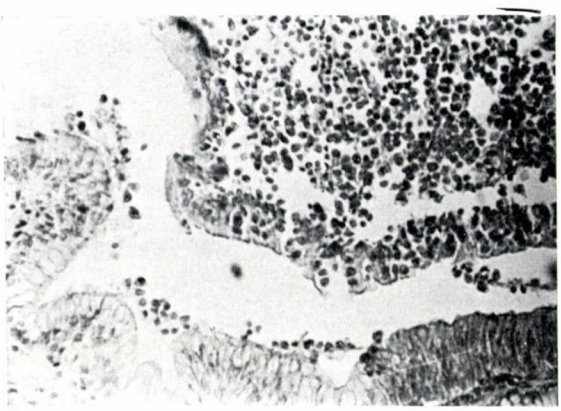

Fig. 2. Inflammation infiltration in intestinal epithelium H and E ×240

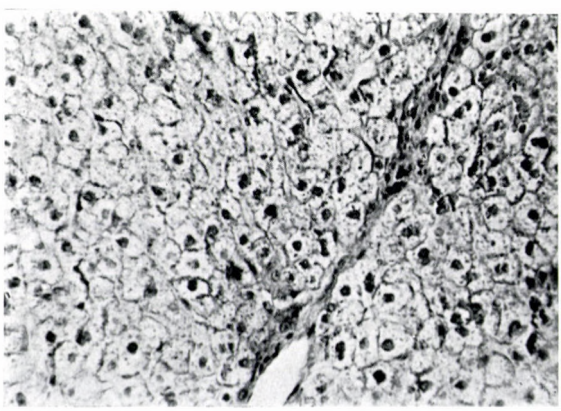

Fig. 3. Vacuolated degeneration and microfocal necrosis in liver. H and E ×240

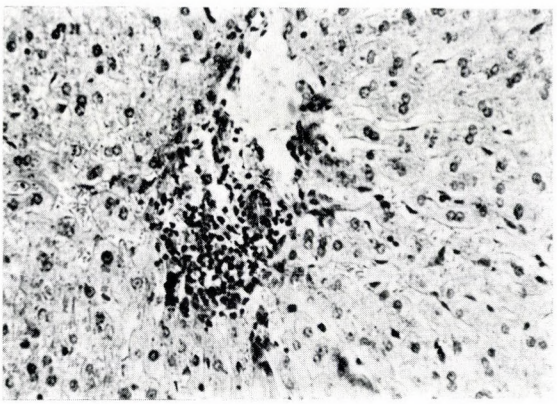

Fig. 4. Inflammation infiltration by mononuclears and single macrophages in liver. H and E ×240

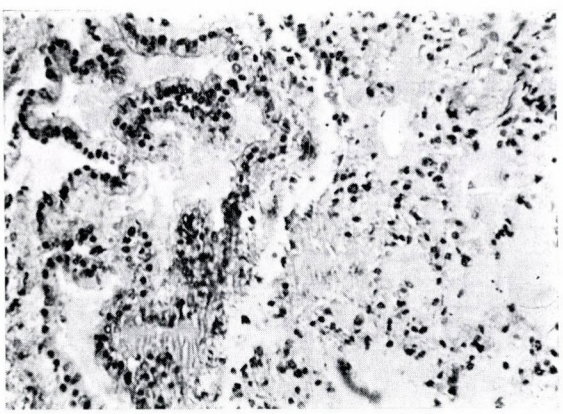

Fig. 5. Inflammation changes in lung tissue, leukocytes and desquamated epithelial cells in bronchi. H and E ×240

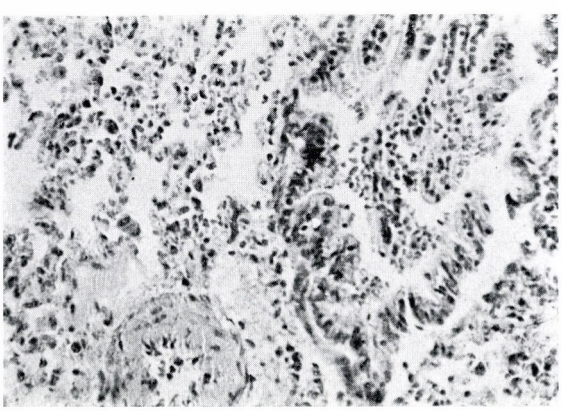

Fig. 6. Inflammation changes in lung tissue with alveolar exudate. H and E ×240

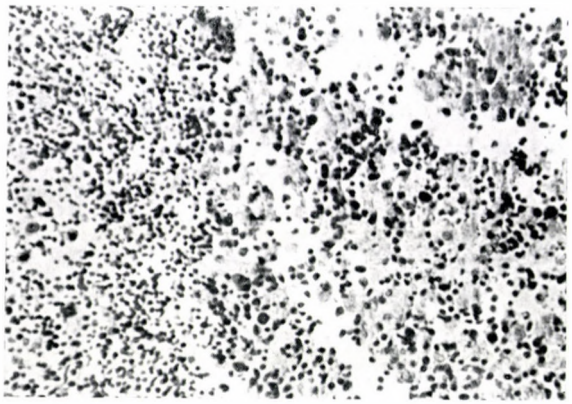

Fig. 7. Infiltration by mononuclears plasmocytes and macrophages in the wall of lymph sac. H and E ×240

