MECHANISM OF DAMAGE TO THE PANCREATIC ISLETS IN DITHISONE DIABETES

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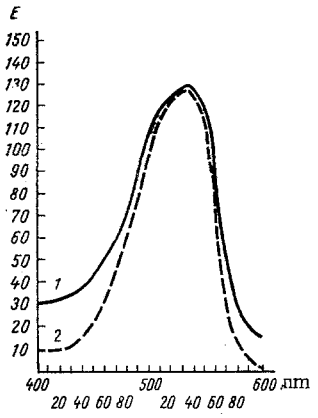
In experiments on 23 rabbits and 18 albino mice, intravenous injection of dithisone (50-100 mg/kg) was followed by the appearance of bright red granules of zinc dithisonate in the pancreatic islets and by their complete disappearance 1-2 h later. In anesthetized rabbits 10 min and 2 h after injection of dithisone, a piece of the pancreas was excised. Zinc dithisonate was found only in the 10-min fragments. The pattern was the same after a further injection of the compound. If the rabbits were killed immediately after the injection of dithisone, the positive dithisone reaction persisted not less than 7 h in the rabbits, compared with 3 h in mice. It is postulated that two zinc fractions exist in the B cells, one of which, bound with insulin, interacts reversibly with dithisone. The other, located in the active centers of the enzymes, can be irreversibly blocked by dithisone, as a result of which insulin synthesis is disturbed.

While developing histochemical methods of determining various metals in the tissues, Okamoto [6, 7] found large quantities of zinc in the islets of Langerhans. Intravenous injection of the reagent for zinc into animals led to the production of dithisone pancreatic diabetes [8]. He explained the mechanism of development of the disease by a "zinc theory" [7], according to which the powerful chelating compound dithisone combines with zinc in the islets as chelates. These produce destructive changes in the B cells, as a result of which permanent insulin deficiency arises. Later, indirect evidence was obtained of the possible role of zinc blocking by dithisone in the pathogenesis of this experimental diabetes [2, 4, 9, 10].



Absorption spectra of chloroform extract from the pancreas after intravenous injection of dithisone (1) and of a solution of chemically pure zinc dithisonate in chloroform (2).

Up till now, however, there has been little evidence of the distribution of zinc dithisonate in the islets, its conversions, or the duration of its action. Information on all these aspects would shed considerable light on the pathogenesis of dithisone diabetes and the mechanisms of development of insulin deficiency, for there is still little direct confirmation of this very attractive "zinc theory". The investigation described below was carried out in an attempt to elucidate some of these problems.

EXPERIMENTAL METHOD

Experiments were carried out on 23 noninbred rabbits and 18 albino mice which received intravenous injections of diabetogenic doses of dithisone (50-100 mg/kg) in 0.25% ammonia solution under various conditions. After the animals had been killed by air embolism the pancreas was removed and frozen sections cut to a thickness of 5 μ, which were examined in the dark ground of a Zeis micro-

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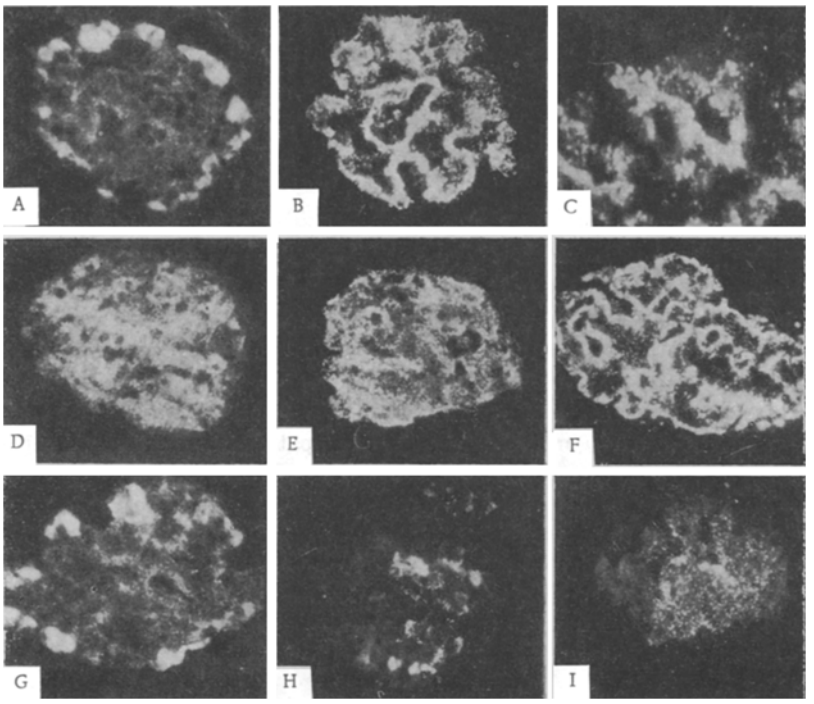

Fig. 2. Sections through islets of rabbits and mice under dark ground illumination: A) islet of an intact rabbit; B) islet after injection of dithisone; C) the same, under high power (800×); D) islet of intact albino mouse; E) islet of mouse after injection of dithisone; F, G) islet of rabbit 10 min and 2 h respectively after injection of dithisone; H) islet of rabbit killed 7 h after injection of dithisone; I) islet of mouse killed 3 h after injection of dithisone; 280×. Thickness of sections, 5μ.

scope and photographed. The formation of zinc dithisonate in the islets was confirmed by the SF-4A spectrophotometer. For this purpose, after injection of dithisone into the animal, the gland was minced in liquid nitrogen, and the dithisonate, formed only in the islets, was extracted with chloroform from the resulting fine powder. Its absorption spectrum coincided with that of synthetic chemically pure zinc dithisonate (Fig. 1).

