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*SYNTHESIS OF SERUM PROTEINS BY A CELL-FREE SYSTEM FROM RAT LIVER**

BY M. CLELIA GANOZA, CURTIS A. WILLIAMS, AND FRITZ LIPMANN

THE ROCKEFELLER INSTITUTE

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The observations that microbial and mammalian cell-free systems incorporate amino acids into certain well-defined proteins[1-3] suggest the possibility that a variety of other proteins could be formed in such systems. In particular, the rat liver cell-free system offers a suitable source for such studies. Using this system, Campbell and his associates[1, 4] have shown that amino acids are incorporated into a protein which is immunologically and chemically similar to serum albumin synthesized *in vivo*. They also observed an apparent increase in the amount of serum albumin formed when microsomes were used in the incubation mixture in place of ribosomes obtained by deoxycholate (DOC) treatment.[5]

In the present studies the capacity of rat liver cell-free extracts to produce other serum proteins has been examined. These proteins, however, are synthesized in relatively small quantities *in vivo*. Formation of these components in cell-free extracts has been facilitated in part by the preparation of a highly active microsomal fraction obtained by gentle homogenization. In addition, more sensitive methods of detection have been applied.

In other studies, autoradiography of fingerprints was employed to detect incorporation of labeled amino acids into specific proteins.[2-4] More recently, autoradiography of immunoelectrophoretic patterns has been used to detect the synthesis of serum albumin in cell-free extracts[6] and of other serum proteins by mouse liver slices.[7] As a result of the sensitivity and accuracy of this technique, it has been possible in these studies to identify several serum proteins labeled by cell-free rat liver preparations.

Materials and Methods.—Preparation of liver microsomes, ribosomes, and 105,000 × *g extract:* The livers of 150-gm Sprague-Dawley male rats were cut into small pieces and homogenized in a glass Potter-Elvehjem homogenizer with a smooth Teflon pestle attached by rubber tubing to a constant-speed stirring motor. The homogenizer vessel was held in a rigid position by use of a wooden clamp. In a typical experiment, 4 gm of liver were suspended in 10 ml of a medium containing 0.01 *M* $MgCl_2$, 0.025 *M* KCl, 0.035 *M* Tris pH 7.8, and 0.25 *M* sucrose. Samples were homogenized at 1000 rpm for 1 min at 5°. During homogenization the pestle was mechanically raised and lowered six or seven times. After centrifuging the homogenates twice at 15,000 × *g* for 10 min, the upper two thirds of the supernatant fraction was carefully collected and centrifuged at 105,000 × *g* for 90 min. The supernatant fluid was collected and used as the 105,000 × *g* extract. The microsomal pellet was resuspended by gentle manual homogenization in 1 ml of the same buffer solution. Ribosomes were prepared as described by Wettstein *et al.*,[8] except that 0.6% DOC was added to the second 15,000 × *g* supernatant fraction.

Amino acid incorporation: The incubation mixtures contained rat liver microsomes, the 105,000 × *g* extract, ATP, and an ATP-generating system as described by Campbell *et al.*[1] L-leucine-C^{14} was added to a final concentration of 10 mμmoles per ml with a specific activity of 198 μc per μmole. Incubation was carried out under N_2 for 50 min at 37°. Excess leucine-C^{12} (10 μmoles per ml) was then added and the mixture was centrifuged at 105,000 × *g* for 90 min at 0° to sediment the microsomes. The supernatant fraction (IMS) was dialyzed and lyophilized. The microsomal pellets were suspended in 2 ml of 0.9% NaCl, sonicated for 2 min in a Branson LS-75 sonifier, and centrifuged at 105,000 × *g* for 90 min. The supernatant fluid was dialyzed overnight against several changes of distilled water, lyophilized to dryness, and suspended in 0.2 ml of 0.05 *M* veronal buffer pH 8.2. This fraction is designated SON. Sonication generally releases 15% of the labeled protein associated with the microsomes. Prolonged sonication yielded slightly greater release of radioactive material, but this material sedimented on standing.

