binding, and intracellular reception of these preparations. Since the neuropharmacologic drugs used in this investigation are known to specifically disturb regulatory functions of "prenerve" transmitters (monoamines), participation of endogenous cyclic nucleotides in realization of the functions of biogenic monoamines can be postulated in early sea urchin embryos. The unique character of this participation, compared with that established for cell differentiation in multicellular animals is that both biogenic monoamines and cyclic nucleotides exert their regulatory functions within the same cells.

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CHANGES IN SUBMAXILLARY GLANDS AFTER ISOPROTERENOL INJECTION INTO INTACT AND DEPANCREATIZED MICE

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After injections of the β-adrenomimetic isoproterenol (IP) into rats and mice an increase in weight of the submaxillary glands (SG), hypertrophy of the acinar cells and, in many cases, a decrease in size of the cells in granular zones of the salivary tubules are observed [1, 11, 15]. The character of changes in SG cells depends on the doses of IP, times after injection, age of the animals, and other factors [1]. In healthy animals injections of IP have also been shown to stimulate secretion of B cells of the pancreatic islets [12]. After intravenous injection of IP into diabetic patients the plasma immunoreactive insulin level rises [10, 14]. The question arises whether the formation of insulin-like proteins (ILP) of nonpancreatic nature is stimulated by injection of IP. It has been shown that SG of animals and man contains ILP [4-9]. In organ culture of mouse SG, synthesis of ILP in these organs has been found [2, 3]. The writers have postulated that injections of IP, inducing hyperfunction of SG, can also intensity production of ILP in them, and in diabetes, this can lead not only to in-

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TABLE 1. Relative Weight of Mouse SG (P = 95%)

Series of experiments	Experimental conditions	Ratio of weight of pair of glands (in mg) to animal's body weight (in g)
Control	intact animals	4,56±0,7
I	Injection of IP	5,95±1,3
II	Depancreatization + injection of IP	7,02±0,9
III	Depancreatization	4,99±1,0

TABLE 2. Areas of Cells of Mouse SG (P = 99%)

Test object	Areas of granular and acinar cells, μ^2	Changes in areas of cells, percent of control
Acinar cells of		
intact mice	248	
depancreatized mice receiving IP	370	49,1
depancreatized mice	279	12,5
Cells in granular zones of		
intact mice	451	
depancreatized mice receiving IP	394	—12,6
depancreatized mice	472	4,7

creased synthesis, but also to increased extrusion of this protein, which can be used for partial compensation of insular insufficiency. To test this hypothesis, the investigation described below was undertaken with the following aims: to discover whether the relative weight of SG and area of different types of SG cells increase during injections of IP into mice, and during the action of IP against the background of diabetes caused by depancreatization; to study ultrastructural changes in the mouse SG reflecting their functional activity under the above-mentioned experimental conditions; to discover how the concentration of immunoreactive ILP (IRILP) in SG changes under the same experimental conditions.

EXPERIMENTAL METHOD

Submaxillary glands of male CBA/C57 black mice weighing 20-23 g were used. In the experiments of series I the mice were given an intraperitoneal injection of IP in a dose of 23 mg/100 g body weight. These animals were killed on the 6th day after injection. Total depancreatization was performed on some of the mice. Animals subjected to this operation were divided into two groups after 1 week.

Mice of one group received an injection of IP in the above dose (series II of experiment) and the animals were killed on the 6th day after injection (i.e., on the 13th day of the experiment). Animals of the other group received no further treatment (series III). These animals also were killed on the 13th day of depancreatization. Glands of intact mice served as the control. Before sacrifice all mice were deprived of food for 24 h.

SG at the light-optical level was studied in material fixed by Carnoy's method, embedded in paraffin wax, sectioned, stained with hematoxylin and eosin. Material for electron-microscopic study was fixed in 2.5% glutaraldehyde solution and postfixed in 1% OsO_4 solution. Dehydration of the tissues and embedding in Epon were carried out by the standard method. Ultrathin sections were stained with lead citrate. For radioimmunochemical assay of IRILP in SG, the protein was extracted beforehand from SG by the method [13] devised for obtaining insulin from the pancreas. Dried extracts were diluted 2-4 times and used for determination of their IRILP content by RIA kits (Hungary) for radioimmunochemical detection of insulin. The IRILP concentration was expressed in microunits/g wet weight of SG.

EXPERIMENTAL RESULTS

Determination of the relative weight of SG (ratio of weight of the gland in milligrams to body weight of the mice in grams) under experimental conditions yielded results (Table 1) indicating that after depancreatization or injection of IP there is a tendency for the weight of SG to increase, whereas depancreatization followed by injection of IP into mice leads to statistically significant increase in the relative weight of these organs by 46.1% (P = 99%).

