THE SYNTHESIS OF SERUM PROTEINS ON ATTACHED RATHER THAN FREE RIBOSOMES OF RAT LIVER.

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It has been suggested that secretory proteins are synthesized on ribosomes that are attached to the endoplasmic reticulum rather than on free ribosomes, which may be engaged in the synthesis of non-exportable proteins (Siekevitz and Palade 1960). The experiments reported in this paper support this view, showing that free and attached ribosomes of rat liver are equally active (per mg of RNA) in incorporating radioactive leucine into Trichloracetic acid (TCA)-insoluble protein, but that nascent serum proteins, which are made for export from the cell, are found mostly on attached ribosomes with little on free ribosomes.

Siekevitz and Palade (1960) showed that the in vivo specific activity of α-chymotrypsinogen after 1 minute of injection of D-L-Leucine-1-C^{14} was greater on attached ribosomes than on free ribosomes of guinea pig pancreas. No in vivo study of the synthetic capabilities of specific proteins on free and attached ribosomes of liver has been reported, but the earlier work of Peters (1962A, 1962B) had established that albumin is synthesized on the rough endoplasmic reticulum. Also, Campbell et al (1960) and Ganoza, Williams and Lipmann (1965) had shown that liver microsomes in vitro can synthesize albumin and other serum proteins. Henshaw et al (1963) reported that attached ribosomes were more active in vivo in incorporating arginine into protein than were free ribosomes, but Campbell et al (1965) showed that isolated free polysomes are capable of in vitro protein synthesis and also

suggested that free polysomes synthesize protein for the internal use of the cell.

Materials and Methods. Rats, starved overnight, weighing between 150 and 180 g were injected intravenously with 0.5 mc of L-Leucine-4-5-H^3 (5 curies per millimole) and the livers removed and placed in cold 0.25 M sucrose in TKM (50 mM Tris pH 7.5, 25 mM KCl and 5 mM $MgCl_2$) at specified times after injection. Free and attached ribosomes were isolated by the method of Loeb, Howell and Tomkins (1967) with a few modifications. The liver was homogenized in 3 volumes of 0.25 M sucrose in TKM and centrifuged at 755 x g for 10 minutes. The pellet was resuspended in 2.3 M sucrose in TKM and centrifuged at 54,450 x g for 1 hour to remove the nuclei. The material which floated to the top of the tube was collected with a spatula and washed once by resuspending in 0.25 M sucrose in TKM and recentrifuged (Fraction I). The 755 x g supernatant was then centrifuged at 20,180 x g for 20 minutes to sediment the remaining attached ribosomes together with mitochondria. This pellet (Fraction II) was washed once with 0.25 M sucrose in TKM. The postmitochondrial supernatant contains the free ribosomes. Fractions I and II were resuspended in 0.25 M sucrose, and 0.5 ml of 13% sodium deoxycholate, per gram wet weight of liver, added to each fraction. The fractions were layered on a discontinuous sucrose gradient containing 3 ml of 2.0 M sucrose in TKM and 3 ml of 1.35 M sucrose in TKM and centrifuged at 105,000 x g for 17 hours.

The pellets which sedimented through the 2.0 M sucrose were resuspended in 0.25 M sucrose in TKM and used for determining RNA (Mejbaum 1939), total protein radioactivity (P. Siekevitz, 1952) and bound serum proteins. The average amounts of ribosomal material obtained in each fraction from these experiments are reported in Table 1.

When serum proteins were to be isolated, they were first released from the ribosomes by treatment with puromycin and spermine. Ribosomes (about 0.2 mg/ml) in 0.25 M sucrose in TKM were incubated with 5×10^{-4} M

puromycin and 2 x 10^{-4} M ATP for 10 minutes at 37°. Spermine (5 x 10^{-3} M) was added, and the mixture was incubated for 20 minutes at 37°. The samples were then centrifuged at 20,000 x g for 15 minutes. This treatment released about 10% of total ribosomal radioactivity into the supernatant and was equally effective for all three fractions. Since the serum proteins were isolated by precipitation with a rabbit anti-rat serum, the solution was first treated with chicken serum and rabbit antiserum to chicken serum in order to decrease the amount of nonspecific radioactive coprecipitation in the subsequent step (Campbell et al 1960, Peters 1962A). Serum proteins were then isolated by adding an excess of rabbit antiserum to rat serum together with carrier rat serum and 0.15 M NaCl and incubated at 37° for 1 hour then for 17 hours at 4°. As a control, some of the tubes were treated a second time with chicken serum and rabbit antiserum to chicken serum. The resulting precipitates were washed 5 times with cold saline, dissolved in 1 ml of Hyamine hydroxide and their radioactivity determined in a liquid scintillation counter. Efficiency of counting was determined by adding an internal standard. The radioactivities of the second chicken serum precipitates were subtracted from those of the rat serum precipitates. The radioactivity of the chicken serum precipitates varied from about 10% of that of the rat serum protein radioactivity on the free ribosomes to less than 2% of the radioactivity of the rat serum proteins recovered from the attached ribosomes.