Determination of amino acid incorporation into protein: Aliquots of the reaction mixtures described above were withdrawn at 0 and 50 min and were pipetted directly into 5% trichloroacetic acid (TCA); the precipitates were heated for 15 min at 90°. Lipid was then extracted according to the procedure of Zamecnik *et al.*[9] Samples were collected in 5% TCA and passed through a Millipore filter with an average pore size of 0.45 μ. The Millipores were glued onto planchets, dried, and counted in a thin window, gas-flow Nuclear-Chicago counter (22% efficiency). Protein was determined by the Folin-Ciocalteau method of Lowry *et al.*[10]

Immunoelectrophoretic analysis (IEA): IEA was carried out on microscope slides as described previously.[7] Rabbit antirat serum was purchased from Hyland Laboratories.

Kodak Royal Blue film was exposed for 6 weeks with the dried slides. All exposed films were developed for 5 min at 20° in Kodak D-11 developer.

Results and Discussion.—The effect of homogenization conditions on incorporation was investigated by altering the clearance between the pestle and the wall of the homogenizer vessel.[11] Determinations were made in triplicate since a broad distribution of values is generally obtained in such experiments. The results of a typical experiment are shown in Table 1. The average cpm incorporated per mg of extract protein increased as a function of average pestle clearance up to 0.9 mm during homogenization. Similar results were obtained when the incorporation of amino acids into the ultrasonic extracts (SON fractions) was examined as a function of pestle clearance. In all subsequent experiments a clearance of 0.9 mm was used. The decrease in activity resulting from smaller pestle clearance may be due to the release of degradative enzymes from lysosomes.[12]

TABLE 1

EFFECT OF HOMOGENIZER CLEARANCE ON INCORPORATION

Pestle clearance (mm)	Cpm/mg protein
0.25	590
0.75	1110
0.93	1270
1.15	680

Other conditions of homogenization are described in *Methods*.

Specific rat serum proteins isolated from incubation mixtures were identified by IEA. Radioactive arcs, indicated by autoradiography of IEA slides, permitted the detection of those serum proteins labeled by the cell-free system. The extracts were run with and without rat serum carrier in order to identify these labeled proteins. Figure 1*a* shows the IEA pattern of the proteins in the SON fraction of the cell-free system. The autoradiogram of slide (Fig 1*b*) shows a strongly labeled albumin arc and several weakly labeled components corresponding in electrophoretic mobility to the serum α- and β-globulins and to an unidentified component of very low mobility. Haptoglobin (α_2-H) was tentatively identified in a parallel experiment in which precipitation arcs were examined for peroxidase activity by staining with benzidine peroxide reagent;[13] this arc is faintly visible in the autoradiogram. The more heavily labeled component of low mobility gives a weak but positive re-

action with bromphenol blue verifying the presence of protein; this arc might correspond to the slow liver component in mice,[7] but this has not been positively confirmed. The transferrin (T) arc is also labeled. None of the labeled arcs could be stained for lipid with oil red-O. In addition, another arc is faintly visible in the region of the β-globulins but was not further identified.

Identical analysis was made of the 105,000 $\times$ g supernate (IMS fraction) to detect any release of labeled serum proteins during incubation. Autoradiograms revealed a faint trace of radioactivity associated with the albumin arc but with no other arc in the IEA patterns. This observation indicates that the TCA-precipitable radioactivity found in the IMS fraction, amounting to 20 per cent of the total TCA-precipitable counts in the incubation mixture, could be ascribed largely to nonserum protein and that significant quantities of newly synthesized serum protein chains are not released from the microsomes under the incubation conditions described here.

