RIBONUCLEASE IN EXPERIMENTAL PANCREATITIS THERAPY

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To improve the results of conservative treatment of pancreatitis the search is continuing at present for new effective preparations which would act on a particular pathogenetic stage of this disease. Much attention is being paid to compounds affecting the intensity of protein synthesis and, correspondingly, the secretory activity of exocrine pancreocytes (EP). In particular, much research has been devoted to the use of cytostatics [4, 5, 6, 11, 12], although we know that high blood enzyme levels in pancreatitis are due to extensive necrobiosis of EP, dyscholia, and parapedesis of zymogen granules (ZG), and not to intensification of secretory activity [13]. In acute pancreatitis and in exacerbations of chronic pancreatitis, on the other hand, the latter is depressed [10]. Ribonuclease (RNase) also has been used as a preparation inhibiting protein synthesis, but it has been reported that in certain doses RNase has a regulatory rather than an inhibitory action on secretory processes in EP [1, 3, 8].

The aim of this investigation was to study the effect of a single injection of RNase on the pathogenesis and morphogenesis of experimental pancreatitis.

EXPERIMENTAL METHOD

Experiments were carried out on 113 rats weighing 180-200 g, divided into three groups: 1) intact rats (n = 10), 2) control rats with experimental pancreatitis (n = 58); 3) rats with pancreatitis receiving RNase (n = 45). Pancreatitis was induced by cooling the splenic segment of the pancreas. Crystalline RNase, diluted with sterile physiological saline, was given as a single intraperitoneal injection in a dose of 1 mg/kg 30 min after injury to the pancreas, corresponding to the edematous stage of pancreatitis. The rats were decapitated after starvation for 18-24 h, 1, 3, 6, and 24 h and 3, 7, 14, 21, and 30 days after production of pancreatitis. Paraffin sections through the pancreas, fixed in 10% buffered formalin (pH 7.0) and 4 μm thick, were stained with hematoxylin and eosin, and by the Jenner–Giemsa and Gram–Weigert methods. Pieces of the duodenal (intact) segment of the pancreas were fixed in a mixture of alcohol, acetic acid, and formalin and embedded in paraffin wax. The area of the nucleus and cytoplasm of EP was determined in paraffin sections 4 μm thick, stained with hematoxylin and eosin, and the relative percentage of binuclear EP was counted. The amylolytic activity of the blood was determined as in [9] and blood levels of trypsinlike activity and of trypsin inhibitor were determined by Shaternikov's method [7].

EXPERIMENTAL RESULTS

In the hemorrhagic stage of pancreatitis (1-6 h) the amylolytic activity of the blood was reduced by RNase [8694.4 g/(h · liter) compared with 14545.0 g/(h · liter) in the control], but in the necrotis (24 h – 3 days) and subsequent stages the

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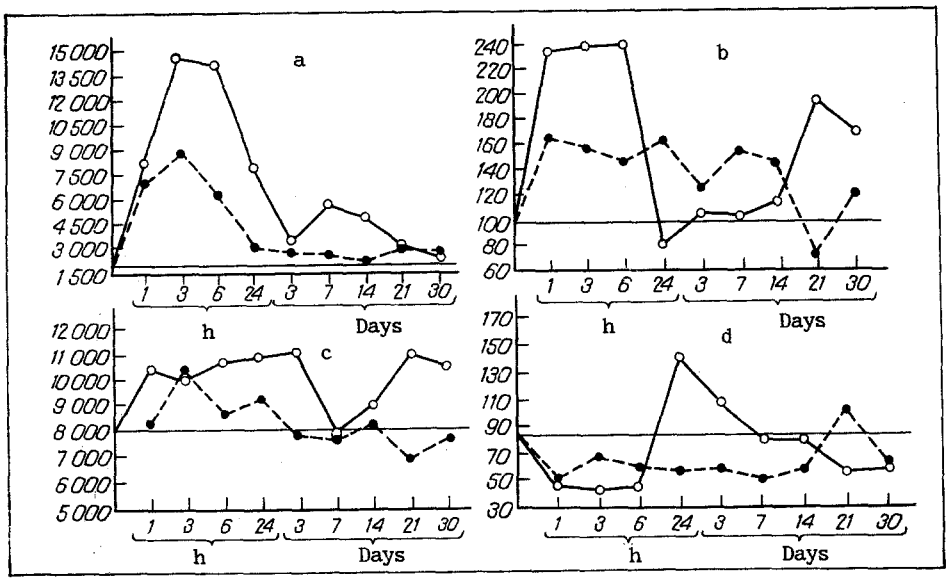

