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**

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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

Penassay broth. Mice were injected intravenously with approximately 2.5×10^7 viable units in 0.2 ml of 0.9% NaCl. It has been shown previously that 8 of 10 mice are likely to survive this dose for at least 10 days.[3] Control mice received 0.2 ml sterile saline. Serum samples of all mice were analyzed by immunoelectrophoresis (IEA) prior to infection and three days following infection. Mice showing atypical serum IEA patterns before or after injection with staphylococci or saline were excluded.

Incubation mixture: Four days after infection the livers of 8–10 mice in each group were homogenized and the microsomal and cell sap fractions were prepared as previously described for the rat liver system,[1] except that KCl was omitted from the suspending medium used for homogenization. Reagent concentrations in the incubation mixture were: 4×10^{-4} *M* GTP, 7.4×10^{-3} *M* glutathione, 2×10^{-3} *M* ATP, 8×10^{-3} *M* phosphoenolpyruvate (PEP), 40 μg PEP-kinase per ml of incubation mixture, 0.056 *M* KCl, 0.025 *M* Tris pH 7.8, 0.006 *M* $MgCl_2$, and 0.1 *M* sucrose. Four μc (0.0047 mg) of C^{14}-amino acids were added per ml in the form of yeast protein hydrolysate (Schwarz BioResearch, Inc.). Polyvinylsulfate was included at a concentration of 4 μg per ml. This concentration increased incorporation 30–50%. Higher levels of polyvinylsulfate were markedly inhibitory. Reaction mixtures were completed by the addition of 3.4–3.6 mg of microsomal protein and 2.6 mg of supernatant protein per ml. The normal microsomal fraction had an RNA/protein ratio of 0.87, and that of the infected mice 0.71. The cell sap fraction from normal livers was used in all incubations. The total volumes were 5 ml for the normal systems and 10 ml for systems made with livers from infected animals. Incubations were carried out at 37° for 50 min.

Fractionation of the incubation mixture: The 105,000 $\times$ *g* supernates of the incubation mixtures (IMS) and the 105,000 $\times$ *g* supernates of the resuspended and sonicated microsomes (SON) were prepared as previously described.[1] The dialyzed and lyophilized IMS fractions were concentrated 12 times over the original incubation mixtures. The SON fractions were concentrated 50 times.

Immunoelectrophoretic analysis: IEA was carried out on microscope slides as described in an earlier report.[3] The antisera were prepared in rabbits using whole mouse plasma as antigen. The antisera contained antihemoglobin antibody but did not contain detectable antihaptoglobin antibodies. Therefore, haptoglobin was detected as the haptoglobin-hemoglobin complex after addition of freshly prepared mouse erythrocyte lysate prior to IEA.

Autoradiograms were obtained from washed and dried IEA slides. Kodak Ortho Process film was exposed for 10–13 weeks in contact with the slides. Images were developed for 5 min at 20° in Kodak D-11 developer.

Assay methods: Amino acid incorporation into protein and protein determinations were performed as previously described.[1] RNA was determined by the method of Mejbaum *et al.*[4]

Results and Discussion.—The specific activities of the various fractions obtained after incubation of mouse liver microsomes are shown in Table 1. Eighty per cent of the label incorporated into protein remained bound to the microsomal fraction of which 12–15 per cent was released by sonication. Prior to dialysis and lyophilization the specific activities of the IMS and SON fractions were approximately 10 and 20 times higher, respectively. Dialysis and lyophilization produced irreversible precipitation or denaturation of material with high specific activity. The specific activities of all fractions obtained with microsomes of infected mice were greater than the specific activities of the corresponding fractions isolated from

TABLE 1

INCORPORATION OF AMINO ACIDS INTO PROTEIN

	Normal System		Infection System	
Fraction	Cpm/ml	Cpm/mg protein	Cpm/ml	Cpm/mg protein
IMS	8,750	200	10,600	250
SON	7,400	360	9,100	630

Values refer to hot TCA-precipitable material of fractions which had been dialyzed, lyophilized, and dissolved in 0.05 *M* veronal buffer pH 8.2.

normal livers. Liver tissues from infected mice incorporate amino acids into several serum proteins at a significantly greater rate than normal liver tissue.[3] We have also shown that amino acids administered intravenously into infected mice are incorporated into the serum globulins at about 10 times the normal rate, and into albumin at half the normal rate.[5] The increased rate of incorporation in the cell-free system appears, therefore, to reflect these quantitative changes in biosynthetic activity.