An experiment was performed to compare the ability of a ribosomal system to

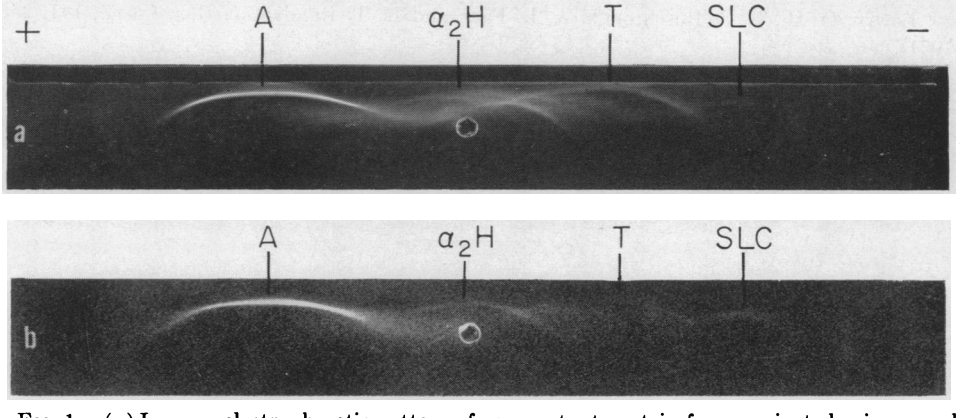

FIG. 1.—(a) Immunoelectrophoretic pattern of supernatant protein from sonicated microsomal fraction (SON) of incubation mixture. 58 μg of SON protein were used. The specific activity of the fraction was 2,600 cpm/mg of protein. (b) Autoradiogram of slide shown in (a). A, albumin; α_2-H, haptoglobin; T, transferrin; SLC, slow liver component (tentative).

synthesize serum proteins with that of the microsomal system. Incorporation into TCA-precipitable protein of the IMS and SON fractions was comparable in the two systems. Autoradiograms of IEA slides showed faint labeling of the albumin arcs in both the ribosomal and the microsomal IMS fractions, and also in the ribosomal SON fraction. Significantly greater incorporation into serum proteins was apparent in the microsomal SON fraction. These findings are in agreement with those of Campbell *et al.*[1, 5]

The radioactivity in the autoradiograms of the IEA patterns appeared to be due to the synthesis of specific proteins and not to the presence of nonspecific radioactive material bound in some manner to the precipitation arcs. The latter possibility is minimized by the sensitivity and specificity of the immunoelectrophoretic technique.[14] In addition, IEA patterns of the IMS fraction which contained a similar distribution of serum proteins as that found in the SON fraction did not show any radioactive arcs except for a slight labeling of the albumin arc in spite of the fact that considerable labeled nonserum protein was present in these fractions.

In conclusion, it may be stated that a cell-free system consisting of the microsomal and supernatant fractions of rat liver is capable of synthesizing albumin, transferrin, and several other serum proteins which are electrophoretically identical to the corresponding proteins found in rat serum.

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*EFFECT OF BACTERIAL INFECTION ON THE SYNTHESIS OF SERUM PROTEINS BY A MOUSE LIVER CELL-FREE SYSTEM**

BY CURTIS A. WILLIAMS, M. CLÉLIA GANOZA, AND FRITZ LIPMANN

THE ROCKEFELLER INSTITUTE

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In the preceding paper we have demonstrated that cell-free rat liver preparations are capable of incorporating amino acids into a number of serum proteins in addition to serum albumin.[1] Of considerable interest is whether such cell-free systems accurately reflect the biosynthetic activity of the tissue from which they were derived.

Certain conditions of stress are known to alter the quantity and normal distribution of serum proteins found in the circulation. Immunoelectrophoretic studies have revealed several such modifications in mouse serum during staphylococcal infections,[2] and it was subsequently demonstrated that many of the changes in serum protein concentrations could be correlated with increased synthesis of these proteins by the appropriate tissues *in vitro*.[3]

The purpose of the experiments reported here is to show that cell-free systems derived from livers of normal and staphylococcus-infected mice incorporate amino acids into serum proteins in a manner similar to that of the corresponding tissue.

Methods.—Staphylococcal infections: Rockefeller NCS female mice were used at 6–8 weeks of age. Cultures of *Micrococcus pyogenes* var. *aureus* (Giorgio) were grown 18–20 hr at 37° in