HISTOCHEMICAL ESTIMATION OF ZINC IN THE PANCREATIC ISLETS OF LANGERHANS IN HEALTHY AND DIABETIC RABBITS

V. A. Eshchenko

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By means of the dithizone test and a specific luminescence reaction, zinc was found in the A and B cells of the pancreatic islets of Langerhans in healthy rabbits but only in the A cells of diabetic rabbits.

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In recent years several models of experimental diabetes have been produced in rabbits in our laboratory by administration of chelating agents [1-3, 5]. It is postulated that the blocking of zinc in the islets of Langerhans is the principal pathogenic factor in the development of this diabetes [4].

In the present investigation the content of zinc in the islets of healthy and diabetic rabbits and its distribution in the A and B cells of the pancreatic islets were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 20 rabbits (10 healthy and 10 diabetic) of both sexes weighing 1.4-3.4 kg. Diabetes was produced by intravenous injection of an ammoniacal solution of dithizone in a dose of 50 mg/kg. The presence of diabetes was established by repeated determination of the blood sugar (Rowe's method) and by histological investigation of the structure of the pancreas. The pancreas was fixed in 70° alcohol saturated with hydrogen sulfide. Deparaffinized sections 5-10 μ in thickness were used for histochemical investigation. The presence of metals in the islets of Langerhans was determined by staining with dithizone by Yoshinaga's method [6], and for comparison, by the specific luminescence reaction using 8-arenesulfonamidoquinolines developed in our laboratory. Differentiation between A and B cells was carried out by staining sections of the gland preliminarily treated in warm Bouin's solution with Gomori's hematoxylin-phloxine. Consideration was paid to the cell composition, the size and number of islets, and the index of function was determined (the ratio between the B and A cells). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Table 1 shows that the diabetic rabbits developed marked hyperglycemia, the number and size of their islets of Langerhans decreased, the number of cells in the islets was reduced, and the index of function fell considerably. All these changes were highly significant relative to the control (healthy rabbits).

In sections of the pancreas stained with dithizone the islets of Langerhans in healthy animals appeared a deep redish purple color and stood out sharply against the pale yellow background of exocrine tissue (Fig. 1c). The islets were round and oval in shape, or less frequently irregular. The borders of the cells were ill defined, and purplish red granules could be seen distinctly in the cytoplasm. Granules of dithizonates were either uniformly distributed in the cytoplasm or concentrated at one pole of the cell (basal or apical).

In sections of the pancreas stained with specific luminescent reagents belonging to the 8-arenesulfonamidoquinoline group and subsequently examined in ultraviolet light, numerous islets showing emerald green luminescence stood out against the dark background. The islets were clearly demarcated from the exocrine tissue, which served as a contrasting background. The nuclei showed almost no

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TABLE 1. Blood Sugar and Cell Composition of Islets of Langerhans of Healthy and Diabetic Rabbits (M±m)

Group of animals	No. of animals	Blood sugar (in mg%)	No. of islets per field of vision of microscope	Diameter of islets (in μ)		Total no. of cells in islet	Index of function
				maximal	minimal		
Healthy	10	109.1±4.6	5.8±0.4	91±2.6	59±1.4	32.6±1.4	4.40±0.30
Diabetic	10	437.7±33.6	2.7±0.3	44±1.4	24±0.6	14.7±0.4	1.07±0.07
P	–	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

