ity of the intracellular structures of the hepatocytes. A marked decrease in the hormone content (following bilateral adrenalectomy) led to an increase in the intracellular inclusions of [^{3}H] corticosterone, to a true increase in the number of tracks above the cytoplasmic structures, and to their appearance above the nucleus.

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ELECTRON-MICROSCOPIC AND AUTORADIOGRAPHIC STUDY OF THE PANCREAS AT DIFFERENT STAGES OF POSTMORTEM ISCHEMIA

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Experiments on dogs showed that the maximal allowable period of normothermic postmortem ischemia of the pancreas must not exceed 45 min, for irreversible destructive changes develop later in the ultrastructural formations of cells of the exocrine parenchyma. Cells of the islets of Langerhans are more resistant to ischemia.

KEY WORDS: *pancreas; transplantation; ischemic injury.*

The degree of injury to cells of an organ allografted during the period of normothermic ischemia, lasting from the time of circulatory arrest in the donor to the beginning of perfusion with the preserving solution, has a marked effect on the function of the organ and its survival. The pancreas is an organ which has received least study in this direction, for the high sensitivity of the cells of its acinar system to hypoxia creates difficulties in the way of the appropriate research.

If the duration of ischemia is 60 min, gangrenous-hemorrhagic pancreatitis regularly arises in the graft, whereas after ischemia for 40 min the course of the postoperative period is smoother [1, 2]. Some writers report that after ischemia for 30 min cells of the exocrine parenchyma develop changes consisting of edema, granular and hydropic degeneration, a sharp decrease in the DNA, RNA, and glycogen content, swelling of the mitochondria, disorganization of the cristae, vacuolation of the ergastoplasm, and a decrease in the number of secretory granules [3, 4, 7]. No such information is contained in most papers on this subject, or their authors merely state the duration of the ischemia [5, 6, 8, 9].

EXPERIMENTAL METHODS

Experiments were carried out on 20 mongrel dogs. The animals were anesthetized and killed by injection of air into the heart. The effect of normothermic ischemia on the pancreatic cells was studied between 5 and 60 min after circulatory arrest. Material for investigation was fixed in 2.5% glutaraldehyde, pH 7.4, and then transferred into 1% osmium tetroxide solution, dehydrated in alcohols of increasing strength, and embedded in Araldite.

To obtain autoradiographs, the pancreas was perfused at the same times through one of its main arteries for 1 min with 25 ml of Hanks' solution containing [^{3}H]leucine in a concentration of 10 μCi/ml. After incubation, pieces were removed from different parts of the pancreas and fixed in Bouin's and Carnoy's fluids. Sections 6 μ thick were fixed to a slide, coated with type M emulsion, exposed for 14 days, processed in amidol developer, and stained

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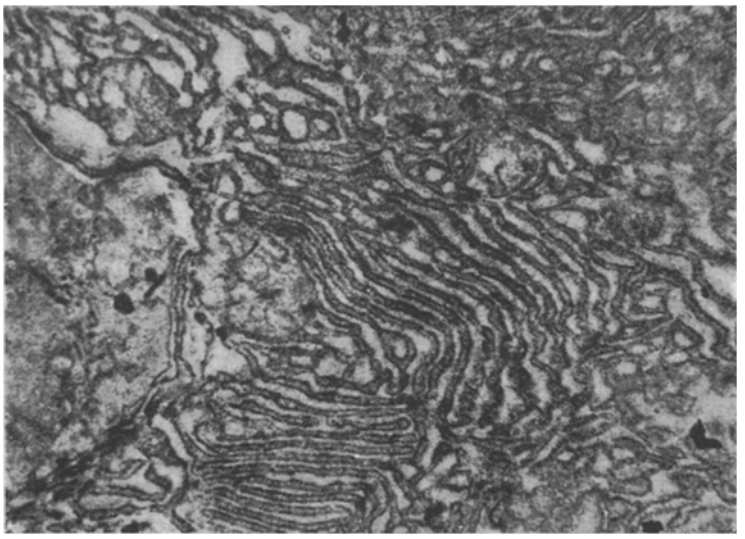

Fig. 1. Pancreatic acinar cells after 5 min of postmortem ischemia. Ultrastructural formations preserved their typical structure; 12,000×.

with hematoxylin–eosin. The number of grains of reduced silver was counted in 50 squares (area of each square 64 μ^2) of the ocular micrometer grid of an MBI-3 microscope, the total number of labeled and unlabeled cells in the area examined being counted. Statistical analysis of the results was carried out by Oivin's method.

