INTRAVITAL DETERMINATION OF DITIZON IN THE ISLETS OF LANGERHANS OF HEALTHY RABBITS AND RABBITS WITH DIABETES

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A method of intravital observation of the islet-cell apparatus of the pancreas after administration of ditizon has been developed. A positive ditizon reaction was found in healthy rabbits and rabbits with diabetes. In the latter, the staining reaction was paler and disappeared more rapidly. Preliminary administration of sodium diethyldithiocarbamate to the animals prevented the appearance of a positive ditizon reaction in the islets of Langerhans.

The development of experimental diabetes caused by administration of ditizon has been shown to be due to selective damage to the islets of Langerhans [1,2,4-6]. However, the mechanism of the direct action of ditizon has not yet been adequately explained.

The object of this investigation was to develop a suitable method of intravital observation of the distribution of ditizon in the islet-cells of the pancreas and to use it to study the dynamics of its entry into and disappearance from the islets of the pancreas.

EXPERIMENTAL METHOD

Experiments were carried out on 68 noninbred rabbits of both sexes weighing 1.8-3.7 kg. Diabetes was produced in 14 animals by intravenous injection of ditizon and derivatives of 8-hydroxyquinoline. Persistent hyperglycemia was used as the criterion of diabetes. The blood sugar was determined by Roe's method with anthrone reagent. The animals were deeply anesthetized with urethane and tied to a frame in the supine position. An incision was made along the lateral line of the abdomen below the costal arch on the left. The pancreas was delivered together with the spleen without disturbing its blood supply, and arranged above a hole through which a beam of light from a 500 W lamp was transmitted from below through a system of lenses. Observation and photography were carried out with the MBS-2 binocular microscope, to which was attached a type MFN-1 photomicrographic adapter with a "Zorkii" camera. Photographs were taken at definite time intervals on KN-3 film (using a green filter) and on LN-5 color film (exposure 0.1 sec). A TSN-250 voltage stabilizer was included in the circuit to ensure a current of constant strength. A 1% solution of ditizon in aqueous ammonia was injected into the rabbits prepared as described in this manner in a dose of 50 mg/kg for 3 min. Between 5 min and 1 h 30 min before the injection of ditizon, 20 rabbits were given an injection of 500-1000 mg/kg sodium diethyldithiocarbamate (DDC). Instead of DDC, control animals were injected with the same volume of physiological saline. After the end of the experiment, the rabbits were killed by air embolism. The pancreas was fixed in Bouin's fluid and sections cut to a thickness of 5 μ were stained with aldehyde-fuchsin by Gomori's method.

EXPERIMENTAL RESULTS

The mean blood sugar concentration of the healthy rabbits was 110.1 $\pm$ 5.33 mg%, and of the diabetic rabbits 353.62 $\pm$ 38.8 mg% ($P < 0.001$).

Ditizon began to appear in the pancreatic blood vessels of the healthy rabbits 1 min after injection, and 2 min after injection it had disappeared from them. The pancreas was covered with purplish-red islets 3 min after injection, and the intensity of their staining reached a maximum after 15-30 min. In the healthy rabbits the islets were numerous, regularly round or oval in shape, and sharply distinguished from the pale yellow background of the surrounding exocrine tissue (Fig. 1a). Subsequently, the islets gradually became

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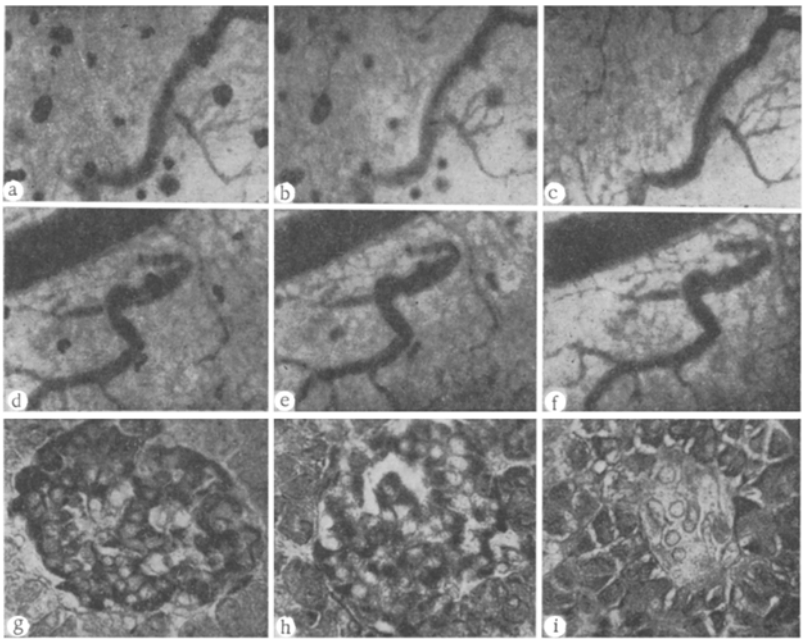