Fig. 1. Dynamics of changes in α-amylase (a) and trypsin (b) activity, trypsin inhibitor (c), and trypsin:inhibitor ratio (d) in experimental pancreatitis (continuous line) and in animals receiving RNase (broken line). Horizontal lines show normal levels. Abscissa, stage of experiment (in h and days); ordinate, enzyme activity (in conventional units of measurement).

blood amylase level returned to normal values (Fig. 1a). The trypsinlike activity of the blood was above the normal level throughout the experiment, except the 21st day (Fig. 1b). Except after 3 h and 7 days the blood level of trypsin inhibitor was lower than in the control (Fig. 1c) and the trypsin:inhibitor ratio was below normal (Fig. 1d).

Macroscopically the pancreas of animals of the control and experimental groups was characterized during the first 6 h of the experiment by marked edema of the damaged segment with hemorrhages of different sizes, amounting in some cases to hemorrhagic saturation. Later necrosis of the parenchyma of the pancreas was formed in both groups, with widespread foci of steatonecrosis, and the dying tissues became encapsulated, with the formation of a pseudocyst. The contents of the pseudocyst consisted of purulent masses (caseous in the control), which were mainly absorbed by the 21st-30th days of the experiment (in the control a pseudocyst was observed after 30 days inclusive).

Microscopic examination of the zone of damage after 1 h revealed disturbances of the complex structure of the acini, eosinophilic and basophilic transformations of the cytoplasm of EP, lysis of their apical zones with widening of the lumen of the acinus, and areas of total lysis and granular degeneration of EP. Focal vacuolation of the basal zones of the cytoplasm of EP, periacinar edema, diffusion of ZG, and signs of parapedesis were observed. Interlobular edema was well marked and rich in fibrin threads. Congestion, stasis of erythrocytes, microthrombosis of vessels of the microcirculatory bed, and thrombosis of veins were noted. Massive hemorrhages were located mainly in the interlobular stroma, beneath the capsule, and less frequently inside the acini. Small concentrations of polymorphonuclear leukocytes (PML) were found. An identical picture also was observed in the control, although intraacinar hemorrhages and hemorrhagic saturation predominated in this case and no PML were seen. After 3-6 h the eosinophilic transformations of the cytoplasm of EP were increased in the experimental group, their granular degeneration and lysis were intensified, and foci of coagulation necrosis appeared. In the necrobiotically changed EP no zymogen was found, and the microcirculatory disturbances described previously were joined by fibrinoid necrosis of the walls of individual vessels. Similar changes were present in the control, but the ZG in the cytoplasm of the necrobiotically changed EP persisted longer and evidence of dyscholia and parapedesis was more marked.

After 24 h and on the 3rd day of the experiment, coagulation necrosis of the parenchyma of the pancreas was complete in both groups, the only difference being that in the experimental group lysis of the necrotic EP was more marked and individual interacinar capillaries were still present, with focal concentrations of PML in and around the zone of necrosis. In the control, against the background of marked perifocal inflammatory infiltration, PML did not penetrate into the zone of necrosis. Formation of the capsule of the pseudocyst was complete by the 7th day of the experiment, with early coarsening and hyalinosis of the collagen fibers in the pancreas of the control group of rats. The structure of the capsule of the pseudocyst in animals of the experimental group was dominated for a long time by cells — macrophages, lymphocytes, and

