MECHANISM OF THE HARMFUL ACTION OF CHELATING AGENTS ON PANCREATIC ISLET CELLS

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With the increasingly wide use of chelating agents in agriculture and different branches of industry and medicine these agents have joined the list of possible etiological factors of occupational and iatrogenic diseases [9, 10, 13]. Despite the numerous investigations into the harmful action of chelating agents on pancreatic islet cells [1, 2, 4, 6-8, 11, 12] the mechanism of this action has not yet been finally explained.

The experimental morphological investigation described below was devoted to the study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 58 rabbits into which the following chelating agents were injected intravenously and separately: 10-100 mg/kg of a 1% solution of dithizone in 0.5% ammonia solution, 10-50 mg/kg of 0.5% solutions of 8-(p-toluenesulfonylamino)-quinoline (8TSQ) and 8-(benzenesulfonylamino)-quinoline (8BSQ) in a 0.1 N solution of caustic soda. The rabbits were killed at various times (from 3 min to 5 days) after injection of the chelating agents. The pancreas was removed and used to prepare frozen sections 10-30 μ thick, and pieces of tissue were fixed in Bouin's fluid, by Timm's method, and in absolute alcohol and acetone. The intravital histochemical reaction was carried out on frozen sections of the pancreas in light and dark grounds of the microscope, in reflected UV light. Luminescence was excited with a UFS-3 filter, and glass ZhS-3 and ZhS-18 protective filters were used. Differentiation between the islet A and B cells in frozen sections was carried out by dark-ground microscopy. Paraffin sections 5-10 μ thick were stained with hematoxylin and phloxine and with aldehyde-fuchsine by Gomori's method, with toluidine blue, and by Brachet's method. Sections also were fluorochromed with acridine orange. Zinc was detected in frozen and paraffin sections by staining with dithizone and 8TSQ by the method described in [3, 5, 8].

EXPERIMENTAL RESULTS

Intravenous injection of chelating agents into rabbits caused the development of an intravital histochemical reaction in the islet cells (Fig. 1a). Most of the intravitally formed reaction products were discovered in the islet B cells. When sections of the pancreas from these animals were stained, the intensity of the histochemical reaction of dithizone and 8TSQ and 8BSQ was approximately equal in islet A and B cells (Fig. 1b). It was shown by spectrophotometric and fluorometric methods that the product of these reactions is a zinc chelate.

The intravital histochemical reaction was positive in the islet cells as early as 3 min after injection of the chelating agents, after which its intensity increased rapidly to reach a maximum after 5 min in the islet A cells and after 10 min in the B cells. The concentration of the zinc chelate in the first cells was considerably lower than in the second. Later staining of the islet became paler and less distinct. In the islet A cells it could no longer be seen after 1 h, and in the B cells after 2 h.

Correlation was observed between the concentration of intravitally formed zinc chelate in the cells, the duration of its stay in the cells, and the severity of the damage caused to

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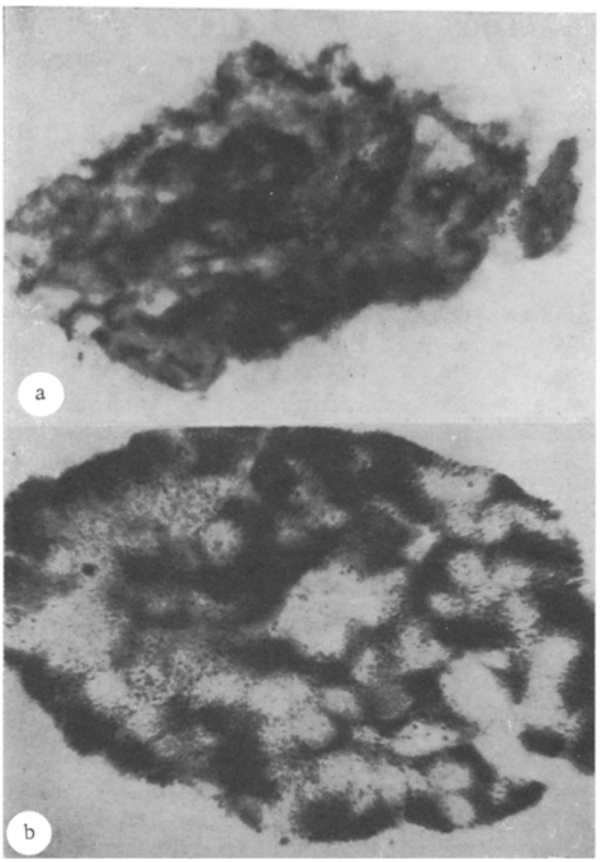

Fig. 1. Histochemical dithizone reaction in pancreatic islets of rabbit after intravenous injection of chelating agent (a) and in paraffin section of pancreas stained with chelating agents (b): a) 280 ×, b) 630 ×.

them. Islet B cells were damaged much more severely by the chelating agents than the islet A cells. Intensification of basophilia, pyroninophilia, and the metachromatic reaction with toluidine blue and acridine orange in the B cells can be explained by increased ability of the denatured substances to take up the dyes. Typical signs of necrosis (pycnosis, karyorrhexis, karyolysis, etc.) were found usually not less than 1-2 h after injection of the chelating agents. Cell debris was absorbed in the course of 1-3 days and islets were deformed and reduced in size.

In severe cases disturbances of zinc metabolism in the islet cells after injection of the chelating agents developed in three phases. A fall in the content of histochemically detectable zinc during the first phase was due to masking of this metal because of its binding by the chelating agents administered. The increase in the concentration of detectable zinc in the islet cells in the second phase took place because of activation of compensatory mechanisms. The third phase arose in cases of considerable damage to the islet B cells and was characterized by a second fall in the zinc content as a result of outflow of materials from the pathologically changed cells.

There is reason to suppose that after administration of chelating agents, saturated and unsaturated complexes with weakly bound zinc may be formed in the islet cells. The relatively low hydrogen ion concentrations evidently promote the formation predominantly of saturated complexes, of low toxicity, in the islet A cells. The hydrogen ion concentration is much higher in the B cell compartments, which accumulate weakly bound zinc, so that mainly unsaturated complexes are formed. These are less lipophilic, they are retained longer inside the cells, and they have a stronger toxic action.

Administration of cysteine and glutathione to the rabbits weakened the cytotoxic action of dithizone, 8TSQ, and 8BSQ. One of the main links in the mechanism of the cytotoxic action

of the unsaturated zinc chelate is evidently its binding with thiol groups, large numbers of which are contained in membrane proteins. Deprotonation of these groups and addition of the chelating agent to them may cause alteration of the proteins and a disturbance of membrane permeability. The outflow of the unsaturated complex from the compartments into the hyaloplasm causes damage to membranes of other intracellular structures. Activation of hydrolytic processes in the cell must be associated primarily with disturbance of permeability of the lysosomal membranes. Damage to the mitochondrial membranes plays a role of some importance. Increased permeability of the plasma membrane facilitates the outflow of materials from the cell. For instance, the hypoglycemic convulsions observed in the rabbits were the result of the outflow of large quantities of insulin from the damaged B cells of the pancreatic islets.

Disturbances of diffusion, osmosis, and active transport, the lowering of concentration gradients, a decrease in heterogeneity, and potentiation of hydrolysis give rise to even more destructive changes, leading to death of the cell. The weak toxic changes in the islet A cells stimulate compensatory mechanisms, which are reflected morphologically in hypertrophy and hyperplasia of these cells and an increase in their zinc concentration.

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