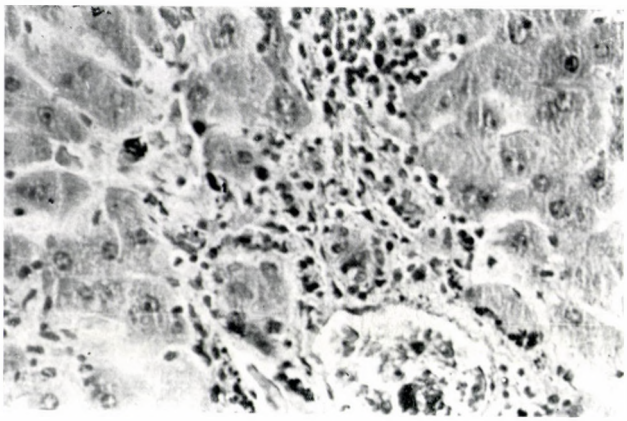

Fig. 8. Vacuolated degeneration and inflammatory infiltration in liver. H and E ×240

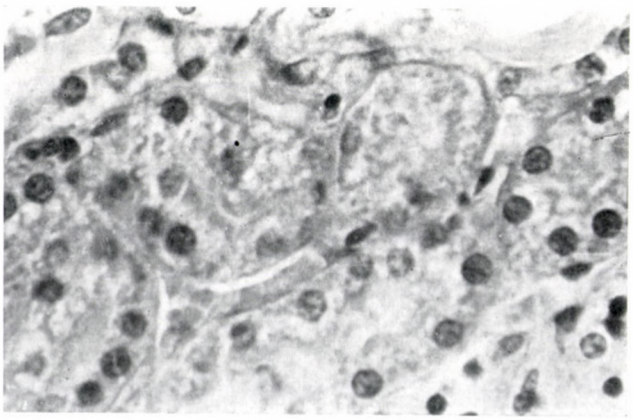

Fig. 9. Parenchymatous degeneration in proximal convoluted tubule. H and E ×400

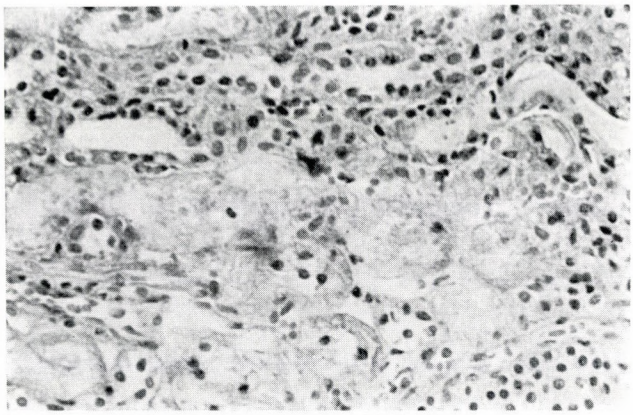

Fig. 10. Necrotic changes in proximal convoluted tubule of kidney. H and E ×240

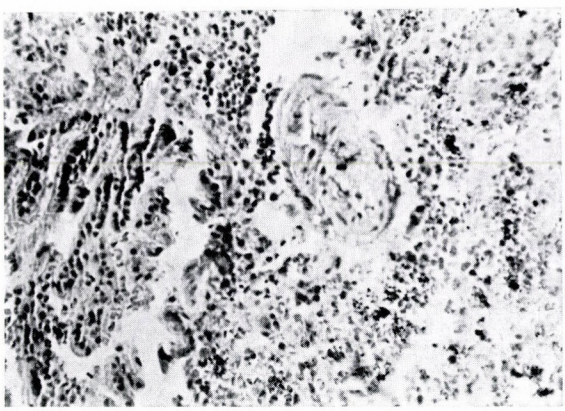

Fig. 11. Inflammation changes in lung tissue and bronchi. H and E ×240

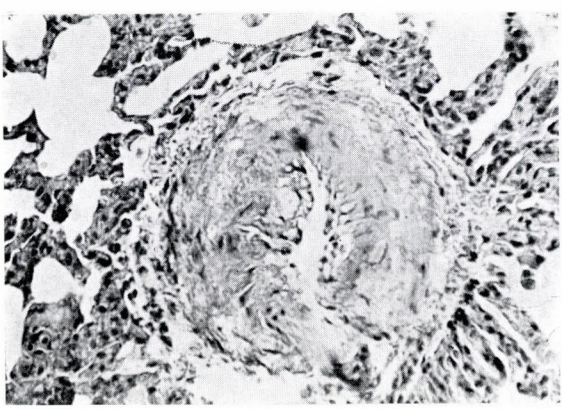

Fig. 12. Endothelial lesion in pulmonary blood vessels. H and E ×240

Rabbits infected with *S. agona* showed changes in the liver, similar to those found by HALL [3] in rats infected with *S. dublin*. GURSEL et al. [2] described liver lesions in the course of human infection with *S. typhi*.

It seems that the changes described above are associated with endotoxin liberated from Salmonella. According to RIETSCHEL [11] lipid A is responsible for the effect of the endotoxin.

In the course of experimental salmonellosis, pathological changes in lungs are rare. BASKERVILLE et al. [1] found them in guinea pigs infected with *S. cholerae* suis and LOBANOWA et al. [5] in hamsters. STORY et al. [12] described bronchopneumonia or unspecific congestion and oedema in human infection with *S. typhi murium*.

In our rabbits there were changes in lungs their aetiology was proved by successful culturing of *S. agona* from the organ.