The IEA patterns of normal mouse serum proteins are shown in Figure 1*a*. The corresponding pattern of infected mouse serum is shown in Figure 2*a*. The sharp

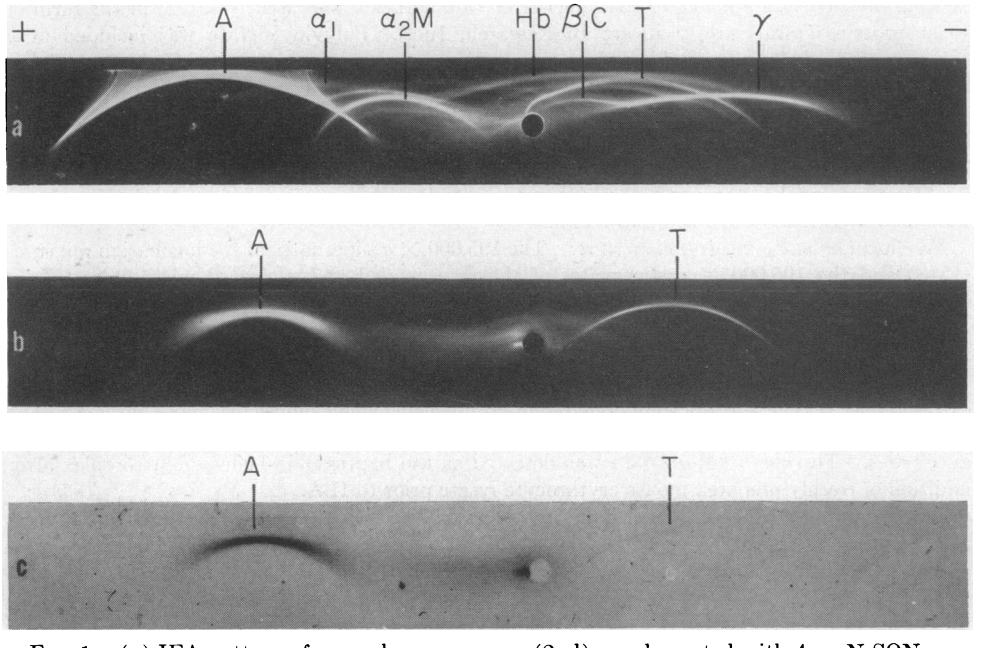

FIG. 1.—(*a*) IEA pattern of normal mouse serum (2 μl) supplemented with 4 μg N-SON protein. (*b*) IEA pattern of 16 μg N-SON protein. (*c*) Autoradiogram of IEA slide (*b*). *A*, albumin; α_2-*M*, macroglobulin; *Hb*, hemoglobin added to serum; β_1-*C*, component of *C*·3 system of serum complement; *T*, transferrin; γ, gamma globulin.

increase in haptoglobin (α_2-H) level, the appearance of the slow liver component (SLC), and the partial conversion of β_1-C globulin to β_1-D globulin are typical of animals subjected to inflammatory stress.[2] SLC is not usually detectable in the serum until a later stage of the infection. In Figure 2*a* the SLC arc is visible because the S-SON fraction containing this protein was added to the serum prior to IEA. Figures 1*b* and 2*b* represent the IEA patterns of the ultrasonic extracts of microsomes from normal (N-SON) and infected (S-SON) mouse cell-free extracts, respectively. In both patterns albumin (A) and transferrin (T) arcs are prominent although the albumin arc in the S-SON fraction (Fig. 2*a*) is diffuse, indicating a reaction of antibody excess. This is consistent with the finding that albumin synthesis in infected mice is markedly reduced.[5] The faint and diffuse arcs in the α and β zones found in the N-SON fraction (Fig. 1*b*) also suggest the presence of excess antibody. In the S-SON pattern there appears to be a greater amount of

the α and β globulins as well as the SLC. Haptoglobin, however, could not be detected in either of the ultrasonic extracts.

The autoradiograms of the IEA patterns of the N-SON and S-SON fractions are shown in Figures 1*c* and 2*c*, respectively. The ultrasonic extracts from normal mice show a heavily labeled albumin arc and a faintly labeled transferrin arc. The diffuse radioactive traces near the starting well could be due to the presence

