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EFFECT OF BONE MARROW TRANSPLANTATION ON RESTORATION OF PANCREATIC MORPHOLOGY AND FUNCTION IN IRRADIATED RECIPIENTS

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It has now been established that cells either in the cell cycle or in a functionally active state exhibit increased radiosensitivity. An increased number of these cells is observed not only in the hematopoietic, immunocompetent, and reproductive systems, but also in endocrine tissues. Structural and functional changes in the endocrine organs developing under the influence of irradiation and disturbances of neuroendocrine regulation arising under those conditions may play an essential role in the development of secondary pathological processes in the irradiated organism [1, 3]. In particular, many investigators [2, 3, 5, 7, 8] have observed considerable changes in the pancreas in an irradiated animal. Considering the important role of insulin in the regulation of metabolism, and also the absence of any sufficiently profound studies of the structural and functional state of the pancreas in lethally irradiated recipients, protected by bone marrow, we decided to study the state of the pancreas in lethally irradiated recipients at different stages after transplantation of native and freeze-dried hematopoietic cells.

EXPERIMENTAL METHOD

Experiments were carried out on 250 inbred (CBA × C57BL)F_1 hybrid male mice aged 2 months. The animals were divided into four groups. Group 1 consisted of lethally irradiated animals, group 2 of lethally irradiated animals receiving native syngeneic bone marrow, the animals of group 3, after lethal irradiation, were given an injection of syngeneic freeze-dried bone marrow, and group 4 was the control (intact animals). Irradiation was given on the RUM-17 apparatus. The conditions of irradiation were: dose rate 39.5 R/min, U = 200 kV, I = 10 A, filter: 0.5 mm Cu + 1 mm Al. The bone marrow was taken from the femora of mice and preserved by the method in [6]. Syngeneic bone marrow obtained from donors, both native and freeze-dried, was injected intravenously in a dose of $1 \cdot 10^7$ cells/ml. The animals were irradiated and bone marrow transplanted at the same time, between 9 a.m. and 12 noon. Modern methods of determination of the blood insulin level do not always give adequate information, and accordingly in practical work, to assess the function of the endocrine part of the pancreas, several parameters of carbohydrate metabolism are studied, and in particular, glucose. Sugar was determined by the method in [4], 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 40, 60, and 90 days after irradiation and bone marrow transplantation. The histologic structure of the pancreas was studied 1, 3, 7, 10, 30, 40, 60, and 90 days after irradiation and transplantation of the bone marrow. Paraffin

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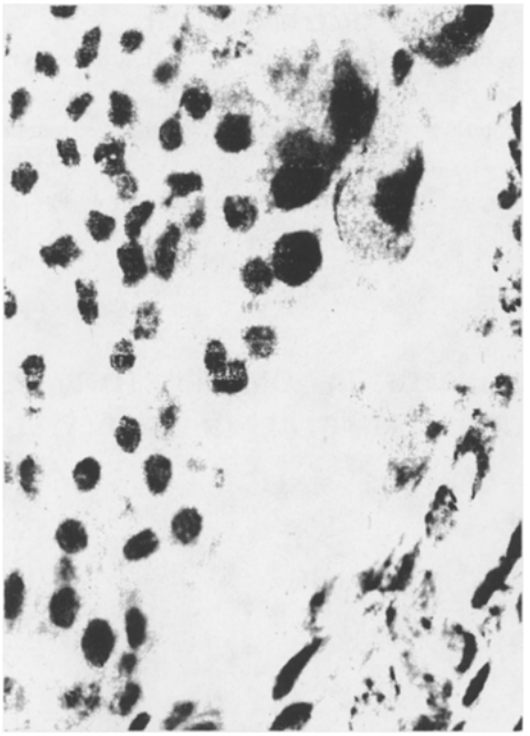

Fig. 1. Widened intercellular spaces. Islet cells with hypo- and hyperchromic nuclei. Giant nuclei of acinar cells and epithelial cells can be seen (3 days after lethal irradiation). Hematoxylin and eosin. 630×.

