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].

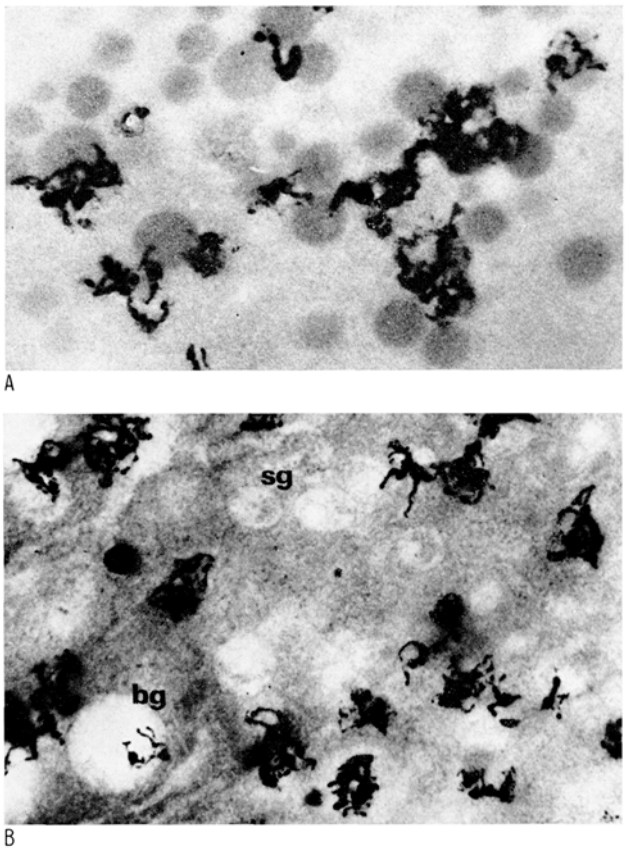

Fig. 3. A) Detail of the first enterochromaffin cell type showing the dense granules of a uniformly round shape. B) Detail of the second enterochromaffin cell type with the 2 types of granules. Small granule (sg); big granule (bg). The silver grains are seen in association with the granules in the cells.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

Sprague Dawley male rats weighing 150–250 g and bearing the Walker 256 carcinosarcoma were injected i.p. with 50 mg tetracycline/kg of body weight 2 and 3 days prior to sacrifice. Groups of rats were sacrificed 24, 48 and 72 h later.

The amount of necrosis in the Walker 256 carcinosarcoma increased with the age and the size of the tumor. Grossly visible tetracycline fluorescence was noted in viable and necrotic tumor, but appeared to be of greatest intensity in areas of early necrosis. Intense fluorescence was never observed in the actively growing margins of the tumor. Other tissues and visceral organs displayed essentially no visible tetracycline fluorescence. Ultraviolet microscopy revealed scattered focal areas of intense tetracycline fluorescence localized solely in necrotic portions of the tumor. No fluorescence was observed on microscopic examination of the other tissues.

Quantitative analysis of carefully separated portions of viable and necrotic areas of tumor uniformly revealed greater tetracycline concentrations in the necrotic regions (Table). Tetracycline concentration was determined in viable and necrotic portions of the tumor and several visceral organs including the liver, spleen and kidney. The quantitative spectrophotofluorometric determination of tetracycline was performed by a recently described technic[7]. The concentrations of tetracycline in the liver, spleen and kidney prove to be high compared to those found in the viable portions of tumor in the same animal. These values are only slightly lower than those found in the necrotic tumor tissue.

No gross or microscopic fluorescence was observed in areas of the tumor where hemorrhage had occurred. However, quantitative determination of tetracycline revealed higher concentrations of tetracycline in areas of hemorrhage than was present in several other tissues including viable tumor, where fluorescence was actually observed. This apparent discrepancy is partially attributable to the 'internal filter effect' of the blood pigments, which absorb the narrow band of the spectrum employed for exciting tetracycline fluorescence (excitation maximum 378 nm).

Abundant evidence already exists that complexing with calcium ion is necessary for tetracycline fluorescence in vitro[8] and it has also been suggested as contributory to fluorescence in vivo[9,10]. We, therefore, examined the role of ionic calcium in determining the apparent fluorescence of tetracycline in the tumor and other tissues.

A homogenate was prepared from portions of necrotic tumor which exhibited gross fluorescence in a pH 9.0 *Tris* buffer without calcium or barbital. The fluorescence was then measured quantitatively and compared with that of a homogenate of the same tissue at an identical concentration but prepared in a pH 9.0 *Tris* buffer solution to which disodium ethylenediamine tetracetate (EDTA) had been added in a final concentration of 0.063 molar. This concentration of EDTA effectively removes unbound calcium ion from solution. The amount of fluorescence observed in the necrotic tissue homogenate in buffer solution alone was significantly higher than that of the same homogenate prepared with buffer containing EDTA. This effect was reversable by the addition of excess calcium ion to the EDTA-containing homogenate, with restoration of fluorescence. Finally, the calcium concentration in necrotic and viable tumor tissue, which had been dried to constant weight and digested in concentrated nitric acid, was determined by atomic absorption spectrophotometry. Necrotic tumor had 8 times the calcium concentration of viable tumor; 6.65 vs 0.76 mg/g dry wt.[11].

Tetracycline concentration in various organs and experimental tumor in rats 24 h after parenteral administration of tetracycline

Rat No.	Tetracycline concentration (γ/g)					
	TC Dose mg	Liver	Kidney	Spleen	Tumor	
					Viable	Necrotic
1	7.5	8.00	10.60	4.40	5.40	18.10
2	3.0	10.75	12.80	–	3.60	13.20
3	5.0	9.80	8.90	–	3.50	12.79
4	12.5	6.95	9.52	4.48	2.16	
5	12.5	15.75	12.30	6.96	2.20	

Zusammenfassung. Quantitative Studien an Karzinomsarkomen von Ratten haben gezeigt, dass lebendes Krebsgewebe Tetracyclin nicht stärker anreichert als normales Gewebe.

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Monoamine-Containing Neurones in Cultures of Rat Brain Stem

Fluorescence microscopic studies have shown that monoamine-containing neurones and nerve terminals are widely distributed throughout the brain stem of the rat[1,2]. We were therefore interested to investigate whether monoamines are also present in brain stem neurones cultivated in vitro.

The explants were prepared from tissue of the medulla oblongata and pons of 2–4-day-old rats, and were grown on a plasma clot on cover slips. The cultures were kept at 37 °C in Falcon plastic tubes filled with nutrient medium consisting of Parker's TC 199, 5% fetal calf serum, 10% bovine serum, glucose and antibiotics[3].