REPARATIVE PROPERTIES OF "HYDROPROT" PROTEIN HYDROLYSATE IN EXPERIMENTAL ALLOXAN DIABETES

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The evolution of structural alterations and the regeneration of pancreas in experimentally induced alloxan diabetes has been studied by V. G. Baranov, I. M. Sokoloverova (1972); E. V. Kuleshov, A. B. Shechter (1972).

In earlier studies (I. Popdimitrov, K. Velikov, N. Tarkolev), we established a nearly 50 mg % reduction of the hyperglycemia provoked, and an increase in insulinemia by about 1 U upon intravenous and oral introduction of the protein hydrolysate "Hydroprot" in rabbits. The quoted results and the frequently established evidence (K. Demireva, 1974; K. Demireva, I. Popdimitrov, 1975) of marked methionine accumulation, respectively hydrolysate, in the pancreas warrant the assumption that in alloxan diabetes protein hydrolysate may also exert a regenerative effect on the Langerhans' cells.

It is the purpose of the present research to assay histological changes in the pancreas and liver in experimentally induced alloxan diabetes in rats, treated with protein hydrolysate.

Material and methods

Twelve white rats with body weight ranging from 150—200 grams were used in the experiment. Blood and urine sugar level was studied in all the animals before the experiment. Diabetes was induced through intraperitoneal injection of alloxan, at dose 170 mg per kg of body weight. The rats were divided up into three groups of four animals each: group I — clinically healthy rats served for control purposes, group II — with diabetes, receiving 1 ml/100 g body weight physiological saline each, and group III — similarly with diabetes, receiving 1 ml/100 g body weight hydrolysate, injected subcutaneously daily over a period of 84 days. During the experiments, hyperglycemia and glucosuria were followed by the orthotoluidine method at 21, 28, 49, 77 and 84 days. After sacrification of the animals pancreas, liver and kidneys were removed for histomorphological investigation. The material for histomorphological study was fixed in Bouin's and Carnoy's solution, and additionally processed after the paraffin method. The preparations with section thickness measuring 5—10 microns were stained with hematoxylin-eosin, after Gomori for demonstrating alpha- and beta-cells in the Langerhans' islands of the pancreas, with azan for connective tissue, PAS-reaction and control with amylase, and Brachet's reaction for RNA.

Results and discussion

The pancreas from control animals is with preserved structure, the Langerhans' islands show a clearcut differentiation and rounding, the cytoplasm is homogeneous, and upon staining after Gomori, the alpha- and beta-cells are clearly distinguished (Fig. 1).

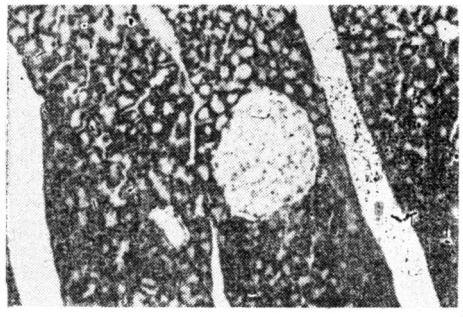

Fig. 1: A Langerhans' island from a healthy animal. Staining Hem. — eosin, magnif. 10×20

Animals injected with alloxan and treated with physiological saline display dystrophic, necrobiotic and necrotic changes in the Langerhans' cells. The islets are made up of a smaller number of cells, and that's why they present a loose appearance. In some of the cells the picture of vacuole dystrophy is noted — their cytoplasm is more abundant than in the control animals, and displays a foamy or reticular pattern, with pale eosin staining. In other cells the nuclei are poorly outlined or completely absent. The changes described are rather marked in the central part of the islets, and in some of them the structure is completely deleted (Fig. 2). Hence it is difficult to differentiate alpha- from beta-cells, while the destruction of the island layer is clearcut.

In the other internal organs moderately pronounced dystrophic changes are present with the character of protein dystrophy. The liver cells contain a scarce amount of PAS positive substances, disappearing upon amylase control, and therefore they were taken to be glycogen.

