IDENTITY OF ZINC- AND INSULIN-CONTAINING GRANULES IN CELLS OF THE ISLETS OF LANGERHANS

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Dithizone and aldehyde-fuchsin granules are located in the same (secretory) parts of the B cells of the pancreatic islets, but the content of each varies differently following administration of glucose and of sodium diethyldithiocarbamate and in the course of development of diabetes. Zinc and insulin, although located in the same structures of the B cells, are evidently incorporated into different systems.

If the B cells of the islets of Langherhans are strained with aldehyde-fuchsin and dithizone, two types of granules are revealed. The first reflects the insulin content of the cells [4], and the second the zinc content. The problem of the identity of these two types of granules remains unsolved: data in the literature are indirect and conflicting [5, 7]. To obtain direct evidence, the method of comparing several histochemical reactions in the same section through the pancreas was used.

EXPERIMENTAL

Experiments were carried out on 35 rabbits, 6 rats, 9 mice, 5 cats, and 6 dogs.

Diabetes was produced in the rabbits by intravenous injection of dithizone in a dose of 50 mg/kg body weight as a 1% solution in aqueous ammonia. The criterion of diabetes was persistent hyperglycemia, detected by repeated determination of the blood sugar by the Hagedorn–Jensen method.

Five rabbits received intravenous injections of a 10% aqueous solution of sodium diethyldithiocarbamate in a dose of 500 mg/kg, while another five received 10 g/kg glucose as a 40% solution (two rabbits were given a single injection, and three received 6 separate infusions of the same dose at intervals of 4 h). Healthy intact rabbits and rabbits receiving the same volume of physiological saline acted as controls.

The animals were sacrificed 1 h after receiving the injection of glucose, 30 min after injection of carbamate, and 24 h and on subsequent days after injection of dithizone. The pancreas was removed, fixed by Timm's method (in 70% ethanol saturated with H_2S), and in Bouin's fluid. The histochemical reaction for zinc was obtained by treating sections of a pancreas stained by Timm's method by means of a 0.2% solution of dithizone in aqueous ammonia and a 0.01% acetone solution of 8-(p-toluenesulfonylamino)-quinoline [1]. Staining with aldehyde-fuchsin was carried out on sections of the pancreas fixed by both methods. The specific reaction for insulin consisted of treatment of sections from the pancreas fixed in Bouin's fluid by means of pseudoisocyanin as described by Schiebler and Schiessler [6]. For comparison of these two reactions the following combinations were used on the same section: quinoline fluorescence with dithizone, dithizone with aldehyde-fuchsin, pseudoisocyanin with aldehyde-fuchsin. A and B cells in the islets were differentiated in control adjacent sections stained with hematoxylin-phloxine [2].

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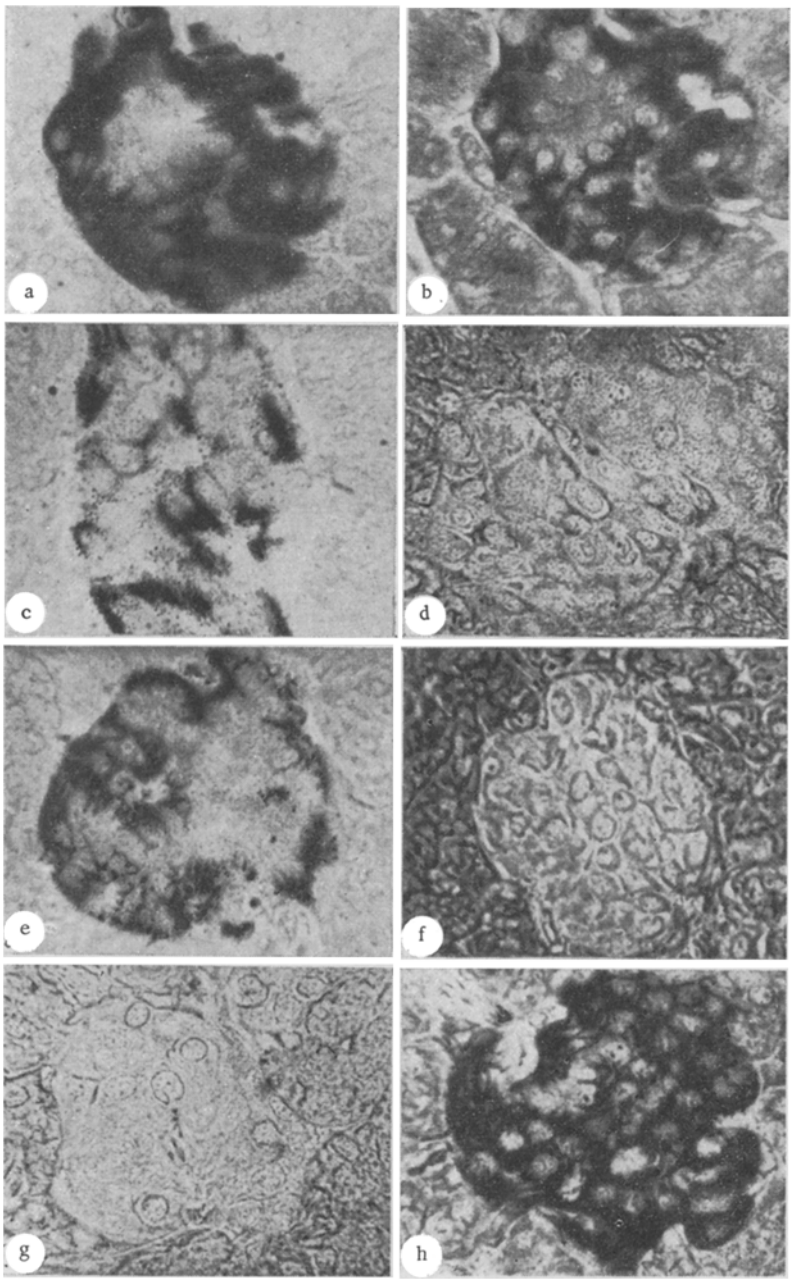