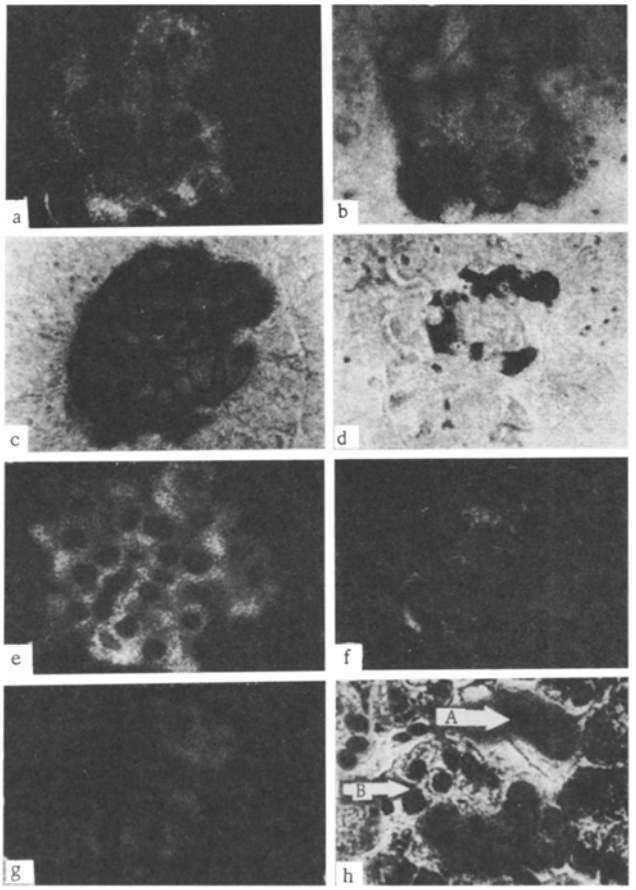

Fig. 1. Histochemical reactions in islets of Langerhans in rabbits. a) Luminescence reaction in islet of a healthy rabbit; b) dithizone reaction in the same islet; c) dithizone staining in normal pancreas; d) ditto in diabetes; e) luminescence of islet of a healthy rabbit; f) the same in a diabetic rabbit; g) luminescence reaction in islet of a diabetic rabbit; h) the same islet stained with Gomori's hematoxylin-phloxine. Arrows point to A and B cells. 420×.

luminescence; they were round in shape and located in the center of the luminescent cells (Fig. 1a and e). The luminescent granules due to the presence of zinc in the islets showed a surprising resemblence in their distribution to the dithizone granules (Fig. 1a and b), obtained by staining with Yoshinaga's method [6]. This is because the islets contain large quantities of zinc, much more than all other metals reacting with dithizone at the pH of the internal medium.

Granules demonstrated by these two methods (luminescence and dithizone) in the islets of Langerhans of healthy rabbits were present in both the B and A cells. This was shown by staining the same section of the pancreas with the luminescence reagent and with Gomori's hematoxylin-phloxine in succession.

Examination of sections from the pancreas of diabetic animals revealed a picture which differed from that described above. In sections stained by Yoshinaga's method, few islets were seen; they were small, deformed, and paler than those in healthy rabbits (Fig. 1d). Purplish-red granules of dithizonates appeared in only a few cells located mainly at the periphery of the islets as a border. In diabetic rabbits the luminescence was paler than in healthy animals (Fig. 1f). Its localization was the same as that of the granules obtained by dithizone staining.

By comparing luminescence of the cells with their appearance on staining with hematoxylin-phloxine in the same section it was found that zinc was present in the A cells of the islets of Langerhans of diabetic rabbits (Fig. 1g and h). Similar results were obtained by comparing parallel sections passing through the same islet and stained with dithizone and with hematoxylin-phloxine. Dithizone granules were found in those parts of the islet where the A cells were situated.

By the use of two histochemical reactions the presence of zinc was thus demonstrated in the A and B cells of the pancreatic islets of Langerhans in healthy rabbits. The development of dithizone diabetes is accompanied by death of the overwhelming majority of B cells while the A cells are left intact. Zinc detectable in the diabetic animals is present in the A cells.

LITERATURE CITED

1. V. A. Eshchenko, Glycemic Reaction and State of the Islets of Langerhans in Animals of Different Species Following Administration of Dithizone, Candidate Dissertation, Karaganda (1965).
2. Ya. A. Lazaris and V. A. Eshchenko, Probl. Éndokrinol., No. 1, 65 (1966).
3. Ya. A. Lazaris and A. Ya. Lazaris, Probl. Éndokrinol., No. 2, 75 (1967).
4. Ya. A. Lazaris and A. Ya. Lazaris, Byull. Éksperim. Biol. i Med., No. 7, 45 (1967).
5. E. V. Naumenko, Experimental Dithizone Diabetes, Candidate Dissertation, Karaganda (1958).
6. T. Yoshinaga, Y. Shinji, T. Katoyama, et al., Acta Histochem. (Jena), 21, 276 (1965).