Electron Microscope Autoradiography of the Mouse Fundic Mucosa after Injection of Tritiated L-DOPA

The presence of 5-hydroxytryptamine (5-HT) in the enterochromaffin cell system has been known since long[1]. In addition there are cells in the gastrointestinal tract which lack endogenous occurrence of 5-HT, but have the capability to accumulate, and convert to the corresponding amine, the injected monoamine precursors dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP)[2]. This process has hitherto been studied with fluorescence microscopy and light microscopic autoradiography[3,4,16].

In the electron microscope it is possible to distinguish several types of enterochromaffin cells characterized by different appearance of the specific granules in the cytoplasm[5–12]. In the light microscope it is, however, impossible to identify the different enterochromaffin cell types.

In the present investigation, the fundic gland area of the mouse has been chosen for an attempt, by means of electron microscope autoradiography, to establish in which cells a monoamine-accumulation takes place after the injection of DOPA. The fundic gland area has been shown to contain cells with the monoamine precursor accumulating capability[2].

Two male albino mice (NMRI-strain) weighing 20 g were injected through the caudal vein with 1.5 mCi ring-2-5-6-^{3}H-labelled L-3(3,4-dihydroxyphenyl)alanine (L-DOPA), spec. act. 28 Ci/m*M* (Radiochemical Centre, Amersham, England). The dose corresponded to 0.54 mg L-DOPA/kg body weight. The mice were killed 2 and 4 h after the injection and small pieces of the stomach from the fundic gland area were rapidly removed and

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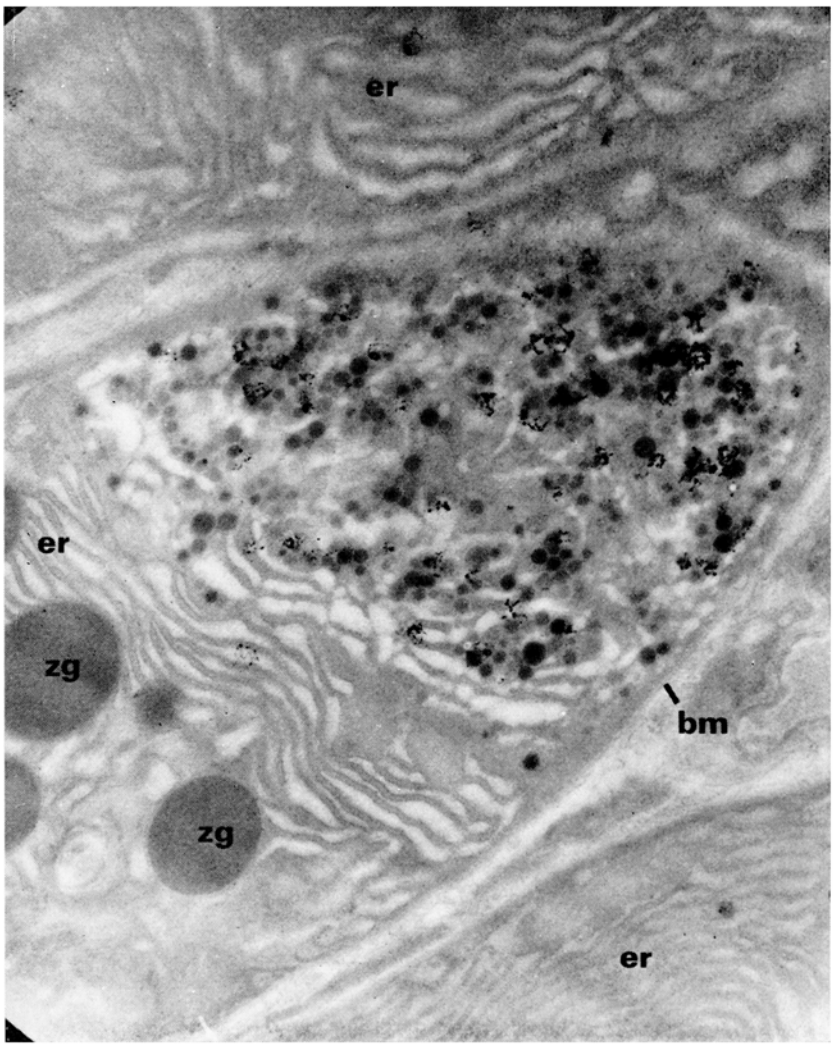

Fig. 1. Enterochromaffin cell of the first type from a fundic gland lying on the basement membrane (bm). Silver grains are seen predominantly over the round dense granules in the cytoplasm. Zymogen granules (zg) and endoplasmatic reticulum (er) of adjacent chief cells. ×13,000.

immersed in a cold 2.5% glutaraldehyde solution in a Tyrode medium pH 7.4. After 2 h of fixation the specimens were rinsed in Tyrode solution and transferred to a cold isotonic solution of osmium tetroxide buffered to pH 7.4 for an additional fixation period of 2 h. After dehydration in ethanol, the tissue pieces were embedded in Epon. The procedure for electron microscopic autoradiography has been described by earlier authors[13]. The sections cut were 400–600 A thick. Ilford L 4 emulsion was used. The exposure time was 8 weeks. The specimens were studied in a Siemens Elmiskop I.

