BIOCHEMISTRY AND BIOPHYSICS

INTERCONVERSION OF LIVER AND SERUM PROTEINS IN HOMOGENATES OF RATS' LIVER

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In a previous paper [4] it was shown that after intravenous injection of rats with isogenous serum proteins labeled with methionine-S^{35}, radioactivity is found in the proteins of the liver, including the insoluble ones, very rapidly. The conversion of serum proteins into liver proteins takes place mainly without the preliminary breakdown of the injected proteins into amino acids. At almost the same time as ourselves, a number of workers [1, 5, 9] from analogous experiments on animals and also with tissue cultures [7, 8, 10, 12], came to the same conclusion.

In order to make a further study of the mechanism of this conversion, it was important to find out how the same process takes place in isolated liver tissue, and in particular in homogenates of the liver.

EXPERIMENTAL METHOD

Experiments were performed on white rats. The proteins were labeled in vivo by injecting the donor rat with 0.5 mC of glycine-1-C^{14}. The labeled serum proteins of the donor were subdivided by the accepted laboratory method of electrophoresis on starch [2] in a veronal buffer (pH = 8.6, μ = 0.18) into 3 fractions: albumins, α-globulins and β + γ-globulins. The soluble liver proteins of the donor were extracted by a method described previously [3], and were also subjected to electrophoresis on starch. As a preparation of "liver globulins" we used proteins whose electrophoretic mobility was close to the electrophoretic mobility of the β- and γ-globulins of the serum. A test of the homogeneity of the serum and liver protein fractions obtained showed, by means of paper electrophoresis, that the serum albumins contained no traces of globulins and that the β- and γ-globulin fractions contained no traces of albumins. The α-globulin fraction of the serum was found to be nonhomogeneous and so was not used for further investigation.

Incubation of the labeled proteins with liver homogenates was carried out in Ringer-bicarbonate buffer (not containing Ca^{++} ions; pH = 7.4) in an atmosphere of 95% O_2 and 5% CO_2 for 2 hrs at 37°C. Before incubation the labeled proteins were dialyzed and the nonprotein label did not exceed 0.6%. The amount of homogenate in each test was equivalent to 1.5 g of liver. The radioactivity of the added proteins varied from 150,000 to 300,000 imp/min per test. To each test, in addition, was added 3 mg of ATP and 10 mg of glucose.

After incubation the proteins were extracted with physiological saline and separated into albumins and globulins as described above. The separated protein fractions were extracted from the starch and precipitated with trichloroacetic acid, and after the usual treatment [4] their radioactivity was determined by means of a gas flow meter.

EXPERIMENTAL RESULTS

As can be seen in Fig. 1, after incubation of the labeled serum albumins with liver homogenates, the radioactivity of globulins extracted from them was markedly higher than before incubation. On adding to the incubation medium a mixture of all the amino acids necessary for protein synthesis, except glycine, the radioactivity in the extracted globulins was considerably increased. The addition of nonlabeled glycine to this mixture (4 mg of the D–L-form per test) reduced the radioactivity, but in this case the radioactivity of the globulins was higher than in the test before incubation. The fall in the radioactivity of the isolated globulins after the addition of unlabeled glycine to the homogenate demonstrates that synthesis of globulins takes place mainly from free amino acids. Nevertheless the fact that in this case the radioactivity remains higher than before incubation gave grounds for considering that part of the labeled serum albumin was converted into liver globulin without undergoing extensive breakdown into amino acids.

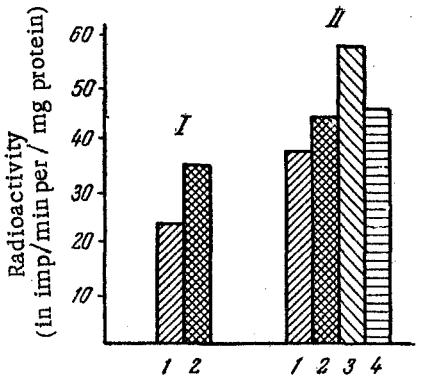

Fig. 1. Radioactivity of the globulins after incubation of liver homogenates from rats with serum albumins labeled with glycine-1-C^{14}. 1) Before incubation; 2) after incubation; 3) after incubation and in the presence of a mixture of the following amino acids: tryptophane, arginine, threonine, lysine, leucine, valine, histidine, isoleucine, phenylalanine, tyrosine, methionine, glutamic acid, aspartic acid, alanine, serine, proline and cystine (at the rate of 2 mg of the L-form of each amino acid per test); 4) after incubation and in the presence of an amino acid mixture including unlabeled glycine (4 mg of the D–L-form). Radioactivity of the added albumins: I) 173,000 imp/min in the test; II) 290,000 imp/min in the test.