The kidneys are also involved in infection with *S. agona*. STORY et al. [12] could not find any changes in the kidney of patients infected with *S. typhi murium*. The localization might depend on the serotype of Salmonella. NORDSTOGA [7] found distinct electronmicroscopic changes in the kidney of pigs infected with *S. cholerae suis and* defined the changes as a Sanarelli-Shwartzman reaction.

The changes found in the kidney of our rabbits were however not characteristic of the Sanarelli-Shwartzman reaction.

Summing up, in the course of infection with *S. agona* pathological changes should be excepted not only in the lymphatic system of intestines but also in the liver, lungs, kidney, suprarenal glands and the spleen. It should be stressed that the changes observed by us were different from those described by other authors in infections with other serotypes of Salmonella.

REFERENCES

1. BASKERVILLE, A., DOW, C., CURREN, W. L., HANNA, J.: (1973) Brit. J. exp. Path., **54,** 90. — 2. GURSEL, S., KANDEMIR, B., KARACADAY, S., TELATAR, H.: (1973) Amer. J. Gastroent., **59,** 250. — 3. HALL, G. A.: (1975) Brit. J. exp. Path,. **56,** 216. — 4. KRZANOWSKA, H., PREIBISCH, J., KORDA, P.: (1974) Zwierzeta laboratoryjne. PZWL. Warszawa, 196. — 5. LOBANOWA, Z. I., ORLOWSKIJ, W. I., BOCZKOW, I. A.: (1975) Arch. Path. **37,** 20. — 6. MCCONNEL, G. G., KAUFMANN, A. F., GANGAROSA, E. J., THOMPSON, M. A.: (1973) Lancet **2,** 490. — 7. NORDSTOGA, K.: (1974) Acta path. microbiol. scand. Sect. A, **82,** 690. — 8. PROKOPOWICZ, D.: (1975) Przegl. Lek., **32,** 403. — 9. PROKOPOWICZ, D., TOMASZKO, H.: (1968) Arch. Hyg., **152,** 357. — 10. REJNIAK, L., PROKOPOWICZ, D., KURASZ, S.: (1970) Acta morph. Acad. Sci. hung. **18,** 155. — 11. RIETSCHEL, E. Th.: (1975) Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., **287,** 73. — 12. STORY, P., HANBURY, W. J.: (1957) J. Path. Bact., **73,** 443.

BEI EXPERIMENTELLER INFEKTION VON KANINCHEN MIT SALMONELLA AGONA IN DEN EINZELNEN ORGANEN BEOBACHTETE HISTOPATHOLOGISCHE VERÄNDERUNGEN

D. PROKOPOWITZ und L. REJNIAK

Die in 30 Kaninchen durch Infektion mit Salmonella agona ausgelösten Organveränderungen wurden analysiert. Pathologische Abweichungen ließen sich nicht nur in den Gedärmen, sondern auch in der Leber, in den Lungen, in der Milz, in den Nieren und in den Nebennieren nachweisen. Es scheint, daß diese Veränderungen von den durch andere Serotypen der Salmonellagruppe ausgelösten Veränderungen abweichen.

ГИСТОПАТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ, НАБЛЮДАЕМЫЕ В ОТДЕЛЬНЫХ ОРГАНАХ В ХОДЕ ЭКСПЕРИМЕНТАЛЬНОГО ЗАРАЖЕНИЯ КРОЛИКОВ МИКРОБОМ САЛМОНЕЛЛА АГОНА

Д. ПРОКОПОВИЦ и Л. РЕЙНИЯК

Обсуждаются изменения органов, вызванные у 30 кроликов заражением животных микробом Salmonella agona. Патологические изменения наблюдались не только в кишках, но и в печени, в легких, в селезенке, в почках и в надпочечниках. Кажется, что эти изменения отличаются от изменений, вызванных другими серотипами группы сальмонел.

Dr. Danuta Prokopowicz
Dr. Leopold Rejniak

Akademia Medyczina
Katedra i Klinika Chorób Zakaznygh
Bialystok, ul. Zurawia 14., Polska

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Third Department of Medicine Semmelweis University Medical School, and National Institute of Traumatology Budapest, Hungary

MESENCHYMAL REACTION AND SERUM GLYCOPROTEIN CONCENTRATION IN CHRONIC HEPATITIS AND LIVER CIRRHOSIS

J. Fehér, L. Jakab, L. Józsa, I. Szilvási and Gabriella Papp

(Received September 5, 1975)

In liver biopsy specimens of 45 patients with chronic persistent hepatitis, chronic aggressive hepatitis and liver cirrhosis the number of lymphoid cells and fibroblasts as well as in the sera of the same patients the concentration of IgG, IgA, IgM, alpha-2-macroglobulin and coeruloplasmin have been studied. The number of lymphoid cells and fibroblasts, was significantly elevated in chronic aggressive hepatitis and liver cirrhosis; a close correlation could be demonstrated between the number of the lymphoid cells and the IgG concentration; the serum alpha-2-macroglobulin level changed parallel to the number of liver fibroblasts in chronic aggressive hepatitis and liver cirrhosis.

It has been shown earlier that in chronic hepatitis the concentration of the carbohydrate components of the serum glycoproteins as well as the immunoglobulins, and the level of alpha-2-macroglobulin and coeruloplasmin underwent changes [3, 4, 6]. Serum hexose, hexosamine, sialic acid and seromucoid concentrations were higher than the control values. The degree of elevation changed according to the type of chronic hepatitis. In the aggressive form associated with an active mesenchymal reaction the concentration was significantly higher than in inactive chronic hepatitis. In persistent hepatitis the elevation of IgM, whereas in aggressive hepatitis that of the IgG concentration was characteristic, although both the IgA and IgM values increased considerably. In liver cirrhosis the level of all the three immunoglobulin classes was elevated. In chronic aggressive hepatitis and liver cirrhosis increased concentrations of serum coeruloplasmin and alpha-2-macroglobulin were found, whereas they showed no significant change in chronic persistent hepatitis.