Measurement of the areas of SG cells showed that under different experimental conditions the areas of cells of the acini and granular ducts changed in different ways (Table 2). The areas of the acinar cells increased after depancreatization and after injection of IP against the background of diabetes. Areas of cells of the granular ducts increased only slightly after depancreatization, and after injection of IP into diabetic mice they actually decreased.

The increase in area of the acinar cells after depancreatization can be attributed to intensification of synthesis of digestive enzymes necessary to compensate for the absence of pancreatic enzymes. Accumulation of secretion in the acinar cells, leading to an increase in their size, also is linked with the state of hunger of the mice before sacrifice, when there were no stimuli for extrusion of secretion. The increase in areas of cells of the granular ducts after depancreatization can also be explained from the standpoint of their participation in external secretory (digestive) function, inhibited by hunger. Meanwhile, the less marked hypertrophy of these cells can be connected with the continued (or even intensified by injection of IP) incretion, or secretion followed by incretion, of certain hormonal factors whose release is unconnected with food stimulation. In the writers' view, after depancreatization there is increased incretion of ILP. Injection of IP against the background of diabetes evidently induces such active release of biologically active substances (including ILP) from the granular ducts that their areas decrease appreciably.

Electron microscopy revealed a basal arrangement of the Golgi complex (GC) in cells of the granular ducts of the control and experimental animals, indicating the possibility of basal release of secretion by these cells. In the control animals the rough endoplasmic reticulum (RER) in these cells had the appearance of spherical cisterns. The secretory granules were discrete in character, and large electron-dense granules were more frequent than more electron-transparent granules. After depancreatization dilatation of the cisterns of RER was observed, so that they often appeared like confluent irregularly shaped lacunae; GC also underwent hypertrophy. The perinuclear spaces widened. These changes are evidence of intensification of secretion formation in cells of the granular ducts. After injection of IP similar changes were observed, evidence of hyperfunction of the protein-synthesizing system in these areas. Those cells of the granular ducts which belonged to the "dark" category (with a more electron dense matrix than in "pale" cells) showed particularly marked changes in this situation. After injection of IP into depancreatized mice, GC and RER underwent very marked hypertrophy. Often the perinuclear spaces were greatly widened, so that the outer nuclear membrane formed large, irregularly shaped outgrowths. The secretory granules frequently merged with each other and many small granules appeared. The apical parts of many cells of the granular ducts swelled and opened into the lumen of the ducts together with their secretion. It could often be seen that not only mature secretory granules and secretory products fused into a single mass passed into the lumen, but also dilated cisterns of RER. Some cells under these circumstances were partly or completely destroyed. Predominance of apocrine and holocrine secretion in the last series of experiments can evidently explain the decrease in area of cells of the granular ducts. Absence of hypertrophy of these cells was thus evidence not of their weak secretory activity, but of the converse.

The question of in which direction hormonal products are secreted from cells of the pancreatic gland ducts is still undecided and is under discussion in the literature [15]. In our view both endocrine and exocrine pathways of release of hormones, including ILP, are possible. During stimulation by IP, extrusion of ILP into the saliva is evidently intensified.

Determination of IRILP in extracts of SG (Table 3) on the one hand confirmed previous data showing a marked decrease in the ILP content in SG under diabetic conditions [5-7, 9] and on the other hand showed that after injection of IP the IRILP content in the glands rises to almost twice that found in the control and three times that observed in the glands of pancreatectomized mice. The considerable increase in the IRILP content in mice after injection of IP can be explained by intensification of synthesis by IP accompanied by limited release, on account of absence of the physiological need for an excess of ILP in healthy animals. The

TABLE 3. Radioimmunochemical Assay of IRILP in SG of CBA/C57 Black Mice

Group of animals	Quantity of substance, in micro-units/g wet weight	Level of significance, percent
Intact	685±50	
Depancreatized	302±66	99
Injected with IP	1125±125	99
Depancreatized and injected with IP	914±120	89

somewhat reduced IRILP content in SG of depancreatized mice receiving injections of IP (compared with that in the glands of animals receiving IP alone) was evidently linked with its active extrusion and increased production, as is confirmed by electron-microscopic observations of hypersecretion by cells of the granular ducts.

After injection of IP synthesis and accumulation of ILP in cells of the granular ducts of the submaxillary glands of mice are thus activated, whereas injections of IP under diabetic conditions stimulate both increased synthesis and increased extrusion of ILP. Injection of IP into diabetic animals evidently enables a stronger compensatory response of SG through increased synthesis and release of ILP, thus reducing to some extent the deficiency of hypoglycemic factors in the body.

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