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EFFECT OF SECRETORY ACTIVITY OF THE SALIVARY GLANDS ON MORPHOLOGY AND FUNCTION OF THE PANCREATIC ISLETS

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Chronic experiments on dogs showed that the loss of saliva by the animals through exteriorized ducts of the parotid, submandibular, and sublingual salivary glands causes morphohistochemical changes in the pancreatic islets indicative of reduced functional activity of the β cells.

KEY WORDS: *Salivary glands; islets of Langerhans; β cells of islets.*

The endocrine function of the salivary glands has for a long time attracted the attention of investigators [9, 13, 14]. One aspect of its influence on the body is its role in the regulation of carbohydrate metabolism [1, 2, 10]. It recently became known that not only the endocrine but also the exocrine activity of the salivary glands influences the state of homeostasis [4, 5]. The writers showed previously that loss of saliva by animals through ducts of the salivary glands exteriorized by Glinskii's method and through esophageal fistulas constructed from metal T-tubes is accompanied by lowering of the organism's tolerance to exogenous glucose and by the appearance of a negative Staub–Traugott effect after administration of a second dose of sugar [6].

In the investigation described below the effect of removal of the products of secretory activity of the parotid, submandibular, and sublingual salivary glands on the morphology and functional state of the islet–cell system of the pancreas was studied.

EXPERIMENTAL METHOD

In experiments on 22 male dogs weighing from 8 to 12 kg the ducts of the parotid, submandibular, and sublingual salivary glands were exteriorized by Glinskii's method so that their saliva was lost. The animals were divided into three groups with five dogs in each group: I) dogs losing saliva for 1 month (from the day of operation), II) for 3 months,

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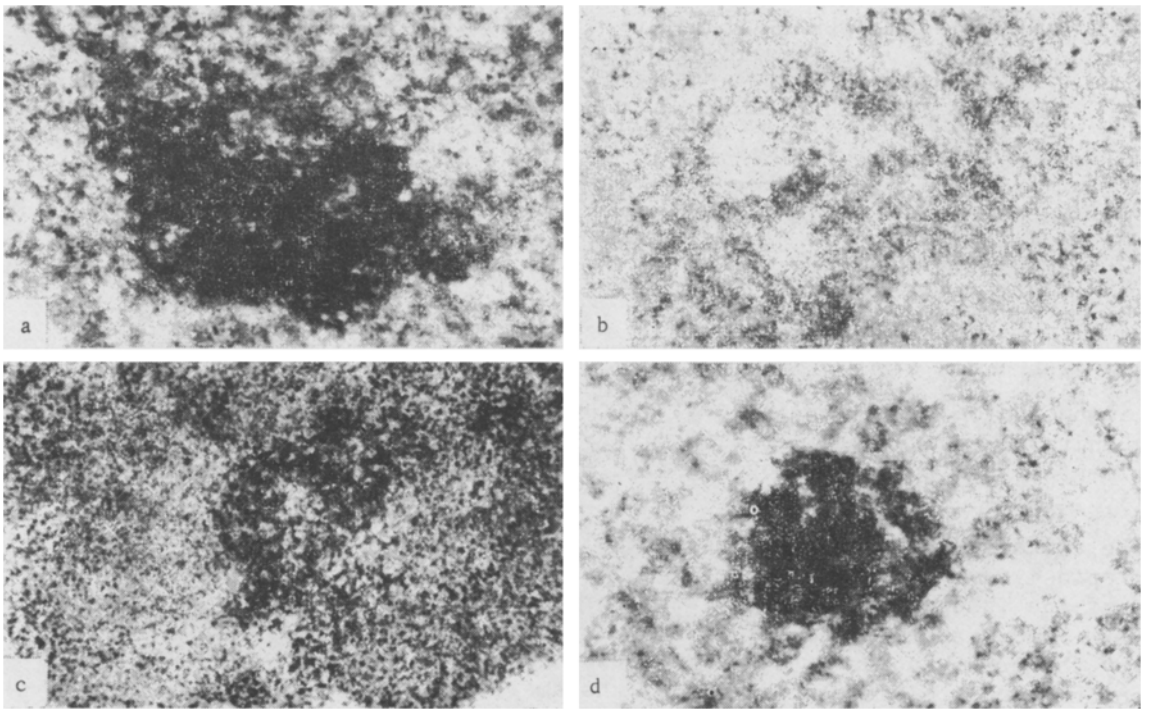

Fig. 1. Dog pancreas, islet of Langerhans. G6PD activity: a) activity of enzyme in islet of control animal; b) sharp decrease in activity of enzyme in islet cells of animal of group I; c) some increase in enzyme activity in islet of group II animal; d) restoration of normal enzyme activity in islet of group III animal.

and III) for 6 months. The control group consisted of seven animals, in three of which the tissues in the region of the salivary glands were divided and sutured under pentobarbital anesthesia. The free plasma insulin level of the experimental and control animals was determined by an immunoradioactive method [15] three times during the last week of the experiment, before and 30 min after administration of glucose (1.85 g/kg body weight). Functional stabilization of the pancreas was produced by starvation for 24 h with no restriction on fluid intake. The dogs were killed by destruction of the medulla at the same time of day (10 a.m.).

Pieces of tissue from all parts of the pancreas were fixed in 10% neutral formalin, Bouin's fluid, and acetone. Sections 5-7 μ thick were stained with Ehrlich's hematoxylin and eosin, specific granules (of insulin) were detected with aldehyde–fuchsin and counterstaining by Halmi's method, and zinc by the dithizone method [3]. Activity of acid phosphatase (by Burstone's azo-coupling method), cytochrome oxidase (CO), succinate dehydrogenase (SD), hexokinase, and glucose-6-phosphate dehydrogenase (G6PD) also was determined.