In the present study we have attempted to establish whether in chronic hepatitis and liver cirrhosis there was any correlation between the number of inflammatory cells and elevation of the serum glycoprotein level.

Materials and methods

The material consisted of 45 patients (21 men and 24 women) treated at the 3rd Department of Medicine. Their age ranged from 18 to 62 years. The diagnosis was based on the history, clinical symptoms, laboratory data and liver biopsy. Ten patients had chronic persistent hepatitis, 20 patients chronic aggressive hepatitis and the group of 15 patients had active liver cirrhosis. As a control group for the serum values of 25 healthy subjects were included. There

were no controls for the fibroblasts and lymphocytes in the liver, since no biopsy was performed in the healthy subjects.

IgG, IgA and IgM as well as alpha-2-macroglobulin and coeruloplasmin were determined by radial immunodiffusion [10]. For control, reference sera (Human, Hyland) were used.

The number of the cellular elements participating in the mesenchymal reaction, namely the lymphoid cells (lymphocytes, plasma cells) and fibroblasts (including the histiocytes found in the liver tissue) was determined in histological sections prepared from the biopsy specimens under 400fold magnification; each cell was counted in 20 visual fields (2.50 mm^2). The results obtained were related to 1 mm^2. The cell count in 1 mm^3 was determined by the Floredus formula [5].

Statistical analysis was performed by the t test, the correlation coefficient (r) and the linear regression equation.

Results

Results are presented in the Figures and Tables. In aggressive hepatitis and cirrhosis the number of hepatic lymphoid cells was significantly higher ($p < 0.001$) than in chronic persistent hepatitis (Table I), IgM was considerably increased in chronic persistent hepatitis, whereas in chronic aggressive hepatitis and liver cirrhosis the concentrations of all the three immunoglobulins were significantly higher than the normal values.

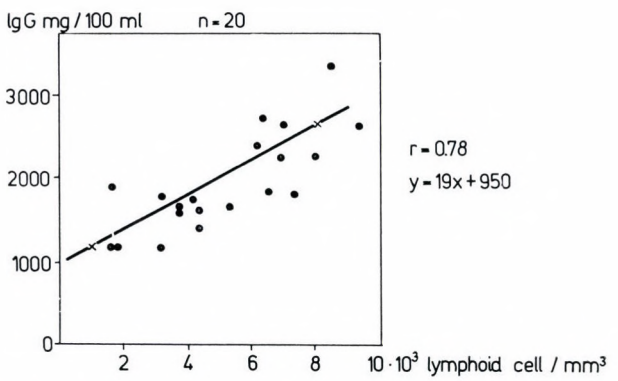

Fig. 1. Serum IgG level and hepatic lymphoid cells in chronic aggressive hepatitis

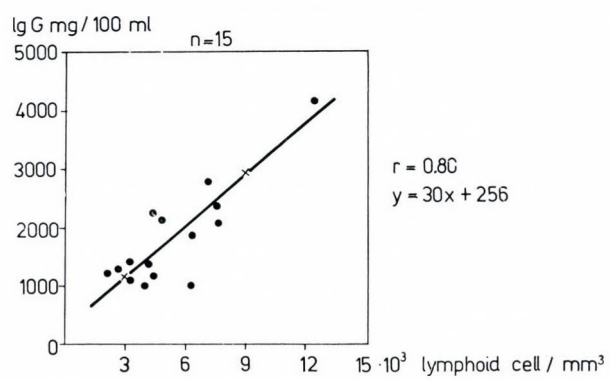

Fig. 2. Serum IgG level and hepatic lymphoid cells in liver cirrhosis

Table I

Number of inflammatory cells in the liver and serum glycoprotein concentration in chronic hepatitis and liver cirrhosis

		Control	Hepatitis, chronic persistent	Hepatitis, chronic aggressive	Liver cirrhosis
Number of lymphoid cells/mm³	n		10	20	15
	$\bar{x}$		2230	5205	5300
	s		269	53	607
	p			<0.001	<0.001
Number of fibroblasts /mm³	n		10	20	15
	$\bar{x}$		950	1760	1940
	s		51	101	111
	p			<0.001	<0.001
IgG mg/100 ml	n	25	10	20	15
	$\bar{x}$	1200	1430	1939	1846
	s	56	163	131	218
	p		>0.05	<0.001	<0.02
IgA mg/100 ml	n	25	10	20	15
	$\bar{x}$	210	267	289	449
	s	16	45	23	58
	p		>0.05	<0.02	<0.01
IgM mg/100 ml	n	25	10	20	15
	$\bar{x}$	130	248	191	254
	s	11	240	18	331
	p		<0.05	<0.001	<0.01
Coerulo-plasmin, mg/100 ml	n	25	10	20	15
	$\bar{x}$	31	38	57	58
	s	2.2	4.2	4.3	6.2
	p		>0.05	<0.001	<0.001
Alpha-2-macro-globulin mg/100 ml	n	25	10	20	15
	$\bar{x}$	244	251	351	453
	s	12	14	26	57
	p		>0.05	<0.01	<0.01

n = number of cases; $\bar{x}$ = mean; s = standard error; p = degree of significance against control, or in the case of cell count, against the chronic persistent hepatitis group

In chronic persistent hepatitis there was no correlation whatever between the absolute number of lymphoid cells and the immunoglobulin levels (the correlation coefficient was in every case below 0.25). In aggressive hepatitis there was a close correlation between the serum IgG level and the number of lymphoid cells ($r = 0.78$; Fig. 1), but none in relation to IgA and IgM ($r < 0.25$). Finally, in the patients with liver cirrhosis there was also a close correlation between the lymphoid cells and the serum IgG ($r = 0.80$; Fig. 2). With IgA the correlation coefficient was 0.41 and with IgM, 0.58 (Table II).