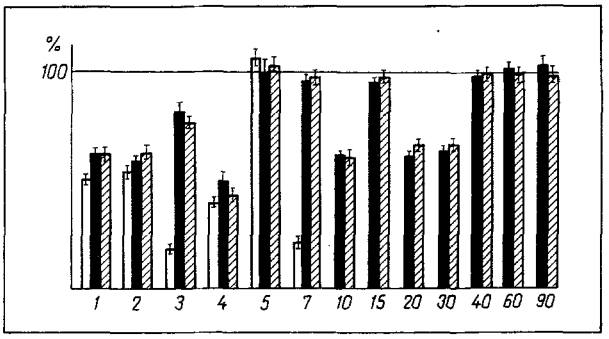

Fig. 2. Changes in dynamics of blood glucose level in lethally irradiated mice, protected and not protected with bone marrow. Unshaded columns — irradiation, obliquely shaded — irradiation, black columns — irradiation and transplantation of native bone marrow, obliquely shaded — irradiation and transplantation of freeze-dried bone marrow. Control (intact animals) taken as 100%. Abscissa, days after irradiation.

sections 4-6 μ thick were stained with hematoxylin and eosin and by Van Gieson's method. The results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The pancreas in the control animals (group 4) was surrounded by a loose connective tissue capsule, from which connecting layers were given off on the inner side, dividing the gland into lobules and forming its stroma. Inside the lobules there were tightly packed acini, consisting of adjacent acinar cells, pyramidal in shape and with strongly basophilic cytoplasm, and containing round nuclei with one or two large nucleoli. Among the pancreatic lobules pancreatic islets could be identified, separated from the acinar tissue by connective tissue. The islets consisted of concentrations of irregularly shaped cells. The cells were diffusely arranged throughout the islet They were either round or oval in shape. Adjacent cells were separated by a narrow light space. The nucleus occupied the central part of the cell.

In the lethally irradiated animals not protected with bone marrow (group 1) histologic changes were observed as early as on the 1st day after irradiation. The whole vascular system was involved in the pathological process. Capillaries and blood vessels were dilated and congested, and stasis was present. The vascular reaction was accompanied also by changes in their walls, in the form of swelling of the endothelium and pycnosis of the nuclei in these cells. Desquamation of endothelial cells into the lumen of the ducts was observed. The acini were reduced in size. No visible morphological changes were found in the islets.

On the 3rd day after irradiation, besides the morphological changes already noted, a disturbance of the exocrine part of the pancreas was observed, namely absence of its usual lobular structure, or even death of individual gland cells with the formation of multiple foci of micronecrosis. The cytoplasm of most acinar cells was swollen and vacuolated and the nuclei were enlarged. Giant nuclei appeared in some cells, and some cells were destroyed. Giant nuclei appeared also in epithelial and connective-tissue cells. The most marked destructive changes in the acinar parenchyma were observed on the 7th day. However, besides degenerative changes in the acini, binuclear acinar cells also appeared at this period. Widened intercellular spaces and cells with hypo- and hyperchromic nuclei could be identified on the 3rd day in most islets of Langerhans (Fig. 1). Around the periphery of the islet tissue connective-tissue cells were clearly outlined. On the 7th day after irradiation some islets could be seen to have lost their distinct outlines.

Thus the histologic analysis shows that on the 1st day after lethal irradiation of mice the most marked destructive changes affected the exocrine part of the pancreas, whereas a lesion of cells of the insular apparatus was observed on the 7th day after irradiation. However, the trend of the sugar level in the lethally irradiated mice suggests a marked disturbance of functional activity of the islet cells. For instance, the blood sugar level in the lethally irradiated animals fell during the 1st day almost by half compared with the control, and reached a minimum on the 3rd and 7th days, whereas on the 5th day it was significantly higher than in the control (Fig. 2). Incidentally, on the 7th day mass death of the lethally irradiated animals was observed and, as shown by data in the literature, at the time of death insulin secretion was inhibited in the irradiated mice [1].