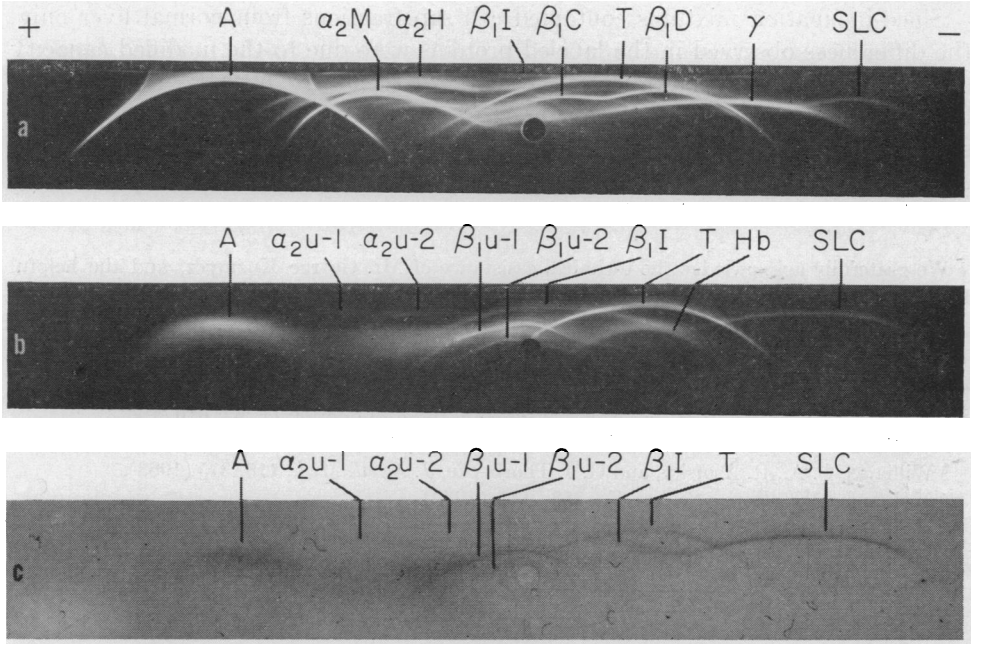

FIG. 2.—(*a*) IEA pattern of serum pooled from infected mice supplemented with 3 μg S-SON protein. (*b*) IEA pattern of 12 μg S-SON protein. (*c*) Autoradiogram of IEA slide (*b*). α_2-*H*, haptoglobin; β_1-*D*, conversion product of β_1-*C*; *SLC*, slow liver component; α_2*u*, β_1*u*-1, and β_1*u*-2 are unidentified serum components. Other abbreviations are the same as in Fig. 1.

of β-globulin in antibody excess or to partially denatured, agar-bound material. In contrast, the ultrasonic extracts obtained from microsomes of infected mice (Fig. 2*c*) contain several labeled proteins. These include albumin, transferrin, β_1-I, SLC, two unidentified α_2-globulins, and two unidentified β_1-globulins. Haptoglobulin was not labeled in these systems in the presence or absence of serum carrier. Since haptoglobin was detectable only if complexed with hemoglobin, a protein or peptide unable to bind hemoglobin would not be precipitated in the α_2-H arc and would therefore be removed prior to autoradiography. The labeling of the slow liver component by the infection system is of particular significance, however, since this protein is produced in detectable quantity by the liver only during infection.[3] Therefore, the results show qualitative changes due to infection.

Autoradiograms of the IEA patterns of the IMS fractions were completely negative suggesting that the newly synthesized serum proteins were not released in significant quantity from the microsomes during incubation. These fractions did contain other labeled protein, however, and were shown to contain the same serum proteins found in the SON fractions. In view of this and the fact that the

distribution of label in the IEA patterns of the SON fractions was not affected by the addition of serum carrier, it seems unlikely that nonspecific binding of radioactive material to serum proteins occurs to any appreciable extent. There is the possibility that highly radioactive material in the SON fraction migrating with the same electrophoretic mobility is trapped and concentrated in the immune precipitate. This is unlikely, however, because of the dynamics of diffusion and precipitation in gels.

Since incubation mixtures contained cell sap fractions from normal liver only, the differences observed in the labeled proteins were due to the modified capacity of the microsomal fraction to direct the synthetic activity. The striking differences between the labeled IEA patterns of cell-free systems obtained from normal and staphylococcus-infected mice closely parallel the differences observed *in vivo*[5] and *in vitro*.[3] We conclude, therefore, that under suitable conditions, cell-free systems reflect the activity of intact tissues.

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*REGULATION OF RNA SYNTHESIS IN ISOLATED NUCLEOLI BY HISTONES AND NUCLEOLAR PROTEINS**

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Although nucleoli have long been recognized as major components of cells and nuclei and have been extensively studied,[1, 2] their precise biological role remains incompletely determined. Recent improvements in techniques for the characterization of nucleic acids and for the isolation of morphologically intact nucleoli from a wider range of animal[3–5] and plant[6] cells have helped provide strong evidence that the nucleoli function in the synthesis of ribosomes.[7–9] In addition, the methylation of RNA,[10, 11] and a synthesis of nuclear proteins[6] have been specifically attributed to nucleoli.

Further evidence that ribosomal RNA is synthesized in the nucleoli has been provided by work on the synthesis *in vitro* of RNA in intact, isolated nuclei[12] and nucleoli.[13, 14] Nucleoli were prepared from rat tumor and liver by procedures designed to permit retention of RNA-synthesizing ability. Incorporation of P^{32}-labeled ribonucleotides into nucleolar RNA was found to be dependent on the