COMPARISON OF RIBONUCLEASE ISOLATED FROM THE LIVER AND PANCREAS OF BURNED AND INTACT RATS

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Alkaline RNase was isolated from the pancreas and liver of intact and burned rats and purified 1400 and 2000 times, respectively. The pH-optimum of enzyme activity was 7.6-7.8. Biosynthesis of RNase under normal conditions and after burns was studied by incorporation of labeled amino acids. Inhibition of synthesis of alkaline RNase was found in both organs of the burned animals. The half-life of RNase from the liver of intact rats was 65 min and from the liver of burned animals 100.8 min. Enzymic activity of preparations of alkaline RNase from the liver and pancreas of intact and burned animals did not differ significantly.

KEY WORDS: *rat liver and pancreas; ribonuclease; burns.*

Investigation of nucleic acid metabolism in burns has shown that in some intact tissues the ribonuclease (RNase) activity is reduced [1].

To study the causes of this phenomenon, alkaline RNase was isolated from the pancreas and liver, and the intensity of biosynthesis of the enzyme, its half-life (reflecting the rate of its renewal), and catalytic activity were determined, and the results obtained were compared in healthy and burned animals.

EXPERIMENTAL METHOD

Experiments were carried out on intact and burned rats. Each group contained 15-20 animals. A 3rd-degree burn (20% of the body surface) was produced by a flame. Biosynthesis of RNase was estimated from incorporation of lysine-C^{14} or glycine-C^{14} (injected intraperitoneally in doses of 200,000 and 250,000 counts/min/g body weight respectively) *in vivo*. Incorporation of glycine into liver RNase was investigated 7-120 min after injection. The results were expressed in counts/min/mg protein.

Alkaline RNase was isolated from the pancreas and liver by means of fractional precipitation and chromatography on Amberlite IRC-50 [5], DEAE-cellulose [6], and carboxymethylcellulose [2], and by gel filtration of Sephadex G-50 and G-25. The RNase isolated by these methods did not contain protein impurities [3]. RNase activity was determined spectrophotometrically [4].

EXPERIMENTAL RESULTS AND DISCUSSION

The sample of RNase isolated from the pancreas and liver of the intact and burned rats gave a single peak on electrophoresis and had a clearly defined pH-optimum of action at 7.6-7.8. During purification the activity of the enzyme per milligram protein was increased by 1400 times for the pancreas and 2000 times for the liver. The specific activity of the

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TABLE 1. Specific Radioactivity (in counts/min/mg protein) of RNase and Its Half-Life

Time (in min)	Labeled precursor	Liver			Pancreas		
		control	burns	change in % of control	control	burns	change in % of control
7	Glycine	9236	3882	—58	—	—	—
20	Glycine	11 250	5700	—50	—	—	—
60	Glycine	8833	5100	—43	—	—	—
120	Glycine	4350	3220	—26	9900	7540	—24
120	Lysine	2460	1890	—24	8585	6345	—26
Half-life	Glycine	65	100,8	+55%	—	—	—

RNases isolated from the liver and pancreas of burned and intact animals did not differ significantly.

However, a marked decrease was observed in the rate of incorporation of labeled amino acids (lysine-C^{14} and glycine-C^{14}) into RNase of the liver and pancreas of the burned animals (Table 1). The fall in the level of utilization of labeled amino acids in these animals was about the same for both organs, and for each amino acid it averaged 25% (2 h after injection of the precursor); this points to the slowing of RNase biosynthesis in the liver and pancreas of the burned animals.

Difficulties arise in the study of the formation of specific proteins in the intact organism, for the specific radioactivity of a protein after incorporation of labeled amino acids *in vivo* can vary not only as a result of renewal of the protein, but also through changes in the reserves of free amino acids. To rule out this source of error, the rate of incorporation of label was investigated over a period of time and the specific activities of the liver RNase of the control and burned animals were compared 7, 20, 60 and 120 min after injection of the labeled glycine. For instance, 20 min after injection of isotope, when the highest percentage of utilized glycine was found in the protein, RNase synthesis in the liver of the burned animals was reduced by 50%, i.e., in the early periods of the investigation the difference between the levels of radioactivity was greater.

The reduction in RNase biosynthesis after burns may be manifested as a decrease in the total activity of the organ.

The half-life for liver RNase, calculated by the formula in [7], was 65 min for intact and 100.8 min for burned animals. Besides inhibition of the biosynthesis of alkaline RNase in the liver after burns, a lengthening of its circulation is thus also observed.

One of the main reasons for the decrease in the initial RNase activity in the liver tissue after burns is thus inhibition of the synthesis of the enzyme itself. Since after heat injury RNase biosynthesis is delayed not only in the liver, but also in the pancreas, this suggests that in this pathological process there are common mechanisms which affect the protein-synthesizing system of this enzyme.

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