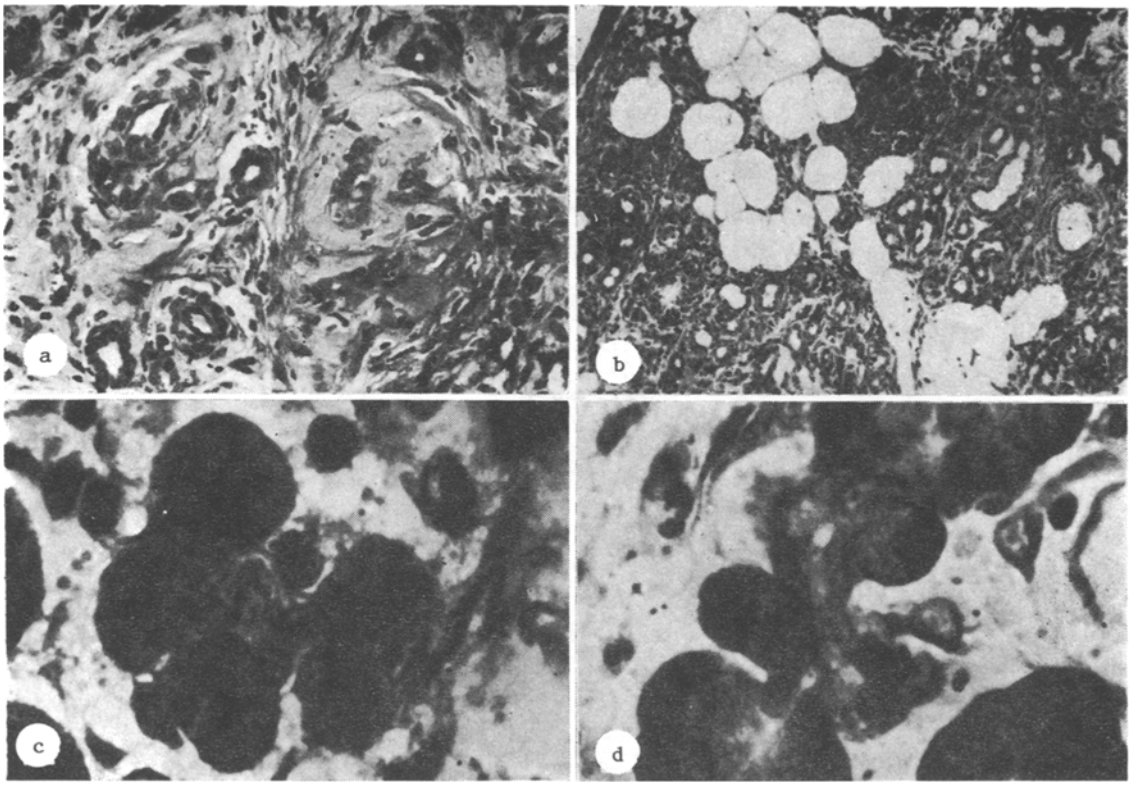

Fig. 2. Effect of RNase on pancreas of rats with pancreatitis. a) Zone of injury after absorption of pseudocyst (21 days). Efferent ducts and tubular complexes, "immured" in hyalinized connective tissue. Stained by Jenner–Giemsa method. 200×; b) Boundary zones on 3rd day. Foci of lipomatosis against a background of tubular transformation of acini. Stained by Jenner–Giemsa method. 100×; c) Boundary zone on 7th day. Concentration of isolated BP, containing zymogen, around cells resembling centroacinar cells; acinus formation. Stained by Gram–Weigert method. 1000×; d) Boundary zone on 7th day. Adhesion of isolated EP by apical poles to small efferent duct. Stained by Jenner–Giemsa method. 1000×.

labrocytes; microabscesses were found, and focal hyalinosis of collagen fibers was observed on the 21st-40th days of the experiment. In cases when the pseudocyst was completely absorbed, fatty and hyalinized connective tissue appeared in the zone of damage to the pancreas, with vessels and degenerating tubular complexes included in it (Fig. 2a).

