# Electron Microscopic Cytochemistry of 5-Hydroxytryptamine (5-HT) in the Beta Cells of Guinea Pig Endocrine Pancreas

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**ABSTRACT.** In order to localize 5-hydroxytryptamine (5-HT) stores in the islet of Langerhans of guinea pig pancreas, a cytochemical procedure for the ultrastructural differentiation between catecholamines and indoleamines was used. Islet tissue of animals receiving reserpine was similarly analyzed. The results obtained with the cytochemical reactions were correlated with those of pancreatic amine assays performed before and after reserpine administration. Dense reactive granules were observed in β cells of normal guinea pigs with the cytochemical procedures. These granules correspond to those normally observed in β cells and known to store insulin. After reserpine administration, the reactivity of the granules disappeared and simultaneously a marked decrease in pancreatic 5-HT content was observed. The possible participation of insulin was discarded in view of results drawn from *in vitro* experiments. Light and electron microscopic observations also suggested that the granular population of β cells, reflecting their insulin content, was not markedly affected by reserpine administration. The evidence obtained indicates that 5-HT present in β cells of guinea pig endocrine pancreas is localized within the same cytoplasmic granule in which insulin is stored. (*Endocrinology* **83:** 917, 1968)

IDENTIFICATION of the storage site of biogenic amines in some endocrine organs is of considerable importance in understanding the physiological significance of these compounds. Monoaminergic mechanisms have been described recently in the endocrine pancreas with the fluorescence histochemical method of Falck and Hillarp (1–4). Several mammalian species were found to store monoamines in their islets of Langerhans, and in those species in which no amines could be detected histochemically the mechanisms involved in monoamine synthesis and metabolism have been demonstrated following the administration of precursors of those compounds (3, 5).

The characteristics of the fluorescence normally observed in the endocrine pancreas of the adult guinea pig suggested the presence of a tryptamine derivative, probably 5-hydroxytryptamine (5-HT) in some islet cells (1). Results obtained combining fluorescence microscopy with differential staining indicated that the monoamine containing cells in guinea pig islets were β cells (2, 3). The distribution of fluorescence in fine cytoplasmic granules, and the previous findings that insulin is synthetized in β cells and stored in its characteristic cytoplasmic granules (6–8), raised the possibility of the coexistence of the amine and the hormone in the same cytoplasmic organelle (2).

To investigate this problem, the fine structural localization of 5-HT in the endocrine pancreas of the guinea pig was studied with a cytochemical reaction that differentiated between catecholamines and indoleamines at the electron microscope level (9). The effects of the administration of reserpine, a potent depletor of monoamine stores, on the cytochemical pattern exhibited by the islets as well as on the monoamine content of guinea pig pancreas were analyzed.

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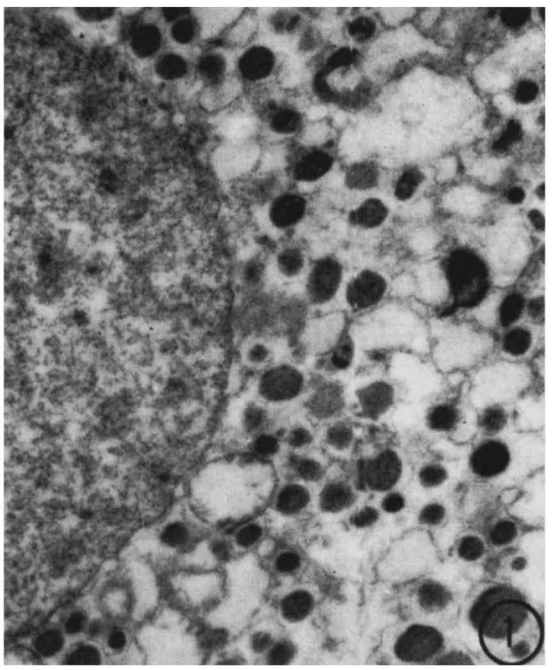

FIG. 1. Electron micrograph showing a portion of the cytoplasm of a $\beta$ cell from the endocrine pancreas of a normal guinea pig. The cell exhibits the characteristic granular population. (Karnovsky's fixative and osmium tetroxide, ×12,500.)