EXPERIMENTAL RESULTS

The ultrastructure of the pancreatic cells 5 min after the heart stopped beating was indistinguishable from normal (Fig. 1). An abundant ergastoplasm and 1 or 2 spherical nuclei with numerous pores in their membrane and with a fairly large nucleolus in the center were seen in the basal part of the acinar cells. Many ribosomes were adherent to the outer part of the endoplasmic reticulum. They could also lie freely among the ergoplasmic membranes. The middle and apical parts consisted of a system of membranes, vesicles, and other formations located in the matrix. The mitochondria were concentrated mainly in the basal zones. Zymogen granules were represented by circular osmiophilic formations. Granules not containing zymogen or containing prozymogen also were found.

The canals of the ergastoplasm were dilated in individual acinar cells after 15 min, the number of free ribosomes was increased and the number of membrane-bound ribosomes reduced, and swelling, partial vacuolation, and a decrease in density of the mitochondria were found. About 85% of the cells preserved their typical ultrastructure. After 30-45 min their number fell to 70% (Fig. 2); hypertrophy and destruction of the ergastoplasm, mitochondria, and zymogen granules, detachment of the outer nuclear membrane, condensation and homogenization of chromatin in the nucleus, and sometimes karyorrhexis and complete rupture of the nuclear membrane were observed in the rest. Most of the acinar cells after 60 min showed destructive changes and were in the stage of irreversible disturbances.

Meanwhile the first appreciable changes in the ultrastructure of cells of the islets of Langerhans, the epithelium of the ducts, and connective tissue cells were not found until 45-60 min after the beginning of ischemia, when most cells of the exocrine parenchyma were already nonviable.

The study of the dynamics of accumulation of [^{3}H]leucine and its distribution in the cytoplasm of the cells showed that these processes mainly reflect the dynamics of development of the ultrastructural changes in relation to the duration of ischemia. However, the total number of cells of the exocrine parenchyma incorporating the label did not exceed 45-50%, and cells of the islet tissue, ducts, and connective-tissue stroma were less active still.

The most intensive accumulation of the isotope took place during the first 30 min of normothermic ischemia. Later the number of labeled cells of the exocrine parenchyma remained unchanged and the intensity of the label above them became stabilized. Meanwhile the number

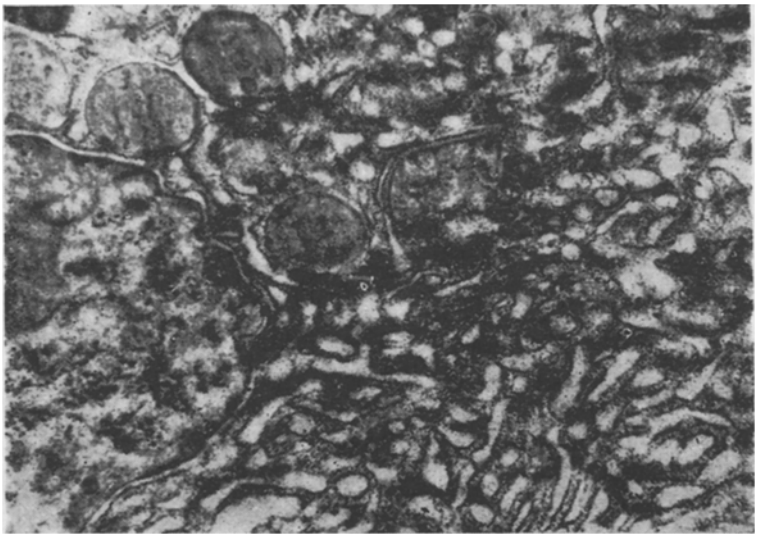

Fig. 2. Slight widening of ergoplasmic reticulum in acinar cell after 30 min of postmortem ischemia; 12,000×.

