SERUM ALBUMIN SYNTHESIS BY ISOLATED RAT LIVER MICROSOMES*

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There is considerable indirect evidence that serum albumin is synthesized by the microsomal fraction of liver cells (Campbell, 1960). Recently, Campbell, et al. (1960) demonstrated clearly the incorporation of radioactive amino acids into serum albumin associated with isolated rat liver microsomes. However, the albumin remained bound, and was not released from the microsomes. We have now found conditions which result not only in the incorporation of amino acids into serum albumin, but also in the release of labeled serum albumin from the particles. This incorporation and release occurs with either microsomes or ribosomes, and the incorporated amino acid appears to be distributed throughout the serum albumin molecule.

Methods. Microsomes were isolated from the livers of approximately 100 g. Sprague-Dawley female rats by a procedure similar to that described by Zamecnik and Keller (1954). The only modification was the use of higher KCl concentration (0.065 M) in the homogenizing medium. Ribosomes were prepared by treating 10 ml. of the microsomal preparation with 1 ml. of 5% deoxycholate at pH 8.6. The resulting ribosomes were sedimented at

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105,000 x g for 60 min., washed in the homogenizing medium, and resedimented at 105,000 x g. The microsomes or ribosomes were incubated in a medium containing 2 μmoles sodium ATP, 10 μmoles potassium phosphoglycerate, 70 μmoles tris(hydroxymethyl)aminomethane-HCl (pH 7.8), 14 μmoles $MgCl_2$, 49 μmoles $KHCO_3$, 91 μmoles KCl, 350 μmoles sucrose, 0.18 μmoles leucine-4-5-H^3 (55,000,000 cts/min.), 30 μg. crystalline adenyl kinase, 1 μmole diphosphopyridine nucleotide, 5 mg. microsomal or ribosomal protein, and 15 mg. non-particulate protein in a total volume of 2 ml. After incubation for 30 min. at 37° C, the reaction mixture was diluted to 12 ml. with cold homogenizing medium, and the particles were sedimented at 105,000 x g for 120 min. Thirty mg. of carrier rat serum albumin were added to each incubation mixture, and the albumin was isolated by aqueous ethanol after precipitation with trichloroacetic acid as described by Debro, et al. (1957).

Carrier rat serum albumin was prepared by the method of Debro, et al. (1957), and purified by chromatography on diethylaminoethyl-cellulose. The albumin exhibited only one electrophoretic component in 0.1 M barbital (pH 8.6) and in 0.1 M tris(hydroxymethyl)aminomethane (pH 7.6). Anti-rat serum albumin was prepared by repeated injection of rabbits with the alum precipitate of the purified rat serum albumin. Anti-bovine albumin was obtained commercially. Hydrolysis of serum albumin with pepsin at pH 2.0 proceeded for 6 hrs. at 20° C. The resulting peptides were separated by paper chromatography with butanol-acetic acid-water (4:1:5) as described by Ryle, et al. (1955). Protein precipitates (reprecipitated from 1 M KOH and three-times washed with 12% trichloroacetic acid) and peptide bands (separated by paper chromatography) were dissolved in 1 M p-(diisobutylcresoxyethoxyethyl)dimethylbenzylammonium hydroxide in meth-

anol and suspended uniformly in toluene containing 2, 5-diphenyloxazole and p-bis(2-(phenyloxazolyl))-benzene. Radioactivity of the samples was then determined with a liquid scintillation counter.

Results. When rat liver microsomes are incubated under the conditions described by Zamecnik and Keller (1954), the microsomes rapidly incorporate amino acid into protein associated with the microsomes, but relatively little labeled protein is released from the microsomes (Table I).

TABLE I

INCREASED LABELING OF MICROSOMAL AND NON-PARTICULATE PROTEINS AS A RESULT OF ALTERED EXPERIMENTAL CONDITIONS

System	Total Radioactivity* (cts/min.)	
	Microsomes	Non-particulate
Original reaction system**	32,880.	5,480.
Phosphoglycerate concentration reduced to 0.005 M	65,760.	6,165.
Adenyl kinase (30 μg.) added	98,640.	8,220.
Potassium ion concentration increased to 0.07 M	246,600.	143,850.
DPN (1 μmole) added	411,000.	308,000.