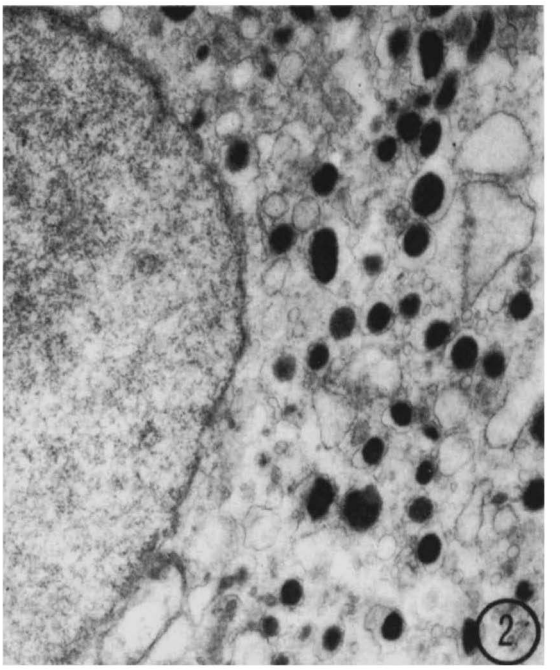

FIG. 2. A $\beta$ cell of a pancreatic islet from a guinea pig receiving reserpine. No marked alterations are observed in the characteristics of the granular population of the cell. (Karnovsky's fixative and osmium tetroxide, ×12,500.)

## Materials and Methods

Adult male albino guinea pigs receiving food and water *ad lib.* were sacrificed by decapitation and the pancreas excised. Animals receiving 1 mg/k of reserpine (Serpasil, Ciba) intraperitoneally during 4 consecutive days were similarly sacrificed 12 hr after the last injection. The pancreas was processed for electron microscopy or extracted for the determination of amines. Six groups of normal and 6 of treated animals, each consisting of 5 guinea pigs, were used in these experiments.

*Electron microscopy procedures.* Islet tissue was microdissected under a binocular magnifier (10) and processed according to the following schedules as previously described (9):

A. Karnovsky's fixative (11) containing glutaraldehyde-formaldehyde in phosphate buffer and post-fixed in 1.5% buffered osmium tetroxide during 90 min, followed by immersion in a 2% aqueous solution of uranyl acetate for 120 min;

B. 3% glutaraldehyde in 0.2M cacodylate buffer pH 7.2 for 4 hr. After washing in 0.15M sucrose in the same buffer, the blocks were transferred to a solution containing 2.5% potassium dichromate plus 1% sodium sulfate in 0.2M acetate buffer pH 4.1 for 4–24 hr. This procedure will be referred to as glutaraldehyde-dichromate (GD) technique;

C. 8% formaldehyde in 0.2M cacodylate buffer pH 7.2 for 24 hr. Washing, post-fixation in glutaraldehyde and treatment with potassium dichromate as in B. This procedure will be referred to as formaldehyde-glutaraldehyde-dichromate (FGD) technique.

All the above-mentioned procedures were carried out at 4 C. Blocks were dehydrated through graded series of ice-cold ethanol and embedded in Epon 812. Thin sections were examined without further treatment in a Siemens Elmiskop I electron microscope.

*Quantitative assay of amines.* Fluorometric amine assays were done according to the methods of Andén and Magnusson (12), Häggendal (13), Carlsson and Waldeck (14) and Green and Erickson (15) for 5-HT, noradrenaline (NA), dopamine (DA) and histamine, respectively. The fluorescence of samples was read in an Aminco Bowman spectrofluorophotometer and plotted by means of an X-Y recorder.

