MODES OF INSULIN RESYNTHESIS IN LIVER

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Liver was established to be able both to resynthesize insulin of its chains A and B preformed in pancreas and to bring about some steps of chain A formation.

As it is known, insulin going from pancreas into the liver undergoes in the latter the action of glutation-insulin-transdehydrogenase. Since the time that enzyme had been discovered in liver (Tomizawa, Halsey, 1959) a great series of studies have been carried out, where glutation-insulin-transdehydrogenase was shown to catalize the reductive breakdown of S-S-bonds of insulin resulting in formation of free chains A and B (Tomizawa, 1962; Katzen, 1962).

Later (Katzen et al., 1963), this reaction was found to be reversible, and in the presence of glutation in oxidized form the mentioned enzyme reduced disulfide bonds causing the resynthesis of biologically active hormone.

Proceed from the above data we considered to be interesting to isolate labelled in vivo insulin from liver tissue with the use of radioamino acid. Such an insulin preparation in the case of comparison between the values of radioactivity in this preparation and in that isolated from pancreas would permit to judge whether resynthesis of that hormone in liver occured from the preformed chains

in pancreas or the resynthesis of the chains of insulin also occured in the liver from smaller precursors.

The proof that the liver tissue splitted the hormone into the chains A and B with subsequent resynthesis of insulin preformed in pancreas would be the changes in the ratios of radioactivity values of the chains A and B of insulin.

At the same time it could be possible to think that if insulin found in liver were the result of resynthesis of the chains A and B formed in enzymatic breakdown of -S-S-bonds of insulin coming from pancreas, the specific activities of the same named chains of insulin isolated from liver and pancreas would have been similar. Discrepancy between these values would suggest that metabolism of insulin chains themselves also took place in the liver.

Methods

To solve the raised problem male rats weighing 200-250 g were intraperitoneally injected with ^{14}C-glycine with specific activity 130 μC/g at a rate of 200 μC for 100 g animal weight. Two hours later the animals were killed by decapitation, tail part of the pancreas was quickly taken out and the tissue batches 3 g in weight were prepared from 6-7 animals. Isolation of insulin was accomplished by acid alcohol extraction (Petting, 1958). 10 mg of crystalline bovine insulin was added to the isolated crude extract as a carrier. Protein material was repeatedly precipitated under isoelectric point of insulin (Morenkova, 1965). Then insulin was oxidated (Sanger, 1949) and splitted into polypeptide chains A and B by means of paper electrophoresis. The chains were located by means of colouration of edge strip of electrophoregram with diazotized sulfanilic acid (Brown, Sanger, Kitai, 1955). Then the chains were eluated with O.OlN HCl, one part of the eluate was

used for measuring the radioactivity with a gas flow 4 counter the other, aliquot part, was taken for determining quantitative values of each chain (Lowry, 1951).

Insulin isolation from liver was accomplished in the following way. Sections of liver tissue 0.6 - 0.7 g for each animal, the total weight amounting to 3.0 g, were grinded thoroughly in 5 per cent trichloracetic acid and dried with mixture of alcohol and ether taken in a ratio of 1:4 and with ether. Then the dried total liver proteins were being extracted with 10 ml 0.2 M solution of NH_4HCO_3 for an hour while slow regular stirring.

Nonsoluble part of protein material was removed by centrifugation; 10 mg of crystalline bovine insulin was added to the supernatant layer and distributed upon a column of Sephadex G-50. 0.2 M solution of NH_4HCO_3 was used as the eluent (see Fig. 1).

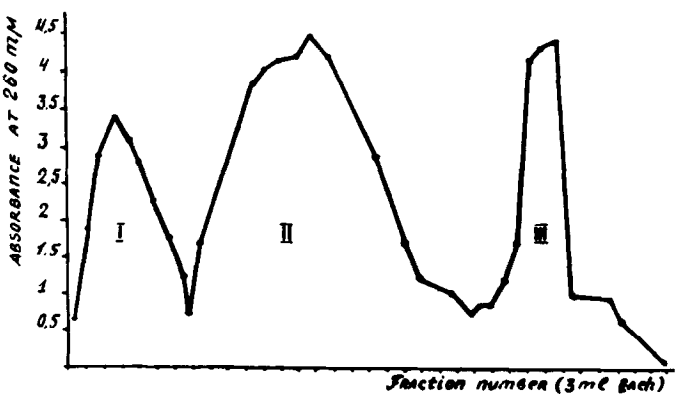

Fig. 1. Column chromatography of soluble liver proteins.

Passing the soluble liver proteins through the column resulted in three peaks with electrophoretic mobility comparable with that for crystalline bovine insulin.

The protein material of the second peak (II) had mobility similar to that of the crystalline bovine insulin. The eluate of that peak was evaporated till 2 ml remained, and the protein

was precipitated by acetone. 6-7 mg of dry protein preparation was oxidated by performic acid (Sanger, 1949) and separated into the chains A and B. The chains migrated with a speed similar to those of crystalline bovine insulin. Radioactivity in the chains and the quantitaty of them were determined in the same way as used for the chains of insulin isolated from pancreas.

Since glycine is converted to serine (Sakami, 1955, Konikova, Morenkova, Kritzman, 1965), we determined radioactivity attributed to serine and glycine residues in insulin (and its chains) isolated from both the tissues. For that purpose insulin and its chain B were subjected to complete acid hydrolysis with consequent radiochromatographic analysis of the hydrolyzates. Radioactivities of serine and glycine residues of the chain A were computed.

Results and discussion

The results obtained are given in table 1.

Table I

Values of specific activities of the chains A and B of insulin isolated from pancreas and liver (c.p.m./ μM amino acid residue of the chain)

NN experiments		Exp. 1			Exp. 2		
Tissue	Amino acid residue	B chain	A chain	B/A	B chain	A chain	B/A
pancreas	glycine	2940	708	4.1	1800	640	2.8
	serine	8820	354	25	5400	320	16.9
liver	glycine	2340	2076	1.1	1560	1440	1.09
	serine	7020	1036	6.7	4680	720	6.5

The results presented show that the specific activity both glycine and serine residues of the chain A from liver is considerably higher than that of the chain A from pancreas and differs insignificantly for the chains B.

If in the liver occurred only resynthesis of insulin of the chains A and B formed as the result of decay of insulin that had come from pancreatic tissue, it would be most probable to expect lowering of specific activities amino acid residues of the chains of liver insulin at the expense of dilution of the "label" by the "pool" chains that had existed in the tissue. It would also be reasonable to suggest the presence of similarities in the "pools" of the preformed chains for both the tissues. In that case the specific activities amino acid residues of the chains A and B of insulin from liver and pancreas would be close to each other.

The experimental results obtained, which evidenced lower specific activity amino acid residues of the chain B of liver insulin in comparison that of the chain B, isolated from pancreas, would allow that insulin degradation in liver consisted only in breakdown of -S-S-bonds, and the formed chains A and B resynthesized again to form biologically active hormone. This is at variance, however, with the results obtained for the chain A of liver insulin the specific activity both glycine and serine residues of which is several times the specific activity that the chain A of insulin from pancreatic tissue.

Such difference in values of specific activities amino acid residues of the chain A suggest that pancreatic chain A is not an immediate precursor of the chain A of liver insulin.

It may be assumed that the synthesis of the chain A of insulin not wholly occurs in pancreatic tissue, but some steps in formation of that polypeptide can be accomplished in the liver tissues as well.

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