The number of fibroblasts and histiocytes found in liver tissue was significantly elevated ($p < 0.001$) in chronic aggressive hepatitis and cirrhosis (Fig. 4, and Table I) against the values for patients suffering from chronic persistent hepatitis.

Table II

Correlation coefficient (r) for the relation of number of lymphoid cells with serum glycoprotein concentration

	Hepatitis, chronic persistent	Hepatitis, chronic aggressive	Liver cirrhosis
IgG	0.07	0.78+++	0.80+++
IgA	0.14	0.10	0.41+
IgM	0.01	0.10	0.58++
Coeruloplasmin	0.00	0.00	0.00
Alpha-2-macroglobulin	0.00	0.00	0.00

+ = weak
++ = evaluable
+++ = high correlation

The serum alpha-2-macroglobulin and coeruloplasmin concentrations were not higher in chronic persistent hepatitis than in the control group, in chronic but aggressive hepatitis and cirrhosis both concentrations were significantly increased (Table I).

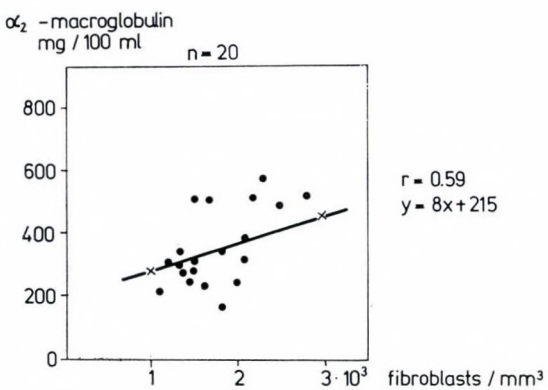

Fig. 3. Serum alpha-2-macroglobulin level and number of hepatic fibroblasts in chronic aggressive hepatitis

In chronic persistent hepatitis no correlation was found between the number of fibroblasts and alpha-2-macroglobulin concentration ($r = 0.05$). In chronic aggressive hepatitis there was no correlation between the coeruloplasmin level and the number of fibroblasts in the liver ($r = 0.13$), whereas an evaluable correlation was detected ($r = 0.59$) between the alpha-2-macroglobulin concentration and the fibroblast count (Fig. 3).

In liver cirrhosis there was no correlation between the number of fibroblasts and the serum coeruloplasmin concentration ($r = 0.09$), but the changes

Table III

Correlation coefficient (r) for the relation of fibroblast number with glycoprotein concentration

	Hepatitis, chronic persistent	Hepatitis, chronic aggressive	Liver cirrhosis
IgG	0.00	0.00	0.00
IgA	0.00	0.00	0.00
IgM	0.00	0.00	0.00
Coeruloplasmin	0.05	0.13	0.09
Alpha-2-macroglobulin	0.02	0.59++	0.75+++

++ = evaluable
+++ = high correlation

of the alpha-2-macroglobulin concentration were proportionate to the number of the fibroblasts and histiocytes (r = 0.75; Fig. 4).

In none of the diseases could a correlation be demonstrated between the number of lymphoid cells and the coeruloplasmin and alpha-2-macroglobulin level, nor between the number of liver fibroblasts and the immunoglobulin concentrations (in each case the r value was 0; Table II and III).

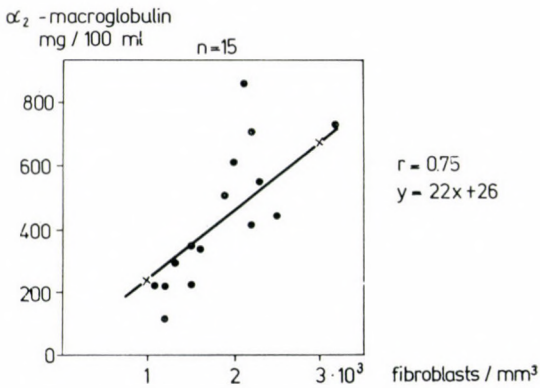

Fig. 4. Serum alpha-2-macroglobulin level and number of hepatic fibroblasts in liver cirrhosis

Discussion

A considerable number of clinical and experimental studies have supported Popper's view concerning the inflammatory processes in the liver. Accordingly, periportal infiltration might be the morphologically characteristic feature of autoaggressive processes. The autoimmune progression of the chronic inflammatory process is maintained by the cellular and humoral occurrences involved

[13, 17]. All the authors attribute a significant role to the lymphocytes present in the liver substance, which are transformed following stimulation and are partially changed into antibody-forming plasma cells. The accumulation of hepatic lymphoid cells has been confirmed by each the pertinent literary data; we have however, failed to find literary references concerning their quantitative evaluation. Studies on the behaviour of the peripheral lymphocyte count and serum immune-globulins were performed by BOLTON et al. [1] in 52 patients suffering from Crohn's disease and 20 with ulcerative colitis, but these authors failed to examine the lymphocyte count and the immunoglobulin concentrations. SKINNER and WHITEHEAD [18] studied the lymphoid cellular elements and the local lymph nodes in surgical preparations of 85 colectomized patients. They interpreted the morphological picture as a proof of the stimulation of the cellular and humoral mechanisms in ulcerative colitis and in Crohn's disease. The results of the present study might be brought into connection with this observation: the behaviour of the immunoglobulins would be the result of the humoral immune response and the changes of the lymphoid cell count of the cellular one. The number of lymphoid cells in the liver and the increase in serum immunoglobulins could be correlated owing to the causal relation of the two factors: the higher serum immune globulin concentration would be a consequence of increased antibody formation induced by the activity of the accumulated cells. On the other hand, it cannot be excluded that the lymphoid elements in the liver are only representing the immunological reaction of the entire organism.