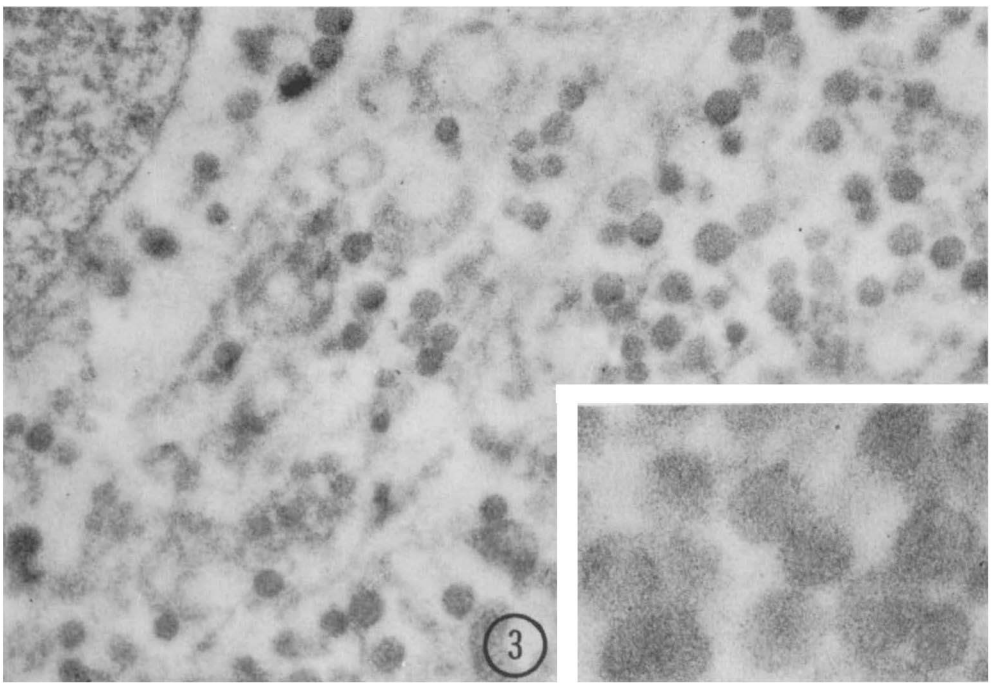


FIG. 3. Portion of the cytoplasm of a β cell of a pancreatic islet of a normal guinea pig, processed with the FGD (formaldehyde-glutaraldehyde-dichromate) technique. Dense reactive granules are observed scattered in the cytoplasm. (×20,000.) In the inset the granules displaying a positive reaction are seen, exhibiting a zone of increased density (×40,000).

In vitro *experiments*. *In vitro* test tube experiments were performed using 5-hydroxytryptamine creatinine sulfate ($5.5 \times 10^{-3}$M) and insulin (80 IU/ml, Eli Lilly & Co.). Both solutions were mixed with equal volumes of formaldehyde (0.03M) and glutaraldehyde (0.3M) both in 0.2M sodium cacodylate buffer pH 7.2 and allowed to stand 2 hr at 37 C. Similar experiments were made with glutaraldehyde alone, omitting the formaldehyde. A similar volume of 2.5% potassium dichromate solution in 0.2M acetate buffer pH 4.1 was added to the test tubes, which were left standing for another 24 hr at 37 C.

## Results

Pancreatic islets of normal guinea pigs fixed in Karnovsky's mixture and in osmium tetroxide exhibit the characteristic islet cell population (16, 17). Fig. 1 shows a portion of a β cell of a normal adult guinea pig in which the presence of numerous granules of different size and density may be observed. Following administration of reserpine, no marked changes were observed in the over-all appearance of the islet nor in the granulation of β cells, as is apparent in Fig. 2. The observation of normal islets processed with the GD or with the FGD procedures gives essentially similar images. Numerous reactive granules corresponding to those observed normally in β cell cytoplasm are seen (Fig. 3). A central area of increased density is apparent in some of these granules (Fig. 3, inset). No other reactive structures are observed either in the islets or in the surrounding acinar cells. Following the injection of reserpine, the density observed in β cell granules disappears, leaving clear structures, in many of which a central denser core is observed (Fig. 4). Similar results were obtained when the GD or FGD reactions were performed.

The concentration of biogenic amines was determined in normal pancreases and in pancreases of reserpinized guinea pigs. In order to assess the effectiveness of reserpine