Fig. 1. Intravital staining for ditizon and histological structure of the islets of Langerhans in healthy and diabetic rabbits. a) Intravital staining of pancreas of healthy rabbit 15 min after injection of ditizon; b) the same 1 h, and c) the same 2 h after injection; d) intravital staining of pancreas of diabetic rabbit 5 min after injection of ditizon; e) the same 15 min, and f) the same 30 min after injection (40 ×); g) islet of Langerhans of a healthy rabbit; h) the same in a healthy rabbit after 4 injections of ditizon; i) the same in a diabetic rabbit (Gomori's aldehyde-fuchsin method. 420 ×).

paler, and this was particularly noticeable 1 h after injection of ditizon (Fig. 1b). After 1.5-2 h, the islets in the gland could no longer be seen (Fig. 1c).

Repeated injection of the same dose of ditizon led to the reappearance of the same staining reaction in the same islets and at the same times. This reaction was repeated after 4 injections of the compound.

A different picture was observed in the diabetic rabbits. Very few islets could be seen, they were small in size, shaped like rings or half-moons, and some of them were irregularly branched. The islets were stained a pale red color which disappeared much more rapidly than in the healthy animals – after 10-40 min (Fig. 1, d, e, f). Similar results were obtained when the injection of ditizon was repeated.

When the intravital observations had been completed, the pancreas was removed and stained with aldehyde-fuchsin. This revealed very little change in the granular appearance of the β-cells in the healthy rabbits, an index of their insulin content (Fig. 1, g, h), while the complete disappearance of granules was observed in the diabetic rabbits (Fig. 1i).

Preliminary injection of DDC, but not of physiological saline, into the rabbits 5-90 min before injection of ditizon prevented staining of the islets. Similar results were obtained previously in acute experiments by Lazaris. In his experiments DDC prevented staining of the islets and development of diabetes after subsequent injection of ditizon [3].

Treatment of sections of the pancreas with ditizon solution with the addition of a special buffer [7] stained the islets a purplish-red color, demonstrating that they contain zinc. The presence of this metal in the islets was confirmed by a highly sensitive specific luminescence reaction.

By using the suggested method of intravital observation of pancreatic islets, it was possible to study the process of formation and deposition of ditizon salts in them. Histochemical reaction revealed the presence of zinc in the islets. Blocking of zinc by the powerful chelating agent DDC prevented the

appearance of an intravital ditizon color reaction, probably because ditizon cannot displace DDC from its stable complex with zinc.

LITERATURE CITED

1. R. S. Gol'dberg, Protein Components of the Blood in Experimental Ditizon Diabetes, Candidate dissertation, Karaganda (1963).
2. V. A. Eshchenko, The Glycemic Reaction and State of the Islets of Langerhans in Animals of Different Species following Administration of Ditizon, Candidate dissertation, Karaganda (1965).
3. Ya. A. Lazaris, Probl. Éndokrinol., No. 2, 77 (1966).
4. Ya. A. Lazaris and V. A. Eshchenko, Probl. Éndokrinol., No. 1, 65 (1966).
5. Ya. A. Lazaris, Probl. Éndokrinol., No. 2, 75 (1967).
6. E. V. Naumanko, Experimental Ditizon Diabetes, Candidate dissertation, Karaganda (1958).
7. M. Mager, W. F. McNary, Jr., and F. Lionetti, J. Histochem. Cytochem., 1, 493 (1953).