Fig. 1. Comparative characteristics of state of dithizone and aldehyde-fuchsin granules in islets of Langherhans of rabbits under different experimental conditions. a) Marked dithizone reaction in islet of healthy rabbit; b) same islet stained with aldehyde-fuchsin: granules located in same parts as when stained with dithizone; c) dithizone reaction persisted in islet after six injections of glucose; d) total degranulations of islet of the same rabbit detected after staining with aldehyde-fuchsin; dithizone granules remained only at the periphery of the islet 24 h after injection of dithizone; f) total degranulation of islet of the same rabbit detected by staining with aldehyde-fuchsin; g) negative dithizone reaction in islet of a rabbit 30 min after injection of sodium diethyldithiocarbamate; h) persistence of all aldehyde-fuchsin granules in islet of same rabbit. Fixation by Timm's method, 630 ×.

EXPERIMENTAL RESULTS

The blood sugar of the healthy intact rabbits varied between 78 and 126 (106.27 ±3.42) mg%, and in rabbits with diabetes between 225 and 740 (476.24 ± 27.71) mg%.

Staining with dithizone revealed granules of irregular shape in the B cells. They varied in size and were purplish-red in color. Individual granules could be distinguished more clearly in areas where their density was least. Granules were most numerous in the apical part of the cell, especially on the side adjacent to the sinusoidal capillary of the islet (Fig. 1a).

After treatment of the sections with 8-(p-toluenesulfonylamino)-quinoline, fluorescent granules were found in the cytoplasm of the B cells in the form of bright green grains. In their character and arrangement they resembled the dithizone granules. Staining with aldehyde-fuchsin clearly revealed dust-like granules of a bluish purple color in the B cells. These granules were located in the same parts of the cell as the dithizone granules (Fig. 1,a and b). In sections of the pancreas stained with pseudoisocyanin, bright brown fluorescent granules resembling aldehyde-fuchsin granules in their distribution and character were found in the B cells.

There is reason to suppose that the luminescent quinoline granules and dithizone (zinc) granules, on the one hand, and the luminescent pseudoisocyanin and aldehyde-fuchsin (insulin) granules, on the other hand, are identical.

For this reason, in the present investigations it was decided to compare only two reactions: dithizone and aldehyde-fuchsin. The luminescent reactions, as more specific but less convenient for fine cytological investigations, were used to verify the presence of zinc and insulin.

In healthy animals of all species, dithizone and aldehyde-fuchsin granules were located in the same parts of the B cells (Fig. 1, a and b). However, their number was not always in the same proportion. For example, extremely little zinc was present in the B cells of the islets in intact rats, while they contained a considerable number of aldehyde-fuchsin granules. According to data in the literature, the zinc content of the B cells in rats is considerably increased by paranephric block and division of the splanchnic nerves [3]. Dithizone granules were always coarser than aldehyde-fuchsin granules, and their shape and size were less constant.

Injection of glucose, particularly if repeated several times, led to a marked decrease in the number of dithizone granules in the B cells, especially those located in the center of the islets. In severe cases, small numbers of granules remained in the apical parts of some cells, mainly close to the blood capillaries (Fig. 1c). Approximately the same changes developed after injection of dithizone, and the degree of degranulation of the B cells was largely determined by the height and duration of the hyperglycemia (Fig. 1e).

In the rabbits receiving sodium diethyldithiocarbamate, the dithizone reaction in the islets was negative (Fig. 1g). The absence of zinc was confirmed in spodogram.

A different picture was found in the pancreas stained with aldehyde-fuchsin. Whereas a single injection of glucose caused hardly any changes in the number of granules in the B cells, 6 injections led to their total degranulation (Fig. 1b). Granules did not reappear in the islets during the 24 h after injection of dithizone (Fig. 1f). The picture in rabbits receiving sodium diethyldithiocarbamate was indistinguishable from that seen initially (Fig. 1h).

The results suggest that zinc and insulin are distributed in the same parts of the cell (its secretory portion), but that they are evidently components of different systems. The granules thus revealed by the histochemical reactions are probably not separate structures, but precipitated masses of reaction product. Electronmicroscopic investigation has in fact revealed granules of only one type (secretory granules) in the B cells [5].

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