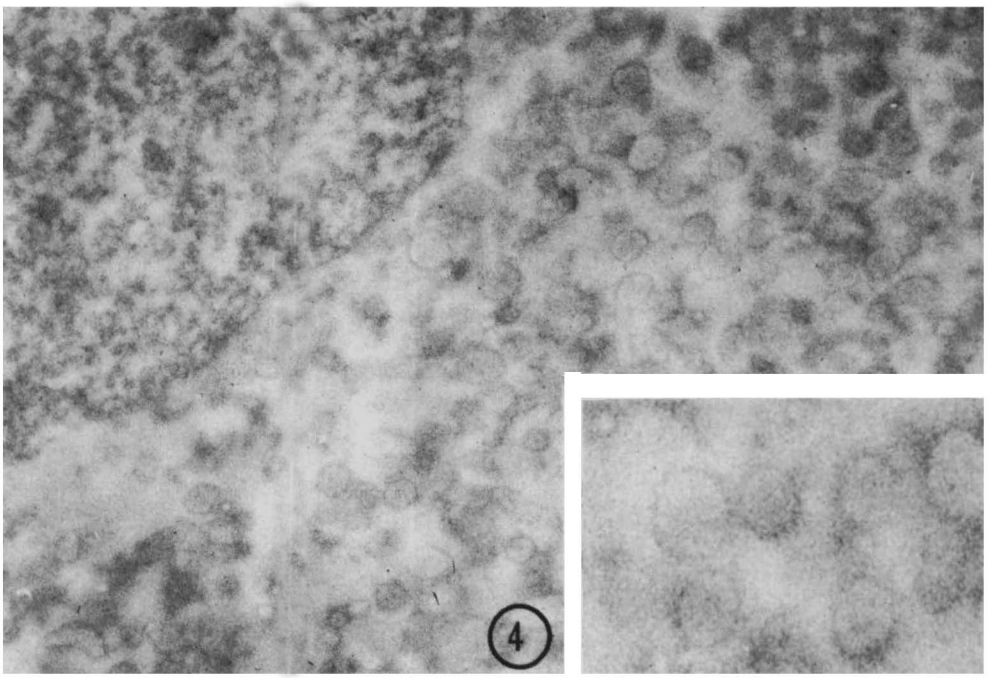

FIG. 4. After administration of reserpine, no reactive granules are observed in the cytoplasm of the $\beta$ cell when processed with the FGD technique, as in Fig. 3. ($\times$20,000.) In the remaining clear structures a central area of increased density is apparent (inset, $\times$40,000).

treatment in depleting 5-HT stores, changes in duodenal 5-HT content were analyzed after administration of the drug. As shown in Table 1, only 5-HT and NA diminished significantly in pancreatic tissue. The decrease of DA content is within the limits of significance. Histamine concentration showed no significant change. Duodenal 5-HT content was markedly diminished by reserpine, indicating that the treatment had been effective in lowering 5-HT concentration.

From *in vitro* experiments, it was concluded that, under the conditions in which the reaction was carried out, only 5-HT gave a metallic precipitate of chromium dioxide after the formaldehyde-glutaraldehyde-dichromate treatment. When the aldehyde was added to the 5-HT solution, a yellow-pink precipitate was immediately formed, indicating the presence of a Schiff monobase (18), whereas in the tube containing insulin a noncolored turbidity was formed, presumably reflecting the action of the aldehyde on the protein. Upon addition of dichromate solution, a brownish-black precipitate was immediately formed in the 5-HT containing tubes, while no reduction was observed in the insulin containing ones (Fig. 5). Similar results were obtained when formaldehyde was omitted and the conditions of the GD reaction (*i.e.*, treatment with glutaraldehyde and dichromate) were reproduced.

## Discussion

The positive reaction given by granules of $\beta$ cells of guinea pig islets with the glutaraldehyde-dichromate (GD) technique indicates that the density observed after GD staining is determined by the presence of an unsubstituted catecholamine or indoleamine (9, 18). Previous studies performed with light microscopy demonstrated that following fixation with formaldehyde prior to chromation no reaction product is present in catecholamine containing structures, while the reaction displayed by struc-

TABLE 1. Content of biogenic amines in guinea pig pancreas and duodenum before and after administration of reserpine (values are expressed in μg per g wet weight ±SE)

| | Controls | Reserpine | % | p* |
|---|---|---|---|---|
| *Pancreas* | | | | |
| 5-Hydroxytryptamine | 0.461 ±0.072 | 0.030 ±0.008 | −93.5 | <0.001 |
| Noradrenaline | 1.215 ±0.059 | 0.138 ±0.028 | −88.6 | <0.001 |
| Histamine | 2.492 ±0.504 | 2.785 ±0.130 | +11.7 | 0.4–0.3 |
| Dopamine | 0.394 ±0.077 | 0.231 ±0.050 | −38.8 | 0.1–0.05 |
| *Duodenum* | | | | |
| 5-Hydroxytryptamine | 6.60 ±0.08 | 0.54 ±0.02 | −91.8 | <0.001 |

* Significances of differences between groups were determined by Student's *t* test.

tures containing 5-HT is not affected (19, 20). The specificity of this reaction that we have recently introduced to the ultrastructural differentiation between catechol and indol compounds has been analyzed in isolated blood platelets (9). The finding that similar images to those observed with the GD reaction could be seen in islet cells after this procedure indicates that 5-HT is present within β cell granules. The disappearance of reactive material following treatment with reserpine confirms this assumption and is in line with the marked drop of monoamine content in the pancreas after administration of the drug. In spite of the fact that amine assays were performed on the whole pancreas, of which the islet cell mass represents a small percentage, the depletion of 5-HT obtained with reserpine treatment must be considered as complete because the fluorescence readings of the treated organs in the biochemical assay were very close to those of the corresponding tissue blanks. These observations confirm that all the possible sources of 5-HT within the organ have been effectively depleted by reserpine administration. Several pieces of evidence support the assumption that 5-HT is localized in the endocrine pancreas. The fluorescence demonstrated with the technique of Falck and Hillarp in islet β cells of guinea pig pancreas disappears following the administration of reserpine (2, 3). The 5-HT content of the pancreas decreases with the administration of sulfonylureas, while other stores of 5-HT remain unaffected, suggesting that the amine is localized in the islet cell mass of the organ (3). In pancreases of species not normally containing fluorescent islet cells such as the mouse and the rat, there appear to be low concentrations of 5-HT (unpublished observations). Moreover, the mast cells, which contain monoamines in some species, can be ruled out as a possible source of amines in the assay performed on the whole organ because they do not store monoamines in the guinea pig (21).

