MORPHOLOGY AND PATHOMORPHOLOGY

HISTOCHEMICAL INVESTIGATIONS OF THE "KUL'CHITSKII CELLS" IN THE GASTROINTESTINAL TRACT OF EXPERIMENTAL ANIMALS

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Peculiar tumors, called carcinoids, develop from the mucosa of the alimentary tract. It has been shown that carcinoids originate in the cells which were described in 1897 by N. K. Kul'chitskii [1]. He found them in the mucosa of the stomach, the duodenum and the large intestine, and showed that they secrete granules, differing in their nature from the granules of the blood cells and of the fat cells of the connective tissue. Similar cells were later described by Yu. M. Lazovskii [2] in the mucosa of the pancreatic duct, and by Detril [7] in the mucosa of the bile ducts. When examined histochemically, the cells studied by N. K. Kul'chitskii were found to have argentaffin and chromaffin properties and to give a positive diazo reaction.

Erspamer and Asero [9] isolated from the metastases of a carcinoid tumor a substance which they called enteramine, since the tumors were derived from the mucosa of the intestinal tract. These authors showed that enteramine was identical with 5-oxytryptamine, which was synthesized by Hamlin and Fischer [14].

Rapport, Green and Page [24] isolated this same substance from the serum of cattle, and called it serotonin, since it possessed a tonic action on smooth muscle.

With regard to the identification of the serotonin present in the enterochromaffin cells with 5-oxytryptamine, the investigations of Benditt and Wong [4] are of great interest; by the use of a histochemical method they showed that 5-oxytryptamine is present in these cells in a 1% concentration. Evidence in favor of the identity of the serotonin of the granules of the enterochromaffin cells with 5-oxytryptamine is given by the variations in the 5-oxytryptamine content after the administration of certain drugs, and also by the 10- to 60-fold increase in the 5-oxytryptamine content in carcinoid tumors [4, 17, 21, 23, 28, 29].

Serotonin is widely distributed in the body; about 99% of the total content is in the intestine, the blood platelets and the brain, especially in the brainstem (cited from Gennes and de Fossey [13]). It causes contraction of the smooth muscle of the internal organs and vessels; the vessels most sensitive to it are those of the kidneys [22]. Reserpine, when given to an animal, causes the disappearance of serotonin from the enterochromaffin cells for several hours, and liberates endogenous serotonin from the brain tissue [20]. The serotonin liberated by reserpine is destroyed by the enzyme monoaminooxidase [25].

Zbinden, Pletscher and Stuber [27] showed that the cells of the enterochromaffin system react differently to the administration of reserpine. In the pyloric cells, for instance, the granules disappear, whereas in the mucosal cells of the fundus of the stomach even lethal doses of reserpine do not lead to disappearance of the argentaffin granules. Evidently the function of the cells of the enterochromaffin system varies in the different parts of the body.

The results of few histochemical investigations of the "Kul'chitskii cells" have as yet been published, in either the foreign or Soviet literature. Insufficient work has been done on their distribution in the gastrointestinal