Results and Discussion. The two fractions of attached ribosomes that were isolated, fractions I and II, are comparable in their ability to synthesize total and serum proteins (Table 1). Fraction I probably contains ribosomes attached to large membranes, while fraction II is from smaller fragments of the endoplasmic reticulum. The free ribosomes incorporate radioactive leucine into total protein to the same extent as attached ribosomes, but synthesize 7 to 8 times fewer serum proteins.

TABLE 1.

Incorporation of H^3-leucine into total protein and serum proteins bound to free and attached ribosomes. Three minutes after an intravenous injection.

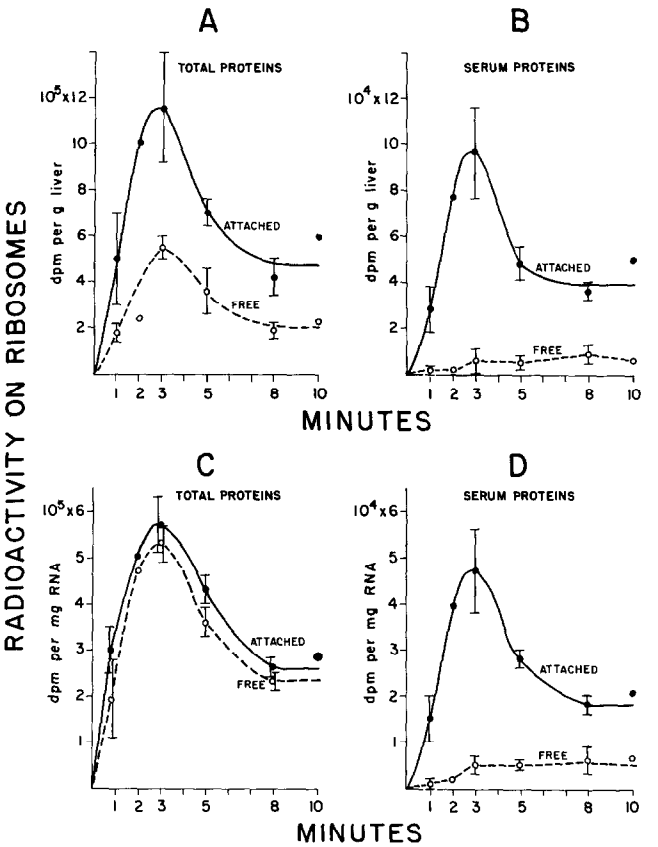
Ribosome fraction	mgRNA/g liver	Protein Radioactivity dpm/mg RNA	
		Total proteins	Serum proteins
Attached I	0.91	557,200 ± 50,900	40,430 ± 13,940
Attached II	0.68	518,566 ± 94,100	34,450 ± 16,590
Free	0.86	500,423 ± 77,700	5,208 ± 4,560

The values represent the average of 3 experiments ± standard deviation. Total protein indicates TCA-insoluble material. The serum proteins were isolated from the ribosomes as described in the text.

The time course of incorporation of radioactive leucine into nascent total proteins and serum proteins is shown in Figure 1. A peak of radioactivity on the ribosome is found at about 3 minutes after the injection, then quickly falls and levels off at about 8 minutes. The time-curves for free and attached ribosomes are similar. The attached ribosomes (fractions I and II combined) synthesize more proteins than the free ribosomes (figure 1A) but this is only because the hepatic cell has more attached than free ribosomes. Figure 1C shows that when the radioactivity is plotted per mg of RNA free and attached ribosomes are equally active. However, when the radioactivities of nascent serum proteins in free and attached ribosomes are compared, more than 90% of the labeled serum proteins is on attached ribosomes at 3 minutes (figure 1B) and this relation holds even when the comparison is made per mg of RNA (figure 1D). The small amount of radioactive serum proteins found on the free ribosomes may be due to contamination of the free ribosomes with loosely attached ribosomes. Homogenization may detach ribosomes from their membranes. The kinetic data indicate that serum proteins are not synthesized on free ribosomes and then later become bound to attached ribosomes. At one minute, after injection of radioactive leucine, nearly all of the nascent serum proteins is on the attached ribo-

FIGURE 1.

Radioactivity of total TCA-insoluble protein and serum proteins bound to attached and free ribosomes at various time intervals after an intravenous injection of H^3-leucine.



The values given, except for the points at 2 and 10 minutes, are the mean of 3 experiments with the standard deviation. The points at 2 and 10 minutes are single observations. Attached ribosomes are fractions I and II combined. Total protein is TCA-insoluble material.

somes. At the later times there is no indication of a transfer of radioactive serum proteins from free to attached ribosomes.

There are some arguments against the hypothesis that free ribosomes are engaged only in making proteins destined for use within the cell. Loeb et al (1967) have shown that treatment of rats with hydrocortisone, which has been shown to induce a number of liver enzymes (Kenney 1962), does not

change the relative amount of free ribosomes. Hydrocortisone may, however, stimulate the rate of synthesis of these enzymes and an increase in the number of free polysomes is therefore not necessary. The present work, however, shows that while free and attached ribosomes contain equal amounts of total nascent proteins, newly synthesized serum proteins are mostly found on the attached ribosomes and not on the free ribosomes. Final proof will await the analysis of a variety of nascent liver proteins bound to free and attached ribosomes.

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