Histochemically Demonstrable Catecholamines of the Newborn Rat Paraganglia after Heavy Hypoxia

The paraganglia are known to contain large amounts of catecholamines (COUPLAND[1], HERVONEN[2-4]) the role of which during fetal life still remains obscure. After the discovery of catecholamine-storing cells similar to the paraganglionic ones, within the sympathetic ganglia too (ERÄNKÖ and HÄRKÖNEN[5,6]), the function of the extra-adrenal catecholamine-storing elements has become a subject of wide discussion (JACOBOWITZ[7], ERÄNKÖ and ERÄNKÖ[8], HERVONEN[2]). The functions suggested for the catecholamine-storing cells, of the sympathetic ganglia, could be classified under 3 main titles: 1. Inhibitory interneuron function was first suggested by ECCLES and LIBET[9] and later this hypothesis has gained support in the ultrastructural studies (MATTHEW and RAISMAN[10]). 2. Endocrine function for the paraganglia had been suggested earlier by several authors (for ref. see HERVONEN[2]) and also demonstrated by BRUNDIN[11] and HERVONEN and KORKALA[12]. Whether the endocrine reactions of intraganglionic catecholamine-storing cells are similar to those of paraganglia or not has not been elucidated (JACOBOWITZ[7]). 3. The nihilistic concept of the functional insignificance of the intraganglionic catecholamine-stores was supported by NORBERG and SJÖQUIST[13] and HERVONEN[2]. To the present authors, it seemed interesting to know whether the catecholamine-storing elements of the rat, the paraganglia and the intraganglionic catecholamine-storing cells, are capable of releasing catecholamines in response to hypoxia or hypercapnia.

The litters of newborn rats were subjected to anoxic conditions in the normal state or after treatment with prednisolone (40 mg/kg for 5 days), which is known to prevent the involution of the rat paraganglia and even induce new catecholamine-storing cells within the ganglia (LEMPINEN[14], ERÄNKÖ and ERÄNKÖ[15]). The animals were kept under heavy hypoxia for 2 h. If respiration ceased, the atmosphere was ventilated and the rats were resuscitated. The preaortic tissue block containing both sympathetic ganglia and the paraganglia was removed and processed for fluorescence microscopy as described by HERVONEN[2].

The paraganglionic tissue, like the intraganglionic catecholamine-storing cells in the coelial ganglion, exhibited bright yellow intensive fluorescence in the animals treated with prednisolone. After hypoxia no changes were detected in the fluorescence intensity or its distribution in the paraganglia and surrounding sympathetic ganglion (ggl coeliacum). After the hypoxia, the colour and the fading velocity of the catecholamine fluorescence also remained unchanged.

The present results suggest that the catecholamines of rat paraganglia and the catecholamine-storing cells of the surrounding ganglia are firmly bound to the cells in

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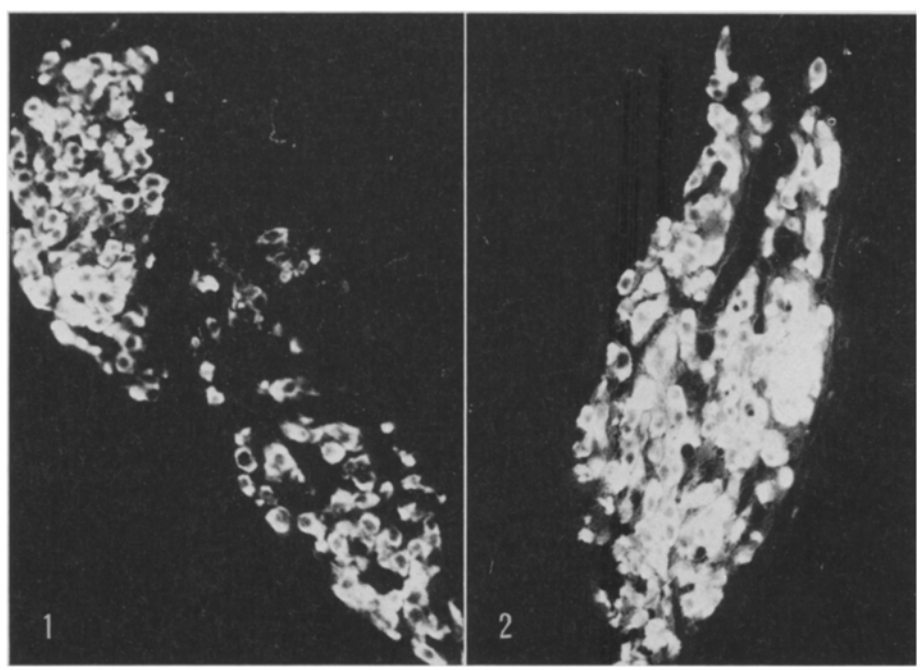

Fig. 1. The main abdominal paraganglion of a 1-week-old rat. The catecholamine-storing cells are all bright yellowish and fluorescent. The rat has received 40 mg/kg prednisolone for 5 days.