It is of particular interest to note the changes in the organs of animals injected with alloxan, and subjected to hydrolysate treatment. Here the Langerhans' islands are large, and made up of a greater number of cells. The lay out of cell elements is irregular with scarce cytoplasm and rounded pyknotic nucleus (Fig. 3).

The borders of the individual cells are not clearly outlined, and are somewhat variable in terms of size and tinctorial properties of the cytoplasm and nuclei. Within the cytoplasm of these cells an abundant amount of RNA is found, which is a proof that it is a matter of young, regenerated cells with an enhanced RNA synthesis. In animals with longer survival terms, a complete restoration of the Langerhans' islands' structure takes place.

Glycogen content in the liver cells and in the epithelial cells of renal ductules does not show variations worthy of notice. In the liver of this group of animals an abundant proliferation of Kupffer's cells with a rich RNA content in the cytoplasm is observed. The changes outlined should be interpreted as an expression of reticuloendothelial system activation, most likely, under the effect of protein hydrolysate administration.

At the end of the experimental term, the body weight shows an increase, as compared to the starting level — by 26 g in the experimental, and by 62 g in the control animals. At the same time, the blood sugar lever in the experimental animals is 113 mg%, and in the controls — 152 mg %.

It is evident from the results obtained that in diabetic animals too, it is a matter of a protein hydrolysate effect on the blood sugar level, similar to that observed after sugar loading of healthy persons. A well manifested regeneration of the Langerhans' islets is also established. At the actual state

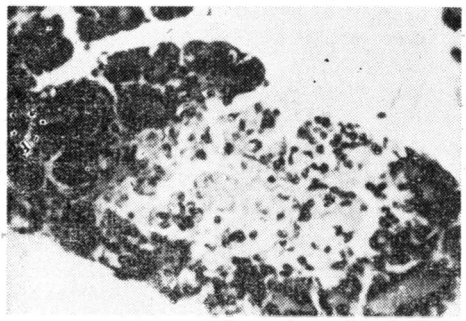

Fig. 2: A Langerhans' island from animal with induced alloxan diabetes, treated with physiological saline. Staining Hem. — eosin, magnif. 10×40.

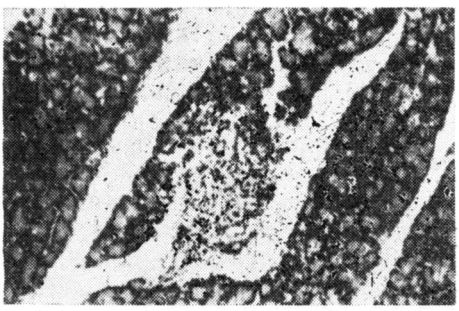

Fig. 3: A Langerhans' island from animals with alloxan diabetes, subjected to protein hydrolysate treatment. Staining Hem. — eosin, magnif. 10×20.

of the research, we are still not in a position to make a definitive statement about the extent to which the regenerated cellular elements undergo differentiation into alpha- and beta-cells.

Although the material is limited and rules out the reaching of categorical inferences, the results obtained warrant the assumption that the protein hydrolysate employed stimulates the reparative and regenerative processes of the pancreas.

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РЕПАРАТИВНЫЕ СВОЙСТВА БЕЛКОВОГО ГИДРОЛИЗАТА «ХИДРОПРОТ» ПРИ ЭКСПЕРИМЕНТАЛЬНОМ АЛЛОКСАНОВОМ ДИАБЕТЕ

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РЕЗЮМЕ

Воспроизведен экспериментальный аллоксановый диабет на белых крысах, дозой 170 мг/к массы. Часть животных третирована физиологическим раствором — по 1 мл/100 г массы, а другая часть — белковым гидролизатом, также по 1 мл/100 г массы. В ходе опыта исследовались: уровень сахара в крови и содержание сахара в моче. После убоя животных проведено гистологическое исследование материала из печени и поджелудочной железы.

Обнаружено, что у животных, третированных белковым гидролизатом, наступает регенерация клеточных элементов островков Лангерганса.