There are many data concerning the glycoprotein-synthetising activity of fibroblasts. FORRESTER et al. [7] observed that the electrophoretic mobility of transformed hamster kidney fibroblasts is connected with the sialic acid concentration of the cell surface. According to SOBEL et al. [18] the glycoproteins and mucoproteins of the connective tissue basal substance are also produced by fibroblasts. KITTLICH et al. [11] observed mucopolysaccharide synthesis in fibroblast cultures. The results of our earlier experiments refer also to the probable role of fibroblasts in glycoprotein synthesis [2, 5, 8, 9]. The present results revealed a correlation between the serum alpha-2-macroglobulin concentration and the number of fibroblasts found in the interstitial substance of the liver in chronic aggressive hepatitis and hepatic cirrhosis. On this basis it seems that the increased glycoprotein synthetising activity of the fibroblasts in the interstitial hepatic substance may induce an elevation of the serum alpha-2-macroglobulin concentration. The observations of JAKAB et al. [10] in patients suffering from acute myocardial infarction also support this hypothesis.

To sum up, significant increase in the number of lymphoid cells and fibroblasts in the liver in chronic aggressive hepatitis and cirrhosis has been verified by quantitative examinations. A close correlation could be demonstrat-

ed between the number of the lymphoid cells and the serum IgG level. The changes in the serum alpha-2-macroglobulin level and in the number of the liver fibroblasts run parallel in chronic aggressive hepatitis and in liver cirrhosis.

Acknowledgement

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REFERENCES

1. Bolton, P. M., James, S. L., Newcombe, R. G., Whitehead, R. H., Huches, L. E.: (1974) The immune competence of patients with inflammatory bowel disease. Gut **15,** 213. — 2. Fehér, J., Jakab, L.: (1972) The role of connective tissue cells in the metabolic changes of glycosaminoglycane and glycoprotein in experimental inflammation. Acta morph. Acad. Sci. hung. **20,** 27. — 3. Fehér, J., Jakab, L., Józsa, L.: (1972) A serum glycoproteidek szénhydrat componenseinek változása chronicus hepatitisben. Orv. Hetil. **113,** 2880. — 4. Fehér, J., Jakab, L., Szilvási, I.: (1973) Immunoglobulins, glycoproteids and Australia, antigen in chronic liver disease. Acta med. Acad. Sci. hung. **30,** 197. — 5. Fehér, J., Jakab, L., Takács, L.: (1970) Effect of liver injury and of induced inflammation on the serum glycoprotein level. Acta med. Acad. Sci. hung. **27,** 57. — 6. Fehér, J., Jennings, E. H., Rannie, I.: (1971) Quantitative changes in the number of the mast cells in experimental inflammation produced by croton oil in rats. Brit. J. exp. Path. **52,** 27. — 7. Forrester, J. A., Ambrose, J. F., Stoker, M. G. P.: (1964) Microelectrophoresis of normal and transformed clones of hamster kidney fibroblasts. Nature (Lond.) **201,** 945. — 8. Jakab, L., Fehér, J.: (1969) Veränderungen in den Glykoproteinfraktionen von Serum und Lymphe bei der von Crotonöl hervorgerufenen Entzündung. Z. ges. exp. Med. **149,** 44, — 9. Jakab, L., Fehér, J.: (1969) Serum and lymph glycoprotein fractions in the healing phase of croton oil induced inflammation. Acta med. Acad. Sci. hung. **26,** 28. — 10. Jakab, L., Fehér, J., Siró, I., Szondy, É., Székely, J.: (1974) Serum glycoproteid concentration changes in myocardial infarction. Magy. Belorv. Arch. **27,** 171. — 11. Kittlich, P. D., Neupert. G., Lümkemann, U.: (1973) Die Wirkung verschiedener Seren auf die Mukopolysaccharid-Synthese in Fibroblastkulturen. Exp. Path. **8,** 194. — 12. Mancini, G., Carbonara, A. O., Heremans, J. F.: (1965) Immunochemical quantitation of antigens by single radial immundiffusion. Immunochemistry. **2,** 235. — 13. Meyer zum Büschenfelde, K. H., Schweiger, J., Konlle, J.: (1970) Zur Isolierung organgebundener Leberproteinen für die immunologische Diagnostik. Verh. dtsch. Ges. inn. Med. **76,** 10. — 14. Popper, H.: (1969) Aktuelle Hepatologie. George Thieme Verlag, Stuttgart. — 15. Popper, H., Mackay, J. E.: (1972) Relation between Australia antigen and autoimmune hepatitis. Lancet **1,** 1161. — 16. Popper, H., Schaffner, F.: (1965) Die Mesenchymreaktion auf Parenchymschädigungen. Zum Problem der chronischen Hepatitis. Ann. N. Y. Acad. Sci. **124,** 781. — 17. Schumacker, K.: (1969) Die Bedeutung immunologischer Reaktionen für Diagnose und Pathogenese der chronischen Hepatitis. Klin. Wschr. **47,** 949. — 18. Skinner, J. M., Whitehead H.: (1974) A morphological assessment of immunoreactivity in colonic Crohn's disease and ulcerative colitis by a study of lymph nodes. J. clin. Path. **27,** 202. — 19. Sobel, H., Hewlett, M., Cohen, R.: (1968) Correlation of loss of hyaluronic acid and chondroitin sulphate in skin of mice with carcass nitrogen loss during starvation. Nature (Lond.) **217,** 1279.