In the animals of groups 2 and 3, histologic changes in the acinar tissue were observed during the first 3 days after lethal irradiation and bone marrow transplantation, the same as in the animals of group 1. Starting with the 3rd day, single mitotically dividing cells with heterochromic nuclei could be seen in the islets (Fig. 3). After 7 days, binuclear cells began to appear in the acinar tissue, whereas in the islets there were no visible histological changes by the end of the observations. On the 10th-20th days, acinar cells with true nuclei were still visible in the exocrine tissue, but beginning with the 30th-40th days, the histologic structure of the exocrine part of the pancreas returned to normal.

The results are evidence that transplantation of bone marrow, irrespective of the type of graft, has no effect during the first 3 days on the character of the histologic changes in the pancreatic tissues, but later, repair processes develop in the recipients of the bone marrow, in the form of cell proliferation in both exocrine and endocrine tissues, and in individual cases the process is completed by the formation of binuclear acinar cells and the appearance of mitotic figures in the islet cells.

Despite preservation of the histologic structure of the insular apparatus in the irradiated animals protected with bone marrow, the blood sugar level during the first 4 days fell sharply, probably due either to increased activity of the insular apparatus or to destruction of cells and the entry of insulin into the blood. On the 5th day the blood sugar rose again to reach the control level, and on the 7th day it remained at that level. In the interval between the 7th and 30th days the sugar level fluctuated, falling significantly on the 10th, 20th, and 30th days, evidently indicating either continuing processes of insular tissue destruction, or hyperfunction of the insular tissue as a result of disturbance of endocrine regulatory processes in the irradiated recipients. Starting with the 40th day the blood sugar returned to normal in animals of both groups, and during the subsequent period of observation (until the 90th day) it remained at the control level, evidence that bone marrow transplantation optimizes carbohydrate metabolism, and thus protects the insular apparatus against excessive strain and functional exhaustion.

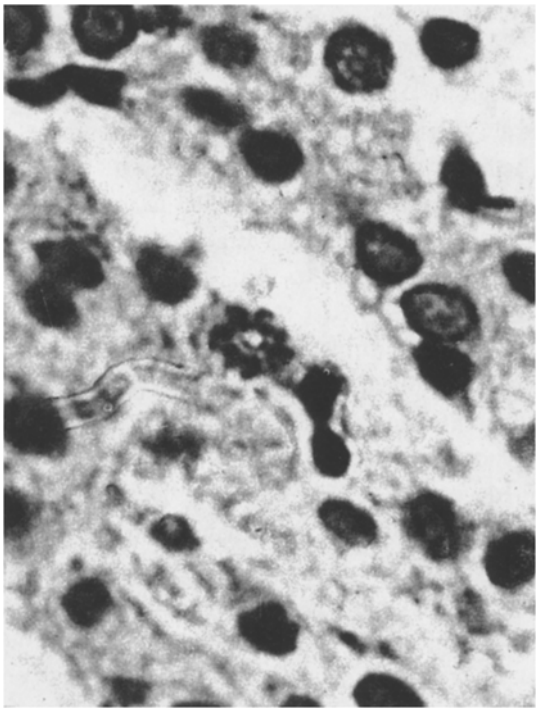

Fig. 3. Heterochromic nuclei of islet cells. Cells in state of mitosis (metaphase, telophase). Third day after irradiation and transplantation of freeze-dried bone marrow. Hematoxylin and eosin. 900×.

The investigations thus showed that both the exocrine and the endocrine tissue of the pancreas is damaged during lethal irradiation of mice, giving rise to a series of destructive changes. Exocrine tissue is more sensitive to the action of ionizing radiation. Acinar cells are damaged sooner than islet cells and pathological changes develop in them more intensively, a fact confirmed by other workers [2, 7, 8]. After bone marrow transplantation into lethally irradiated animals, compensatory and repair processes are observed, and are manifested as mitotic division of the gland cells and the epithelial cells of the ducts. Postradiation reparative processes in exocrine tissue begin in such cases several days later than in the endocrine tissue.

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