EXPERIMENTAL RESULTS

Dithisone, injected intravenously, disappeared from the blood stream after a few minutes [1]. Dithisone combines selectively with only certain tissues. A complex of dithisone with zinc was discovered in the retina, the submandibular salivary gland and the pancreas. In these tissues, bright red and bright orange granules, readily soluble in chloroform, could be seen in the dark ground of the microscope. The absorption maximum of the colored solution corresponded to that of zinc dithisonate, namely 530 nm. Preliminary injection of sodium diethyldithiocarbamate, a more powerful chelating compound than dithisone, was accompanied by the formation of colorless zinc carbamate, and the appearance of the colored granules of zinc dithisonate in the islets was thus prevented.

No granules with the characteristic color of the dithisonate could be found in the sections through the pancreas of intact rabbits (Fig. 2A). Pale blue A cells could be seen clearly at the periphery of the islet and B cells with a dark nucleus and with small grey granules in its central part. After injection of dithisone into the rabbits, the islets were filled with bright red granules, distributed uniformly over the whole surface of the islet (Fig. 2 B, C). The A cells usually could not be seen in these experiments. Finer granules were seen in sections through the gland of the intact mice in the dark field, nuclei of the B cells were less clearly visible, and the A cells were indistinguishable (Fig. 2D). After injection of dithisone into the mice the whole islet was filled with equally brightly colored granules, but they were somewhat smaller than in the rabbits (Fig. 2E). The colored dithisonate granules persisted for a relatively short time.

They decreased considerably in number in the rabbits after 45 min to 1 h, and after 2 h they disappeared completely.

In the mice the color of the islets became pale after 30 min and disappeared completely 1 h after the injection of dithisone.

To make a more detailed study of the fate of the dithisonate formed in the islets, special experiments were carried out. Rabbits were anesthetized with urethane, laparotomy was carried out, and the pancreas was exposed. A solution of dithisone (50.8-50.9 mg/kg) was injected into the auricular vein and, after application of a ligature to the peripheral part of the pancreas, the piece of tissue separated by the ligature was resected 10 min and 2 h after the injection. After each resection the abdomen was closed and the rabbit heated to prevent hypothermia. Islets filled with brightly colored dithisonate granules could be seen in sections from the piece of pancreas taken 10 min after injection of dithisone when examined in the dark field (Fig. 2F). These granules could no longer be found after 2 h (Fig. 2G). The same dose of dithisone was again injected into some of these rabbits which remained anesthetized for a long time, and pieces of the pancreas were again resected 10 min and 2 h later. The same picture was observed as after the first injection. Further confirmation of the possible repeated formation of the colored complex of dithisone with zinc in the islets and its subsequent breakdown and decolorization of the islets was given by experiments on rabbits and mice receiving repeated injections of dithisone and sacrificed 2 h after the last injection. The mice received two injections of dithisone and the rabbits four injections at intervals of 2 h, in doses leading to the appearance of brightly colored granules 10 min later in the sections (mice 100 mg/kg, rabbits 30 mg/kg). Not even traces of dithisonate could be found in sections through the pancreas from any of the animals killed 2 h after the last injection.

Decolorization of the islets after a relatively short period in the pancreas of the living rabbits (i.e., in which the circulation remained intact) can be represented as follows. Zinc dithisonate breaks down rapidly into its components, and dithisone itself is removed from the islets by the blood stream. The possibility cannot be ruled out that the zinc dithisonate complex may also be removed from the islets with the blood stream. In that case, binding of only part of the zinc of the B cells by the small quantity of dithisone entering the islets from the blood stream must be an inevitable condition. It is an essential condition of the first and second hypotheses that the circulation of blood in the islets remains intact; otherwise neither dithisone nor dithisonate could be removed. If the proposed explanation is true, if the blood flow is interrupted, i.e., in the cadavers of animals killed immediately after injection of dithisone and kept under the same temperature conditions, the coloring of the islets with dithisone could not disappear within such short times. In fact, zinc dithisonate could be found even after 7 h in the cadavers of two rabbits killed 5 min after the injection of dithisone and kept at 37°C, by contrast with the living rabbits (Fig. 2H). A similar picture was observed in the islets of the cadavers of three mice killed after the same interval and kept for 3 h at 37°C (Fig. 2I).

These experiments indicate that the diabetic dose of dithisone, causing the appearance of diabetes mellitus after 24-28 h in the animals, clearly does not block all the zinc irreversibly in the pancreatic islets. This is clearly shown by the experiments in which repeated injections of dithisone were given into living rabbits, for after each injection the characteristic color of zinc dithisonate appeared in the islets. The mechanism of development of dithisone diabetes is evidently much more complex than that described in Okamoto's "zinc theory". If dithisone diabetes was in fact due to blocking of the zinc, this cannot in any event be true of the zinc which reacts so easily with dithisone and which is given up by the dithisone just as readily or is partly eliminated rapidly with it from the islets long before the onset of diabetes. Presumably two zinc fractions exist in the pancreatic islets. The larger fraction is stored as an insoluble compound with insulin [4], and it reacts relatively easily and reversibly with dithisone. The other fraction is more firmly bound in the cell with the active centers of enzymes concerned with insulin synthesis. Blocking this zinc fraction by dithisone may be the direct cause of the disturbance of the insulinogenic function of the B cells and of their death. Evidence has recently been published [3] in support of this hypothesis. However, the mechanism of the diabetogenic action of dithisone cannot yet be regarded as solved.

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