DIE MESENCHYMALE REAKTION UND DER GLYKOPROTEIDSPIEGEL IM SERUM BEI CHRONISCHER HEPATITIS UND LEBERZIRRHOSE

J. FEHÉR, L. JAKAB, L. JÓZSA, I. SZILVÁSI und GABRIELLA PAPP

Bei 45 Kranken mit chronischer persistierender Hepatitis, chronischer aggressiver Hepatitis und Lebercirrhose wurde im Biopsiematerial der Leber die Zahl der lymphoiden Zellen und der Fibroblasten, ferner im Serum der gleichen Kranken die IgG-, IgA, IgM-, α-2-Makroglobulin- und die Coeruloplasminkonzentration untersucht und folgendes festgestellt:

1. Bei chronischer, aggressiver Hepatitis und Leberzirrhose läßt sich bei der quantitativen Untersuchung ein signifikanter Anstieg der Zahl der lymphoiden Zellen und der Fibroblasten aufzeigen.

2. Zwischen der Zahl der lymphoiden Zellen und dem IgG-Spiegel ist eine enge Korrelation nachweisbar.

3. Bei chronischer, aggressiver Hepatitis und Leberzirrhose ändert sich der α-2-Makroglobulinspiegel parallel der Veränderung der Fibroblastenzahl in der Leber.

РЕАКЦИЯ МЕЗЕНХИМЫ И КОНЦЕНТРАЦИЯ СЫВОРОТОЧНЫХ ГЛИКОПРОТЕИДОВ ПРИ ХРОНИЧЕСКОМ ГЕПАТИТЕ И ПРИ ЦИРРОЗЕ ПЕЧЕНИ

Й. ФЕХЕР, Л. ЯКАБ, Л. ЙОЖА И. СИЛВАШИ и ГАБРИЕЛЛА ПАПП

У 45 больных персистирующим хроническим гепатитом, агрессивным хроническим гепатитом и циррозом печени авторы изучали в печеночной ткани, полученной при биопсии печени, число лимфоидных клеток и фибробластов и в сыворотке тех же больных определили концентрацию IgG, IgG, IgM, альфа-2-макроглобулинов и церулоплазмина. Они нашли, что

1. при агрессивном хроническом гепатите и при циррозе печени в случае количественного исследования в печеночной ткани наблюдается достоверное повышение числа лимфоидных клеток и фибробластов;

2. между числом лимфоидных клеток и концентрацией IgG можно выявить тесную корреляцию;

3. при агрессивном хроническом гепатите и циррозе печени уровень альфа-2-макроглобулинов изменяется параллельно изменению числа фибробластов в печени.

Dr. János Fehér
Dr. Lajos Jakab
Dr. István Szilvási
Dr. Gabriella Papp
László Józsa

Semmelwei University Medical School,
Third Department of Medicine,
1430 Budapest, Mező Imre út 17.,
Hungary
National Institute of Traumatology, 1430 Budapest,
Mező Imre út 17., Hungary

RECENSIONES

H. KAROBATH: *Der Blitzumfall.* Verlag G. Witzstrock, Baden-Baden, Brüssel, Köln (1975) 106 p., 42 Figures, 7 Tables.

Modern electropathology and research on lightning injuries over its start at the turn of the century Jellinek of Vienna. During the past 75 years several of his statements have become obsolete. Karobath's small monograph now summarizes all up to date knowledge concerning lightning accidents. After a survey of the history of persistent research the meteorology of thunderstorm and the physics of lightning discharge are described. Then the symptoms of the lightning stroke, its effect on the heart and the ECG changes are discussed in addition to individual accidents and the author's animal experiments. In the following chapters, the triad of the lightning syndrome i.e. disturbed consciousness, lightning paralysis, and skin alterations, further the disturbances of sight and hearing as well as the influence on pregnancy of the lightning injury are discussed.

Most physicians know very little about lightning accidents; in this little book they will find everything may ought to know.

E. SOMOGYI

I. OLÁH, P. RÖHLICH, I. TÖRŐ: *Ultrastructure of Lymphoid Organs. An Electron Microscopic Atlas.* Publishing House of the Hungarian Academy of Sciences, Budapest, 1975., and J. B. Lippincott Co., Philadelphia and Toronto, and Masson et Cie, Paris. Pp. 317, 255 illustrations, 28 schematic drawings

In the last decades a great development has taken place in the field of experimental and clinical immunology as well as in the diagnostics and therapy of diseases of the lymphatic system. In a number of diseases of unknown origin functional disturbances of the immune system have been observed and the autoimmune origin of some of these diseases has been proven.