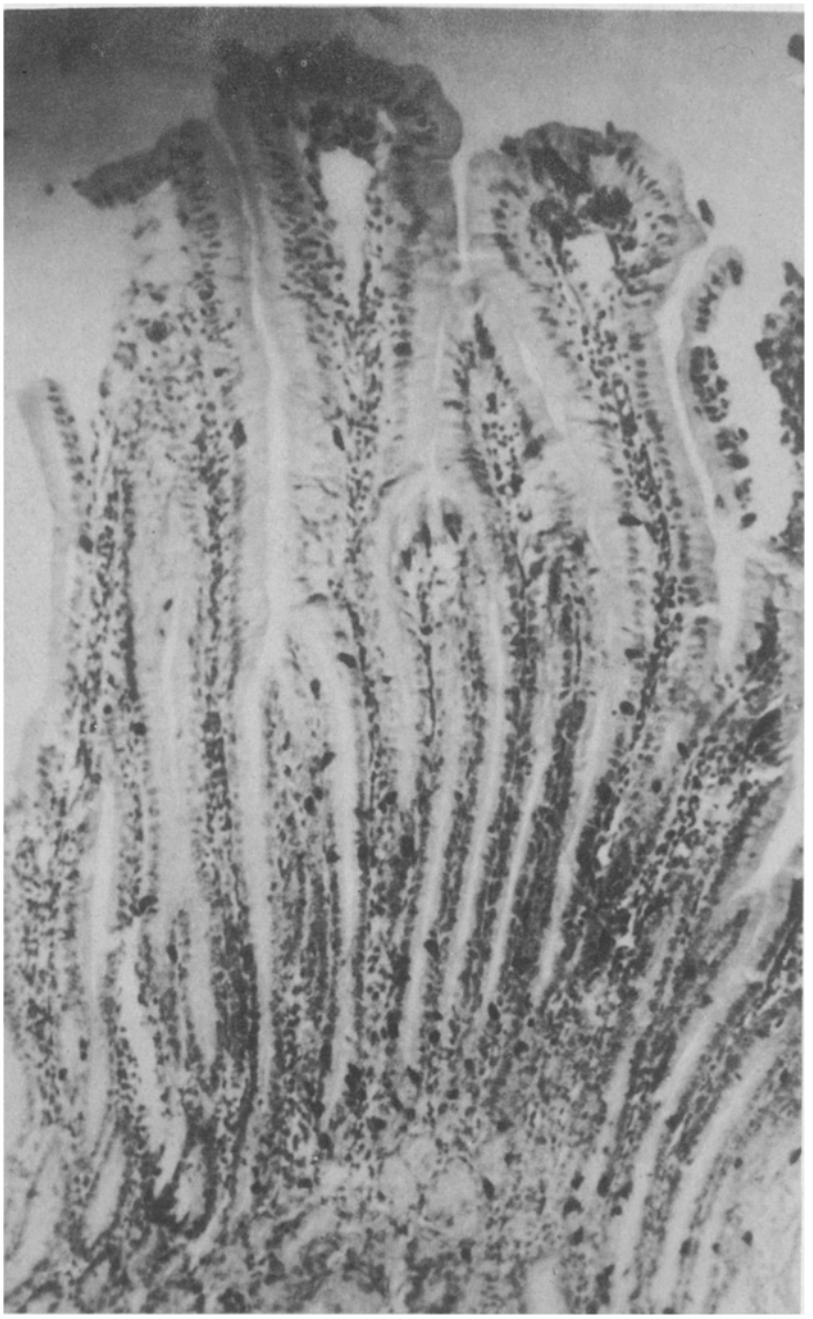

Fig. 1. "Kul'chitskii cells" in the mucous membrane of the duodenum of a guinea pig. Fontana's modification of Masson's method. Magnification: ocular 7×, objective 8×.

tract of different animals, and on the degree of packing with granules in relation to the species of animal and its physiological state.

When detecting serotoning histochemically in the cells of an animal, it must be appreciated that there is still no absolute proof that the granules of the "Kul'chitskii cells" are identical with serotonin. Morphological investigations have to be supplemented by biochemical methods of checking, by color chromatography and so on. No less than three histochemical reactions must be carried out: argentaffin, chromaffin and diazo reactions. Argentaffin cells possess the property of reducing silver on account of endogenous substances. The chromaffin reaction is a specific histochemical test for polyphenols, aminophenols and ortho- or paraphenols. The diazo reaction is used to exclude the presence of lipofuscin, for granules of the latter substance are not stained by diazonium salts nor their decomposition products.