However, the presence of a catecholamine, such as DA, together with 5-HT in the same islet cell must be considered in view of results showing the presence in β cells of the mechanisms responsible for the synthesis and storage of both amines (3, 5). Also, the recent finding that islet cells in newborn and fetal guinea pigs probably store both 5-HT and DA makes this possibility worthy of consideration (4). We

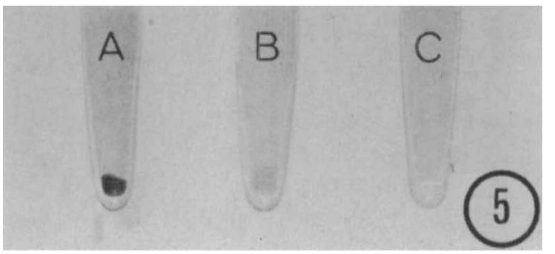


FIG. 5. The test tubes observed in this photograph contain similar volumes of formaldehyde, glutaraldehyde and dichromate solutions. Tube A shows the metallic precipitate given by 5-HT solution upon addition of these reactives, tube B the non-metallic precipitate of insulin, while in tube C, in which no additions were made, no precipitate is observed.

have recently demonstrated the coexistence of NA and 5-HT in the same granulated vesicle in the autonomic nerve endings in the rat pineal gland using a cytochemical approach similar to the one used here (22). Although marked differences exist between amine pools of various organs, it is of interest to consider that an interchange of amines in the storage granules similar to that demonstrated by Bertler *et al.* (23) in adrenomedullary cells may be operating in islet β cells. Since reserpine administration diminishes both pancreatic catecholamines and indoleamines, the presence of another monoamine together with 5-HT in the granules of monoaminergic islet cells cannot be determined at present. Combined pharmacological and cytochemical studies are being conducted with drugs that selectively interfere with or enhance the synthesis of different monoamines.

The possibility that insulin may be responsible for the reaction observed with GD or FGD procedures in normal islets can be ruled out in view of results from *in vitro* experiments showing that insulin fails to give a metallic precipitate in conditions similar to those in which 5-HT gives a strong reaction. The results of control preparations of pancreatic tissue stained with aldehyde-fuchsin performed during these experiments, as well as those of the electron microscopic observations here reported, coincide in demonstrating that the granular population of β cells is not markedly affected by reserpine treatment. Since these granules roughly reflect the insulin content of those cells (7), it can be assumed that the drug does not produce a marked change in pancreatic insulin content under conditions in which amine depletion is maximal. This provides further support to the assumption that the lack of reaction product following reserpine observed in β cell granules is due to the decrease of amine content.

The finding that tolbutamide releases stored insulin and degranulates β cells (7), and also decreases pancreatic 5-HT content (3), gives strong support to the hypothesis that both substances are contained within the same granule. Recent experiments also indicate that insulin has a high binding capacity for 5-HT *in vitro* (Håkanson and Iturriza, personal communication). Studies now being carried out in our laboratory indicate that in islet cells of the mouse, in which no amines can be normally demonstrated, the administration of precursors of amine synthesis results in the formation of amines that are stored in the β cell granule, as revealed by the positive cytochemical reaction observed under the electron microscope in the otherwise nonreactive granules.

The present results demonstrate that the 5-HT contained in the β cell of the islet of Langerhans of the guinea pig is stored within the same granule known to store insulin. It remains to be determined if all the granular population of the β cell behaves similarly with regard to both components and if the morphological variations observed between the β cell granules (24) reflect different storage properties. The capacity to store and synthesize 5-HT has been considered a common mechanism in some endocrine cells producing a polypeptide hormone (25). In the parafollicular cells of the thyroid gland of the sheep, which are known to store 5-HT, we have localized the amine within the same organelle considered as the storage site of thyrocalcitonin (26). These cytochemical results as well as those discussed here demonstrate that the amine and the polypeptide hormone coexist not only in the same endocrine cell but in the same organelle in which the hormone is stored. However, further studies are needed to elucidate the significance of this suggestive association.

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