The density of the aldehyde-fuchsinophilic granules was assessed on a four-point system [8], and the number of islets in a standard area of section in the splenic part of the pancreas was counted. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Examination of sections of the pancreas from the group I animals showed perivascular edema of the islet tissue, vacuolation of insulocytes, peripheral condensation of chromatin, and pycnosis of nuclei. Acid phosphatase activity in the islet cells was lower than normal. Hexokinase activity was depressed: The enzyme was discovered as fairly large, infrequent granules of monoformazan, whereas normally dark blue diformazan granules, closely packed together, are found in the cytoplasm of the insulocytes. Activity of CO, SD, and G6PD in the islet cells also was lower than normal (Fig. 1a, b).

The density of zinc and insulin granules in the β cells of the experimental animals is shown in Table 1.

TABLE 1. Estimation of Density of Zinc and Insulin Granules in Pancreatic Islet Cells of Dogs Losing Saliva

Animals	Zinc	Insulin
Control	++++	+++-
Experimental		
Group I	±	+
Group II	++	+++
Group III	+++	++++

The immunoradioactive insulin level in the blood plasma of the control animals was 15 ± 2.09 microunits/ml before sugar loading and 59 ± 2.14 microunits/ml thereafter, whereas in the experimental dogs the corresponding figures were 5.2 ± 1.07 and 16 ± 1.29 microunits/ml ($P < 0.01$).

In the animals of group II, besides insulocytes in a state of degeneration (vacuolation of the cytoplasm, pycnosis of the nuclei), cells with hyperchromic nuclei appeared at the periphery of the islets. Islets consisting of two or three cells, all β cells, were seen in large numbers, and transformation of periinsular pancreatocytes into islet cells was observed, as shown by the accumulation of aldehyde–fuchsin granules in their cytoplasm; the formation of insulocytes from proliferating cells of the small efferent ducts of the pancreas also was noted. Activity of the above-mentioned enzymes was higher in the β cells of the animals of group II than in those of group I (Fig. 1c), but it did not reach the control level.

In the animals of group III many insulocytes were normal in structure. There was a statistically significant increase in the number of islets per standard area of section: 2.25 ± 0.1 in the control and 5.66 ± 0.42 in experimental group III. CO and SD activity in the cytoplasm of the insulocytes was a little higher than in the control, and acid phosphatase, hexokinase, and G6PD activity in most cases was back to normal (Fig. 1d).

Under these experimental conditions changes were thus found in the insulocytes (hydropic degeneration, a sharp decrease in the zinc and acid phosphatase content in the cytoplasm of the β cells) which, according to Toroptsev and Eshchenko [7], are evidence of depression of their functional activity. Meanwhile, the activity of hexokinase, an enzyme phosphorylating glucose and responsible for transporting polysaccharide from the blood stream into the cell [11], was reduced in the islet cells. A deficiency of phosphorylated glucose leads to inhibition of all pathways of its utilization, including the pentose phosphate shunt, which has a leading role in insulin synthesis [15]. After loss of saliva for 1 month the free insulin concentration in the blood plasma and the density of insulin granules in the β cells were both reduced.

The lowering of tolerance of the body to exogenous glucose, observed in the writers' earlier experiments [6], is accompanied by hypertrophy of the islet system, manifested by the formation of new insulocytes from terminal exocrine structures and cells of the efferent ducts, the appearance of numerous islets consisting only of β cells, and an increase in the activity of acid phosphatase, hexokinase, and enzymes of the Krebs cycle and pentose phosphate shunt. These processes were well marked in the group II animals and evidently lead to an increase in the number of islets and β cells, such as was found in the animals of group III. The morphohistochemical picture of the islets in this group of experimental animals indicated that they were functioning intensively (increased activity of enzymes of the Krebs cycle compared with normal) but not completely efficiently, for the density of zinc granules in the insulocytes was below the control level.

It can be concluded from these results that deviation of the saliva of the parotid, submandibular, and sublingual glands from the gastrointestinal tract leads to marked histological structural changes in the islet–cell apparatus of the pancreas, indicating a disturbance of insulin synthesis by the β cells, which was evidently the cause of the changes in carbohydrate metabolism of the experimental animals.

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IMMUNOHISTOCHEMICAL DETECTION OF THE LOCALIZATION OF MELATONIN AND N-ACETYLSEROTONIN IN ENTEROCHROMAFFIN CELLS

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Melatonin synthesis was identified by an immunohistochemical method with specific antisera against melatonin and N-acetylserotonin in the enterochromaffin cells of the gastrointestinal tract. It is considered that enterochromaffin cells, together with the pinealocytes of the pineal gland and other cells synthesizing melatonin in the retina and cerebellum, form a group of melatonin-producing cells with an important role in the maintenance of homeostasis.

KEY WORDS: *Melatonin; enterochromaffin cells; specific antisera.*

The important role of melatonin in the regulation of metabolic processes has recently been established [4, 7, 8].

Until recently the pineal gland has been considered to be the only organ synthesizing this hormone [9, 14], although it was not quite clearly understood how the relatively small quantity of melatonin produced in the pineal could maintain the course of metabolism at a high enough level.

Recent investigations have questioned the previous view of the leading role of the pineal in melatonin production [10, 11].

Since serotonin is essential for melatonin synthesis [8], the suggestion has been made that melatonin can be formed in the enterochromaffin cells of the gastrointestinal tract

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