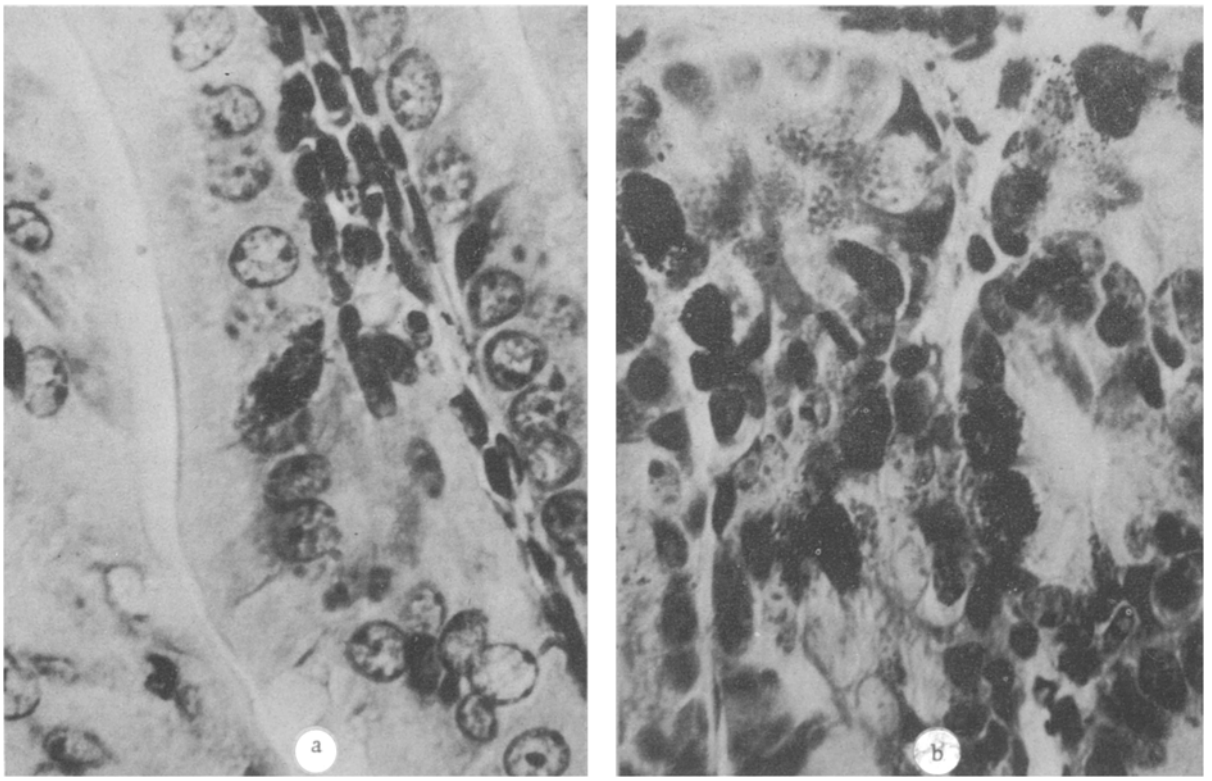

Fig. 2. "Kul'chitskii cells," situated in the crypts of the duodenal mucous membrane of a guinea pig. a) Cells, densely packed with argentaffin granules; b) a similar cell, lightly packed with granules, at the apex of a villus in the same preparation. Fontana's modification of Masson's method. Magnification: ocular 7 x. objective 40 x .

We investigated the content of "Kul'chitskii cells" in the gastrointestinal tract of different experimental animals. The aim of the present research was to examine the distribution and degree of packing of these cells with granules, throughout the gastrointestinal tract of laboratory animals in order to discover the most convenient and economical model on which to study pharmacologically active compounds possessing a reserpine-like action or the property of inhibiting monoaminooxidase (compounds of great importance in the practice of neuropsychiatry).

EXPERIMENTAL METHOD

We examined the gastrointestinal tract of monkeys, cats, rabbits, guinea pigs, white rats, mice, birds and frogs, 120 animals altogether. The animals were kept on the ordinary nursery diet, apart from 20 guinea pigs which were in a fasting condition. The specimens from the healthy monkeys were obtained from the Institute for the Study of Poliomyelitis. In order to examine the effect of different physiological conditions on the degree of packing of the "Kul'chitskii cells" with argentaffin granules, an experiment was carried out on fasting animals. Guinea pigs, weighing 330-400 g, were kept in a specially contructed cage, with a grating over the floor to prevent any possibility of the animals eating their own feces. The guinea pigs received water but no food until they died from starvation. All the animals were sacrificed by decapitation, the starving animals 2, 3, 5, 6 and 7 days from the beginning of the experiment; furthermore some of the guinea pigs died on the 8th day of starvation. The killed and dying animals were examined post mortem, and after inspection of the internal organs, samples of the wall of the stomach, duodenum, jejunum and large intestine were taken for morphological examination. For the argentaffin and diazo reactions, the material was fixed in 12% neutral formalin, and for the chromaffin reaction, fixation was for 24 hours in a mixture of 9 parts of bichromate (3.5%) and one part of neutral 40% formalin. In order to reveal argentaffin granules we used Masson's method, with Hamperl's formula and using Fontana's solution. The chromaffin reaction was carried out by Pfeiffer-Jarisch method (for a description of the methods [3]).