* Amino acid incorporation in the absence of ATP and phosphoglycerate was less than ten per cent of the incorporation in the presence of these substances.

** Reaction system of Zamecnik and Keller (1954).

The relative amount of radioactivity incorporated into microsomal protein is very similar to that observed by Zamecnik and Keller (1954). If the phosphoglycerate concentration in the reaction mixture is decreased to 0.005 M, and if adenyl kinase is added, there is approximately a three-fold increase in the incorporation of amino acids by microsomal proteins, but little increase in the labeling of the non-particulate proteins. However, an increase in potassium ion concentration (to 0.07 M) produces a two-fold increase in amino

acid incorporated into microsomal protein, but a 17-fold increase in incorporation into the non-particulate proteins (Table I). A further two-fold increase in amino acid incorporation is produced by the addition of diphosphopyridine nucleotide. This increased incorporation of amino acid into non-particulate proteins requires the presence of microsomes, and apparently results from the release of labeled proteins from the microsomes. That a portion of this labeled non-particulate protein is serum albumin is indicated by the data of Table II.

TABLE II

RADIOACTIVITY OF SERUM ALBUMIN PRODUCED BY ISOLATED MICROSOMES AND RIBOSOMES

System	Total Radioactivity			
	Microsomal System		Ribosomal System	
	Plus ATP and PGA	No ATP nor PGA	Plus ATP and PGA	No ATP nor PGA
Chemically-isolated rat serum albumin	176,600.	16,400.	188,300.	19,100.
Precipitate obtained by addition of bovine serum albumin plus anti-bovine serum albumin*	15,700.	1,200.	14,300.	1,450.
Precipitate obtained by subsequent addition of anti-rat serum albumin	155,600.	14,100.	169,900.	16,800.

* Further addition of anti-bovine serum albumin resulted in a negligible precipitation of radioactivity.

Not only can labeled serum albumin be isolated from the non-particulate proteins by the trichloroacetic acid-ethanol method of Debro, et al. (1957), but also labeled albumin can be precipitated by the antiserum to rat serum albumin. The data indicate that serum albumin accounts for approximately

50 per cent of the labeled non-particulate proteins produced by the microsomes.

Further evidence for the incorporation of labeled amino acids into serum albumin comes from an examination of the radioactivity of the peptides produced by the action of pepsin on isolated serum albumin. The peptic hydrolysate can be resolved into eight distinct components by paper chromatography as described by Ryle, et al. (1955), and Table III shows that each of these peptide fractions is labeled.

TABLE III

RADIOACTIVITY IN PEPTIDES OBTAINED FROM A PEPTIC HYDROLYSATE OF LABELED RAT SERUM ALBUMIN

Peptide	R_F	Total Radioactivity	
		Microsomes	Ribosomes
1	.07	400.	500.
2	.14	200.	360.
3	.19	75.	600.
4	.23	235.	480.
5	.29	290.	475.
6	.38	235.	350.
7	.70	280.	215.
8	.79	565.	320.

This indicates that the leucine has been incorporated throughout the serum albumin molecule, and is the result to be expected if entire serum albumin molecules are being synthesized and released by the microsomes.

The data of Tables II and III also show that serum albumin is labeled not only by microsomes, but also by isolated ribosomes. Although the pattern of

labeling in the peptides from serum albumin produced by ribosomes is slightly different from the serum albumin produced by microsomes, the important point is that all of the peptide fractions are labeled, indicating a synthesis of entire serum albumin molecules. This suggests that microsomes, as such, are not essential for serum albumin synthesis. However, it would be premature to conclude that the ribosomes have been completely freed of membrane material and that such material is not necessary for protein synthesis.

The amount of serum albumin formed by the rat liver ribosomes appears to be of the same order of magnitude as the amount of hemoglobin formed by the reticulocyte ribosome system described by Schweet, et al. (1958). The synthesis of serum albumin should provide another useful experimental system for the study of the synthesis of a specific protein.

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