In the boundary zones of the pancreas, as a result of the circulatory disorders necrobiotic changes developed similarly in EP. On the 3rd day of the experiment tubular transformation of the acini was observed in the boundary zone, and in the experimental group it was more widespread in character, individual groups of acini being replaced by fatty tissue (Fig. 2b). Attention is drawn to the phenomenon of concentration of isolated EP around cells resembling centroacinar cells, the formation of new acini, and adhesion of isolated EF by their apical pole to a small efferent duct (Fig. 2c, d). Tubular complexes, with a structure similar to that of efferent ducts, appeared in bands of connective tissue; concentrations of PML were often found. Tubular complexes in the pancreas of the control group of rats were mainly dying and replaced by connective tissue, and some of them underwent secondary differentiation.

Acini of the intact segment of the pancreas in animals of the experimental group were characterized until the 3rd day of the experiment by a high content of ZG in the cytoplasm of EP, possible evidence of delay of excretion of the end product. Later marked polymorphism of the acini was observed: acini were seen with a large, moderate, or small number of ZG. Starting with the 14th day of the experiment, foci of necrosis of the cytoplasm and diffusion of ZG were found in some EP, some of the dilated efferent ducts contained albuminous plugs, and foci of lymphohistiocytic infiltration of the stroma were observed. Some increase in the number of binuclear EP was observed under the influence of RNase on the 3rd-14th day, returning to normal on the 30th day (Fig. 3a). The area of the nucleus (Fig. 3b) and cytoplasm of EP (Fig. 3c) exceeded that of the intact rats throughout the experiment, and the nucleocytoplasmic ratio fell after 24 h and on the 30th day as a result of

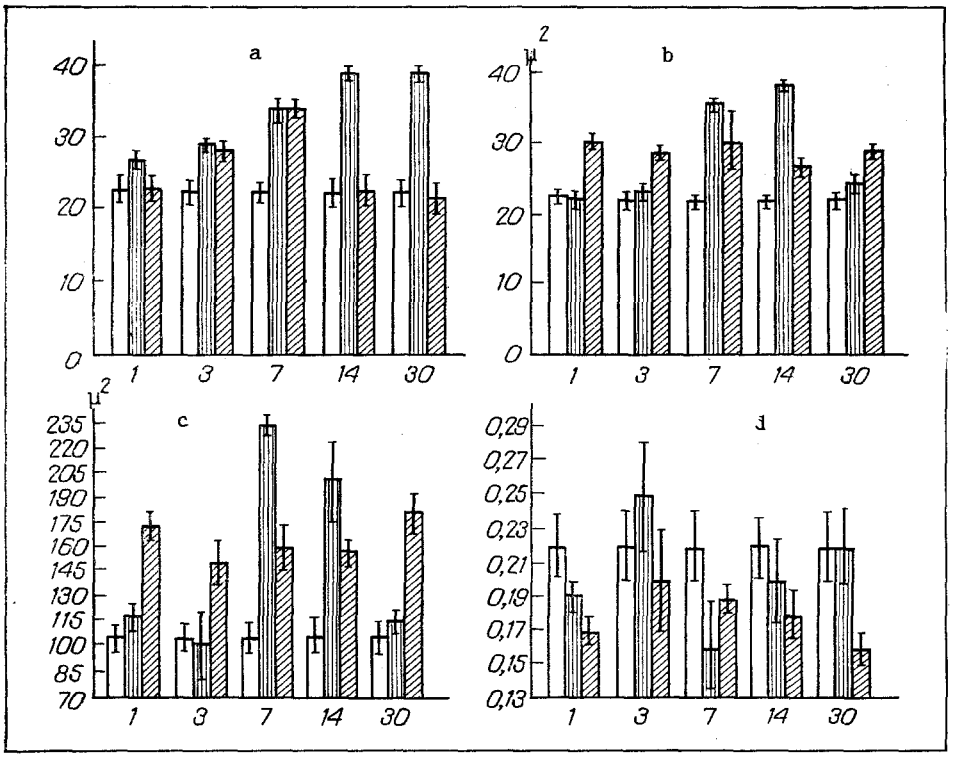

Fig. 3. Morphometric parameters of EP of duodenal (intact) segment of pancreas. a) Relative percentage of binuclear (EP); b) area of nucleus of EP (in μm^2); c) area of cytoplasm of EP (in μm^2); d) nucleocytoplasmic ratios of EP. Unshaded columns — intact rats; vertically shaded — pancreatitis; obliquely shaded — pancreatitis + RNase. Abscissa, times of experiment (in days).