EXPERIMENTAL RESULTS

Histochemical investigation showed that the clearest granules were observed by the use of the argentaffin stain, and less clear by the diazo reaction; the chromaffin reaction gave diffuse staining of the protoplasm of the "Kul'chitskii cells".

In the gastrointestinal tract of the guinea pigs we found an abundance of argentaffin cells, densely packed with granules, mainly in the mucous membrane of the duodenum; considerably fewer of them were found in the mucosa of the fundus of the stomach, the jejunum and large intestine, and rather more at the outlet of the small intestine into the large; a few cells were seen in the mucosa of the hepatic and pancreatic ducts. In the thickness of the mucosa of the duodenum the "Kul'chitskii cells" were distributed unevenly: there were more of them in the crypts, fewer in the villi, and only solitary cells were found at the apex of the villi; there was a falling off also in the packing of the cells with granules as the apex of the villus was reached (Fig. 1).

In a transverse section of the intestine it could be seen that areas of the mucosa situated over lymphatic follicles were almost free from these cells. Other characteristic features were the arrangement of the argentaffin cells in the membrane between the epithelial cells, their triangular or oval shape and the absence of any contact between their protoplasm and the surface of the lumen of the intestine (Fig. 2, a, b).

The content of granules in the cells varied enormously, even in normal animals. In adults, cells were present which were packed with an enormous number of granules, so that the nucleus was invisible; in other cases its outline could be made out and, finally, there were cells in which a few hardly perceptible specks were present. In general, in healthy adult guinea pigs, enterochromaffin cells densely packed with granules were predominant. On transverse section of the duodenum, in different animals, from 450 to 1000 cells could be counted, and in other divisions of the intestine, from 0.25 to 100 cells.

In white rats the number of enterochromaffin cells was considerably smaller and the granules were small and not so numerous; they were sometimes distinct from the surface of the protoplasm in the form of strips, whereas in guinea pigs the granules were scattered over the whole surface of the protoplasm of the cell. In rabbits and mice the number of enterochromaffin cells, and the extent to which they were packed with granules, were also smaller than in the guinea pigs, but the shape of the cells and the distribution of the granules were identical. In monkeys, enterochromaffin cells were also found most often in the mucous membrane of the duodenum, but much more rarely than in guinea pigs. Their shape and distribution were similar to those described in man. We found cells, reasonably well packed with granules, in the mucous membrane of the duodenum in fowls and chicks; in the remaining divisions of the intestine, solitary cells, poor in granules, were seen, and in the wall of the stomach they were absent. In the frog the number of enterochromaffin cells in the mucosa of the alimentary tract was insignificant, and the granularity was represented by a slight accumulation of formations resembling dust.

The diazo reaction gave clear results in cases when the argentaffin granules were well represented, but by the use of the first method the granules had indistinct outlines. With the chromaffin reaction the protoplasm of the cells was dissusely stained and the outlines of the granules could not be distinguished.

In view of the difference in the content of cells of the enterochromaffin system in the animals investigated, we consider that the most suitable for experimental research are guinea pigs and chicks, for in these species it is easier to make quantitative estimations of the changes in the cell composition and in the degree to which the cells are packed with granules.