Different results were obtained after incubation of labeled serum globulins. As can be seen from Fig. 2, in this case the radioactivity of the albumins isolated after incubation not only was no higher than before incubation, but on the contrary was considerably reduced. This fall may be explained by the fact that the incubated globulins contained a small trace of labeled albumins, undetectable by electrophoresis, which was mixed on incubation with the unlabeled liver albumins. If synthesis of labeled albumins from labeled globulins from the serum did not occur, a mixing of this sort must lead to a fall in the radioactivity of the albumin fraction was A marked increase in the radioactivity of the albumin fraction was observed after incubation of liver homogenates with labeled liver globulins (Fig. 3). Both in this case and in that of incubation of labeled albumins, the addition of unlabeled glycine to the incubation medium slightly reduced the radioactivity, but even so left it higher than that of the albumins before incubation. This shows that the process of formation of serum albumin from liver globulins in small quantities in the homogenates may take place without breakdown to amino acids.

The results obtained, that the serum albumins are far more rapidly converted in liver homogenates than the serum globulins, are in full agreement with the recently published findings of Roberts and Kelley [11], who report that serum albumins added to liver sections are oxidized and utilized for glyconeogenesis much more rapidly than serum globulins.

There are points of disagreement, however, between our findings and those of the authors cited on the mechanism of conversion of serum albumin into globulin. From our findings, as pointed out above, this conversion took place in liver homogenates mainly after breakdown of the albumin to amino acids, with subsequent synthesis of globulins. According to Roberts and Kelley the conversion of albumin which takes place in liver sections does so without breakdown into amino acids. It must also be pointed out that in their latest report, Campbell and Stone [6] came to the conclusion that in vivo conversion of injected serum proteins into liver proteins takes place only after breakdown into amino acids.

Thus in the literature so far there are two opposing points of view on the question of the mechanism of conversion of injected serum proteins into liver proteins. Our own findings persuade us that depending on the conditions present in the body, either the first or the second process may take place. One of the main factors determining the course of this process may be the proteolytic activity of the tissues. If this activity is great, as for example in liver homogenates, synthesis takes place mainly from amino acids liberated as a result of breakdown

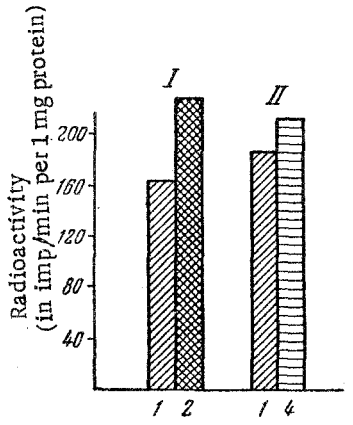

Fig. 2. Radioactivity of albumin after incubation of liver homogenates from rats with serum globulins labeled with glycine-1-C^{14}. Radioactivity of the added globulins in each test 160,000 imp/min. Interpretation as in Fig. 1.

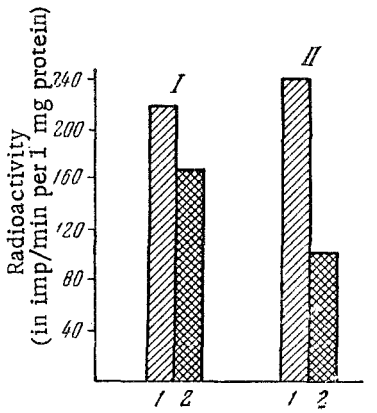

Fig. 3. Radioactivity of albumin after incubation of liver homogenates from rats with liver globulins labeled with glycine-1-C^{14}. Radioactivity of added globulins: I) 163,000 imp/min in the test; II) 230,000 imp/min in the test. Interpretation as in Fig. 1.

of proteins; if however the intensity of proteolysis is not great, then the formation of liver proteins from injected serum proteins takes place mainly by conversion without preliminary breakdown of the injected protein to amino acids.

SUMMARY

It was shown with the aid of proteins labeled by glycine-1-C^{14} in vivo that serum albumin is transformed into globulins in the homogenates of rat's liver. This takes place mostly after disintegration to amino acids of the added albumins. In these conditions hepatic globulins are transformed into albumins. There is no production of albumins from the serum β- and γ- globulins added to the homogenates.

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* Original Russian pagination. See C. B. translation.