In the pathogenesis of various diseases an increasing importance has been attributed to immunoreactions and mechanisms. In addition, examination of the structural and functional changes of lymphocytes, has become a routine diagnostic procedure. This explains why the researchers possessing a classical knowledge of the lymphoid organs felt an increasing need for a work supplying information on the fine structure of lymphoid organs revealed by electron microscopy in the last two decades. This book meets this requirement and illustrates the cellular elements in their micro environments which have an important role in immunoreactions and immunocytological phenomena.

The book is introduced by a brief chapter on the material and the methods of fixation, embedding, staining, etc., used by the authors.

In the first part of the book — anticipating that the reader has the necessary basic knowledge of light microscopy — the components of lymphoid tissue are described: the cellular elements, lymph follicles and the venules with a peculiar endothelial lining present in the lymphoid organs. After a description of the fine structure of the components of lymphoid tissues, separate chapters are devoted to the individual lymphoid organs: thymus, bursa of Fabricius, tonsils, gut-associated lymphoid organs, lymph nodes and their particular form, the so-called heamolymph-nodes and finally the spleen. Their description is illustrative and didactically constructed. The description of each type of tissue and organ is introduced by the presentation of light microscopic pictures of 1000—2000 times magnification, and of illustrative schematic drawings. These are followed by electron microscopic pictures with magnifications ranging from 2000 to several 10000 times. The transitions between the different degrees of

magnification always follow a well-chosen order, allowing an easy orientation also for readers less versed in the structure of lymphoid tissue.

The most important and valuable part of the atlas is the rich illustration material. Apart from some exceptions, the illustrations made from 32 semi-thin sections, and the 223 electron microscopic pictures are of excellent quality and the 28 schematic illustrations are also outstanding. The legends to the figures as well as the explanations to the symbols are found on the page opposite to the figures. The construction allows the reader to familiarize himself with the material and obtain profound knowledge about the fine structure of the cellular elements of the lymphoid tissue and lymphoid organs. The atlas also contains a few montages made up of several pictures which fit precisely. The technique applied in more than one case, when structural properties considered important are rendered conspicuous by clippings or by fat contours is ingenious and didactic. It also contributes to the value of the atlas that the functional importance of each fine structural element is pointed out. Another merit of the book is that the authors do not enter into a detailed analysis of the various hypotheses concerning the single structures, but instead present that knowledge about the lymphoid organs which has found general acceptance. They always find means to call attention to hither to unclarified problems.

The rich original picture material of the atlas is based primarily on the study of the lymphoid organs of the rat, guinea-pig, bat, rabbit, cat and monkey. In studies on the bursa of Fabricius the chicken was the object of examination.

It is unfortunate that the examinations should not involve the lymphoid organs of man. In our days when the reclassification of Hodgkin and non-Hodgkin lymphomas on the basis of the structural changes of the lymphoid system and on the changes of immune function are current, this would have had a particular actuality. It is hoped that the next edition of the book will be complemented with scanning electron microscopy pictures on the cellular components of lymphoid organs.

The literature presented at the end of the atlas and classified according to the chapters comprises 300 important literary sources on the ultrastructure of lymphoid organs, tissues and their cellular elements, also in respect of human material.

The subject of the atlas fills a long felt need. The book will represent a useful source of information for researchers and clinicians interested in the physiology and morphology of the lymphoid system and will be most suitable for teaching purposes.

K. Lapis

King McLelland: *Outlines of Avian Anatomy:* 154 pages with 73 figures, Baillière Tindal and Co., London 1975.

This book is a sketchlike summary of the anatomy of domestic birds. It supplies basic knowledge for beginners and provides guidance for training courses. It is a useful book for zoologists, biologists, for those interested in comparative anatomy and experimental medicine as well as for high school and university education.

The monograph contains 15 chapters. In the first chapter the origin and classification of the birds, in the second the structure of the skin, glands and plumage as well as the development of the latter are summarized, referring also to the skin changes in Marek's disease. Chapter 3 summarizes the organ system of motility, with a somewhat detailed description only of the vertebrae. It makes a digression on the functional role of the pneumatic bones and discusses the species specific characteristic of ossification and also the morphology of bone diseases. Chapter 4 is a precise and clear survey of recent knowledge concerning body cavities: this is the book's most valuable chapter. Chapter 6 discusses the digestive apparatus and the processes of food uptake, transmission, physical and chemical digestion and crop-milk formation. The part concerning the stomach and enternal digestion also belongs among the bests. The more detailed description of the respiratory apparatus in Chapter 7 discusses also the function of the organ of phonation (syrnix), the pleural cavities and the electron microscopic structure of the lungs; pulmonary respiration is also discussed. The sexual organs are briefly summarized in Chapters 8 and 9. A separate chapter discusses the functional morphology of the cloaca and the external sex organs. The functional histology of the endocrine glands (Chapter 10) refers to the most recent data. Together with the description of the blood vessel system (Chapter 11) blood formation (haematopoiesis) and with the description on the lymphatic system (Chapter 12) acquired immunity is also discussed. Chapter 13 dealing with the nervous system is the most detailed one and mainly contains recent data. In Chapter 15 the organs of sense are briefly summarized.

In the single chapters, few comparatíve references to vertebrates are found. At the same time some less known facts and recent knowledge studies are described in more detail.

The chapters which would be important in teaching and practice, namely those dealing with the urinary system and the sexual organs and egg formation, unfortunately occupy a small space in the book.

The book is a successful up-to-date guide serving the English educational system. It supplies in each chapter the basic and most recent reference studies. The easily surveyable and ingeniously constructed figures are excellent and mean a great help in teaching, more than would illustrations presenting more details and supply at the same time perfect directions in the course of the necropsy.

G. FEHÉR

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