On the electron microscope autoradiographs, a strong accumulation of silver grains could be seen over cells of enterochromaffin type, which, on the basis of different appearance of the granules, could be said to be of 2 different types. Silver grains over zymogen cells or over parietal cells were few and seemed to be without any preferential localization. On control sections handled for autoradiography from 2 mice not injected with ^{3}H-DOPA, specific accumulation of silver grains over special cells or cell structures could not be seen.

The first cell type over which an accumulation of silver grains could be seen (Figures 1 and 3A) had round granules with a diameter varying from 150–250 nm and with a homogenous appearance, and a rather high electron opacity. The cells had a basal location in the epithelium of the deeper parts of the gastric glands without any contact with the gland lumen. The appearance of these cells is similar to cells in the mouse fundic mucosa described by HELANDER[5,6], and by him called argyrophil cells. Similar cells have also been described in several other species by other authors[7–12]. These cells show similarities with the α-cells of the pancreatic islets, and it has been proposed[10] that they might be the producer of the intestinal glucagon[14].

The second enterochromaffin cell type over which an accumulation of silver grains could be seen (Figures 2 and 3B) had 2 kinds of granules. Some of them were small (about 150 nm) containing a round or irregular osmophilic body surrounded by a clear space. Other granules were larger (300–600 nm) and seemed to lack content or contained only a small osmophilic body. Also these cells had a basal location in the deeper parts of the gastric glands and they seemed to lack contact with

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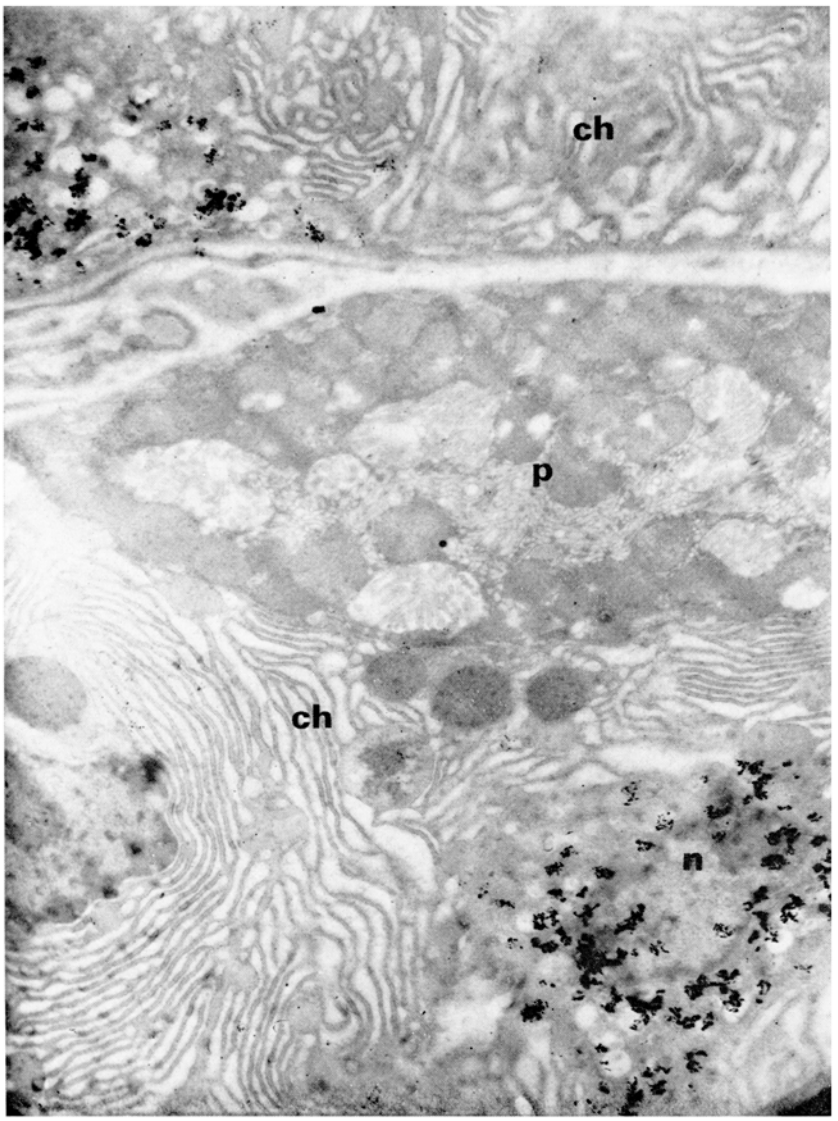

Fig. 2. Silver grains with a selective localization over the cytoplasm of 2 enterochromaffin cells of the second type. Nucleus (n) of an enterochromaffin cell; parietal cell (p); chief cells (ch). ×9,000.

the gland lumen. Cells similar to these have been described in the fundic mucosa of other species[10–12]. They show similarities with cells in the sympathetic trunk[15]. They might in part correspond to the cells which have been called 'enterochromaffin-like' by HÅKANSSON, LILJA and OWMAN[16] and they might then also contain histamin[17].