Fig. 2. The main abdominal paraganglion of a 1-week-old rat after heavy hypoxia for 2 h. The fluorescence intensity and distribution is essentially the same as in Figure 1. The rat has received 40 mg/kg prednisolone for 5 days.

conditions which release the amines from adrenal medulla (COMLINE[16]). VAN ORDEN[17] came to the same conclusion after trying to release the catecholamines with pharmacological agents. However, it should be pointed out that even extensive discharge of catecholamines from the tissue might escape detection if only observation by eye is used. Therefore, judging from the present results, discharge of an endocrine nature cannot be ruled out. The paraganglia of human fetus and newborn rabbit, on the other hand, react clearly to strong hypoxia (BRUNDIN[11], HERVONEN and KORKALA[12]). The catecholamine-storing cells induced by glucocorticoids might be different from normal ones with respect to the endocrine function.

Zusammenfassung. In den paraganglionären Zellen der neugeborenen Ratte wird die Catecholaminfluoreszenz durch schwere Hypoxie nicht beeinflusst.

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Quantitation of the Number of Villi and Crypts in the Intestine of Rodent Animals

In the studies of gastro-intestinal damage induced by radiation, many kinetic analyses[1–5] on the cell population in the epithelium of gut in rodent animals have been made on tissue sections, and it was proved that the epithelial cells were produced in the crypt, transfered upwards to the villus tip, and extruded into the lumen. The explanation has been gained through the studies of 2-dimensional relationship of the crypt to the villus. Accurate information is not always available concerning the 3-dimensional relationship of the crypt to the villus[6–10]. By the routine histological technique using paraffin, it might be rather difficult for the crypts and the villi to be fixed in the desired orientation, e.g., so as to run parallel with each other, so that accurate 3-dimensional relationships between them were not proved.

The present study was undertaken to examine the other from the routine histological technique to obtain accurate measurements of the respective number of crypts and villi per unit area of mucosal surface.

Experimental animals used were mice, rat, and golden hamsters, all 3-month-old adults. From each animal small intestines were removed, cut off at a desired length, and fixed in a 3:1 mixture of 70% ethanol and glacial acetic acid for 1 to 2 h, and then hydrolized in N-HCl[10] by means of standing at room temperature for 1 to 2 days.

The segments were opened by cutting along the long axis of intestine. The mucous membrane of the flattened segments was swept gently with a plastic or bamboo-made knife taking care that the lamina propria was not damaged. It is favorable that the edge of knife is dull. Sweeping was done in a pail continuously supplied with tap water. By such sweeping the epithelial cells of villi were almost completely removed leaving lamina propria and crypts. After sweeping the segment was placed in a Petri dish with a small quantity of water and covered gently with a thin leaden perforated plate.

Through the perforated area in the leaden plate, the crypts and the villi were photographed using a microscope equipped with water-immersion lenses of suitable magnification. Enumeration of the number of crypts and villi in unit surface of mucosa was performed in the photographs. Examples of such photographs are shown in Figures a) and

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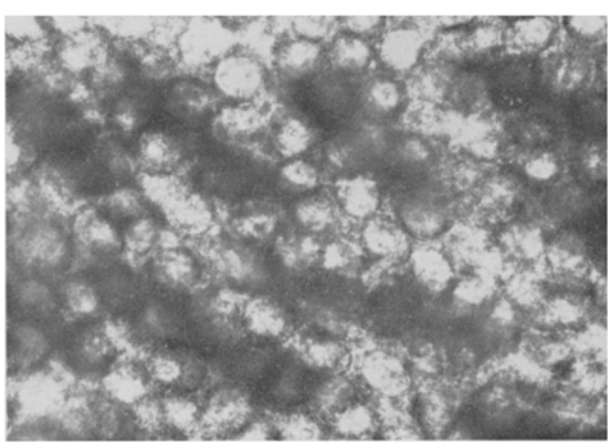
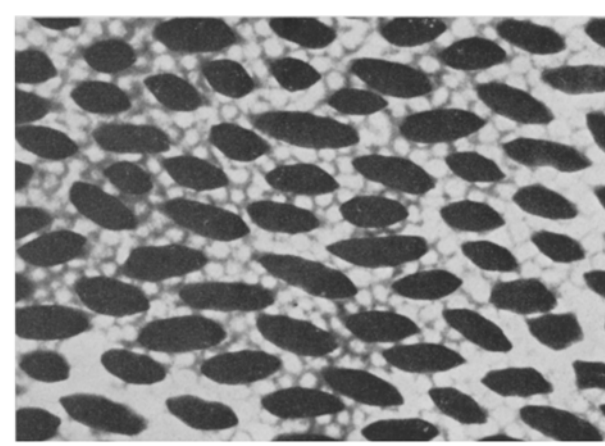


a) Crypts (× 100)



b) Villi (× 40)

Photographs of crypts and villi in intestinal mucosa of golden hamster