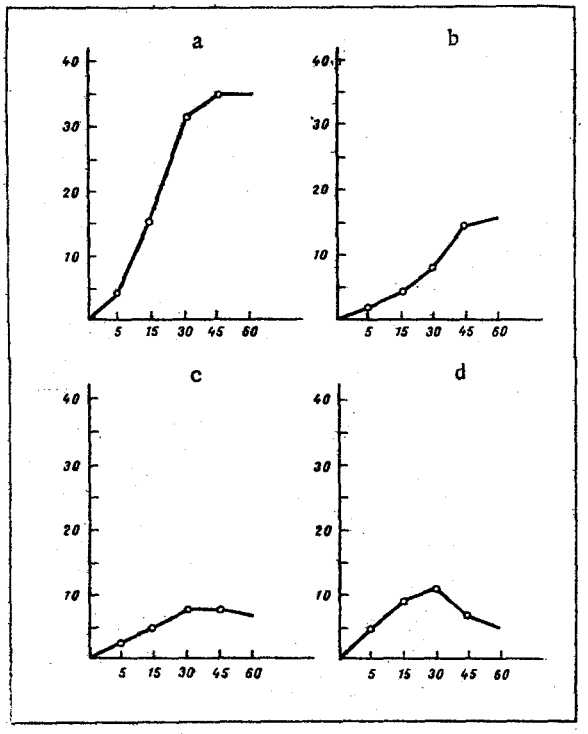

Fig. 3. Dynamics of accumulation of [^{3}H]-leucine in different pancreatic cells during normothermic ischemia. Abscissa) duration of ischemia (in min); ordinate) accumulation of [^{3}H]leucine (in %). a) Cells of exocrine parenchyma; b) cells of islets of Langerhans; c) cells of epithelium of ducts; d) cells of connective-tissue stroma.

of labeled cells of the islets of Langerhans continued to increase, and not until 60 min was a tendency for the process to slow down observed. The number of labeled epithelial cells of the ducts and connective-tissue cells began to decrease after 30 min, which coincided with the development of edema of the connective-tissue stroma of the gland and swelling of the cytoplasm of the acinar cells and fibroblasts (Fig. 3).

It can thus be concluded from these results that the cells of the exocrine parechyma of the pancreas are most sensitive to ischemia, whereas cells of the endocrine apparatus, the epithelial cells of the ducts, and the connective-tissue cells are more resistant to hypoxia. This is evidently connected with specialization of the acinar cells, with their very intensive production of highly active enzymes, activated inside the cell in response to changes in the parameters of the microcirculation and internal milieu and disturbance of outflow from the cell. The experiments showed that these changes reached the threshold level 30-45 min after the beginning of ischemia. From that moment catabolic processes predominate in the cells of the exocrine parenchyma and connective tissue, and autolysis of the gland begins, making it unsuitable for transplantation.

It can be definitely concluded from a comparison of the results of these experiments with data in the literature that one of the chief factors affecting the outcome of allografting of the pancreas in the immediate postoperative period is the degree of injury to cells of the exocrine parenchyma during postmortem normothermic ischemia, the maximal permissible duration of which must not exceed 45 min from circulatory arrest in the donor. The pancreas must therefore be removed and preserved not later than this time.

In the writer's view, the low level of incorporation of [^{3}H]leucine into the cytoplasm of the cells also reflects the degree of their functional activity at the given moment and confirms data in the literature on absence of synchronization of the functions of these cells of the endocrine and exocrine parenchyma.

The data described above may be useful in connection with allografting of the pancreas in experimental and clinical practice.

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