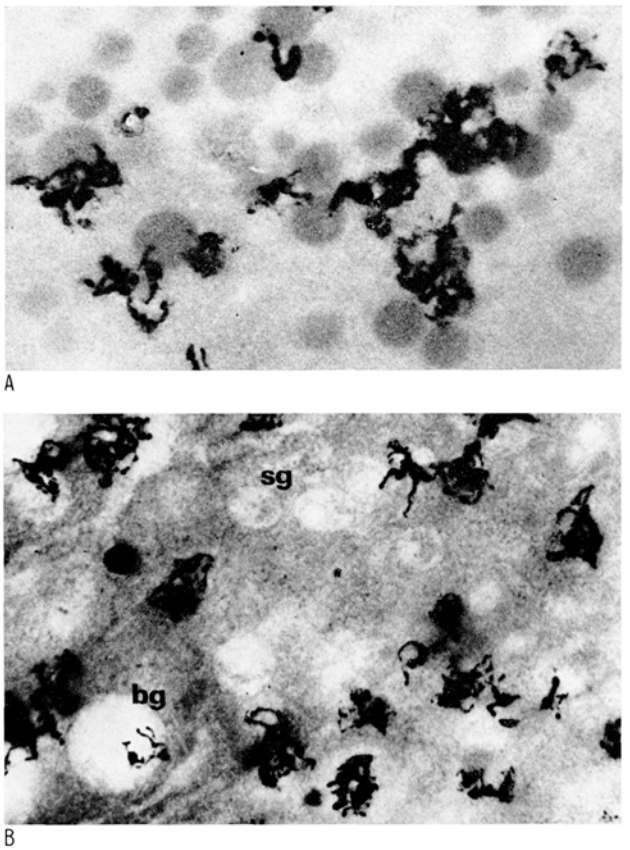

Fig. 3. A) Detail of the first enterochromaffin cell type showing the dense granules of a uniformly round shape. B) Detail of the second enterochromaffin cell type with the 2 types of granules. Small granule (sg); big granule (bg). The silver grains are seen in association with the granules in the cells. ×40,000.

Any contact with the gland lumen has not been observed in the 2 cell types described in the present investigation, or in most earlier investigations where the corresponding cells have been described[6,10–12]. From the morphological point of view, it therefore does not seem probable that they are the producers of the gastric antipernicious principle (the intrinsic factor) as has been proposed[18].

The classical enterochromaffin cell with endogenous 5-HT content is lacking almost entirely in the fundic mucosa of the mouse[16] and could not be observed in the present investigation. Other enterochromaffin cell types, with or without silver grain accumulation, could not be observed either. In the enterochromaffin cells observed, the silver grains were predominantly localized to the specific granules. 5-HT formed after 5-HTP-injections to mice have a similar localization over the granules in the parafollicular cells of the thyroid[19]. The monoamines, which in some species are stored endogenously in the pancreatic islets, and in the parafollicular cells, also have this granule-localization[20,21].

Zusammenfassung. Nach Injektion von ^{3}H-DOPA wurde bei der Maus im Fundus des Magens ultrastrukturell-autoradiographisch eine Markierung von zwei verschiedenen endokrinen Zelltypen festgestellt.

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Quantitative Studies on the Accumulation of Tetracycline in Tumors

Tetracycline fluorescence after oral or parenteral administration of this drug has been reported in a variety of human and experimental animal tumors[1–5]. An implicit assumption in these studies is that there is a selective concentration due to some special affinity of the tumors for the drug. A similar supposition in the case of porphyrin fluorescence of tumors was shown to be erroneous[6]. Proper quantitative studies supporting this assumption would provide a proper foundation for clinical studies and for the investigation of the mechanism involved.

We have examined by quantitative and qualitative methods the distribution of tetracycline fluorescence and the tetracycline concentration in a transplantable rat tumor system. Our conclusions are that the tetracycline concentration in several organs of the rat is considerably higher than that in the viable portions of the tumor, but that the tetracycline concentration is highest in necrotic areas of the tumors. Visible fluorescence in vivo depends upon the formation of a complex with free calcium ion that is abundant in the necrotic tumor tissue. Nonfluorescent tetracycline accumulates in other tissues.

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