the greater increase in size of the cytoplasm (Fig. 3d). The morphometric parameters of EP in the viable regions of the pancreas indicated a mild and stable degree of hypertrophy of EP under the influence of RNase.

Thus, a single injection of RNase in the edematous stage of pancreatitis, while not preventing the development of the hemorrhagic stage, did give rise to some reduction of blood levels of pancreatic enzymes. The trypsin:inhibitor ratio remained below normal throughout the experiment. Under the influence of RNase absorption of the pseudocyst took place more rapidly, due in all probability to delay of maturation of the connective tissue in its capsule and also to intensification of inflammatory infiltration, with liquefaction and elimination of the structural components of the pseudocyst. Ribonuclease stimulates tubular transformation of the acini of the boundary zone while at the same time promoting early lipomatosis and fibrosis of the zones of the pancreas mentioned above. In the intact segment of the pancreas mild hypertrophy of EP developed under the influence of RNase, and against this background the morphological features of chronic pancreatitis were formed.

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EFFECT OF STRENUOUS PHYSICAL EXERCISE ON DESTRUCTIVE AND REPARATIVE PROCESSES IN THE RAT LIVER

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Most physiological, biochemical, and morphological investigations have been devoted to the study of the response of skeletal muscle to physical exercise. However, for a correct training program to be formulated it is necessary not only to study the state of the muscles but also to have some idea on how other vitally important organs and, in particular, the liver, respond to physical exercise. An important role in the solution of this problem must be played by the method of electron microscopy, for the limited light-optical evidence of the state of the liver during physical exercise demonstrates only an increase in the number of binuclear cells and differences in RNA and glycogen levels [2, 5, 6, 10].

EXPERIMENTAL METHOD

Experiments were carried out on 19 male Wistar rats weighing 200-300 g. The rats were trained to run on a treadmill at a speed of 35 m/min, which is regarded as work of high intensity [9]. One group of rats ran on the treadmill 5 times a week for 1 month; the other group for 1.5 months. Animals of the same age and sex, which were untrained, served as the control. All animals received food [9] and water ad lib. Pieces of liver were fixed in 2.5% glutaraldehyde solution in S-colloidal buffer at pH 7.2-7.4, and postfixed with osmium tetroxide. After training for a month material was taken immediately after the last training session (four rats) and 24 h later (five rats); two rats served as the control. In group 2 material was taken (four rats) immediately after the last training session, and four rats served as the control. The material was embedded in Epon and sections were stained by Reynolds' method and examined in the JEM-100C electron microscope. Relative volumes of mitochondria, rough endoplasmic reticulum, peroxisomes, and glycogen were calculated by the use of a random step grid [8].

EXPERIMENTAL RESULTS

A study of the ultrastructure of the liver cells of animals trained for 1 month and killed immediately after the last training session revealed no changes in the nuclei compared with the control. They contained small nucleoli, the heterochromatin was uniformly distributed throughout the nucleus, and some binuclear hepatocytes were seen. Mitochondria were more numerous in all hepatocytes than in the control (Table 1) and the principal changes took place in them and in the rough endoplasmic reticulum (RER). The degree of these changes differed not only in different hepatocytes of the same animal, but also in the cytoplasm of the same hepatocyte, suggesting individual sensitivity at both cell and organelle level.

The mitochondria showed a more or less palely stained matrix with the appearance of myelin figures (Fig. 1). Different parts of the same mitochondrion could react differently. Some mitochondria with swollen matrix and whose cristae

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