Microscopic examination of the alimentary tract of the starving guinea pigs showed the gradual diminution in the granularity of the enterochromaffin cells, although this did not completely disappear, even in the animals dying from starvation. It was also found that after intraperitoneal injection of reserpine, in a dose of 10 mg/kg, the argentaffin cells disappeared in normal animals but persisted in the starving animals. After the same dose of reserpine, given by mouth, the granules did not disappear. It is evident that in the starving guinea pigs, decomposition of the reserpine took place after its parenteral injection.

SUMMARY

The authors conducted a histochemical study of the distribution of "Kul'chitskii cells" and their saturation with the granules in various laboratory animals. The argentaffin and chromaffin reactions were determined

with a parallel diazo reaction. An uneven distribution of "Kul'chitskii cells" along the intestine was revealed. The highest density of the cells and the greatest granule saturation were observed in the duodenal mucous membrane of cats, rabbits, guinea pigs, white rats, white mice, monkeys and chicks. The content of argentaffin granules is slightly decreased in the cellular protoplasma of fasting guinea pigs, but even in animals dead of hunger they do not disappear completely. After intraperitoneal injection of reserpine, argentaffin granules vanish in normal animals, but not in fasting ones.

LITERATURE CITED

[1] N. K. Kul'chitskii, (Kultschitzky N), Arch. microskop. Anatomie u. Entw. 49, 7 (1897).

[2] Yu. M. Lazovskii, Zhur. Éksptl. Med. 1, 2, 93 (1928).

[3] A. Pearse, Histochemistry, 1956 [Russian translation].

[4] E. Benditt and R. Wong, J. Exper. Med. 105, 6, 509 (1957).

[5] B. Brodie, A. Pletscher and P. Shore, Science, 122, 3177, 968 (1955).

[6] C. Clark, H. Weissbach and S. Udenfriend, J. Biol. Chem. 210, 139 (1954).

[7] P. Detril, Presse méd. 64, 49, 1138 (1956).

[8] O. Eber and F. Lembeck, Pflügers. Arch. 265, 6, 263 (1958).

[9] V. Erspamer and B. Asero, Nature. 169, 4306, 800 (1952).

[10] F. Feyrter and Klaus-Unna, Virch. Arch. 298, 187 (1937).

[11] J. Gaddum and N. Giarman, Brit. J. Pharm. 11, 88 (1956).

[12] A. Gebauer and K. Kümelin, Dtsch. med. Wschr. 83, 15, 620 (1958).

[13] L. Gennes and M. de Fossey, Presse med. 64, 46, 1066 (1956).

[14] K. Hamlin and F. Fischer, J. Am. Chem. As. 73, 5007 (1951).

[15] K. Hegglin and H. Langemann, Helv. med. acta 4/5, 463 (1955).

[16] L. Heilmeyr and K. Clotten, Deutsch. med. Wschr. 83, 15, 617 (1958).

[17] O. Hornykiewicz, Acta Neuroveget. 13, 1, 111 (1956).

[18] P. Isler and G. Hedinger, Schw. med. Wschr. 1, 4 (1953).

[19] H. Kahr, Acta Neuroveget. 1, 99 (1956).

[20] H. Langemann, Schweiz. med. Wschr. 85, 40, 957 (1955).

[21] F. Lembeck, Arch. exper. Path. u. Pharm. 221, 50 (1954).

[22] J. Levy and E. Michel-Ker, Compt. rend. séance Acad. sci. 242, 25, 3007 (1956).

[23] B. Pernow and Waldenstrom, Lancet. 267, 951 (1954).

[24] M. Rapport, A. Green and J. Page, J. Biol. Chem. 176, 1243 (1948).

[25] F. Roewer and E. Werle, Arch. exp. Path. u. Pharm. 230, 6, 552 (1957).

[26] S. Udenfriend, E. Titus and H. Weissbach, J. Biol. Chem. 216, 499 (1955).

[27] G. Zbinden, A. Pletscher and A. Studer, Kl. Wschr. 35, 11, 565 (1957).

[28] G. Zbinden, A. Pletscher and A. Studer, Schw. med. Wschr. 87, 22, 629 (1957).

[29] W. Zilka and P. Köhler, Klin. Wschr. 39, 12, 622 (1957).