LABELING WITH ^{14}C AMINO ACIDS OF ALBUMIN-LIKE PROTEIN BY RAT LIVER RIBONUCLEOPROTEIN PARTICLES

ALEXANDRA VON DER DECKEN, Ph.Lic.

From the Wenner-Gren Institute for Experimental Biology, University of Stockholm, Sweden

ABSTRACT

Ribonucleoprotein particles were prepared by treatment of rat liver microsomes with detergents and high concentrations of KCl. They were active in incorporating ^{14}C amino acids into protein when incubated with cell sap together with ATP, GTP, and a system to regenerate the triphosphates. The albumin of the incubation mixture, soluble at 105,000 *g*, and that of the fraction released by ultrasonication of the particles were studied by immunoelectrophoresis in agar gel. When the ribonucleoprotein particles were incubated with cell sap the immunological precipitation lines formed with antiserum to rat serum albumin were highly radioactive as tested by autoradiography. After zone electrophoresis on cellulose acetate, two immunologically reactive albumins were obtained which differed in their electrophoretic mobility from rat serum albumin. Labeled albumin, when purified on DEAE-cellulose columns, retained its radioactivity as tested by autoradiography following immunoelectrophoresis. On cellulose acetate this purified albumin showed an electrophoretic mobility higher than that of rat serum albumin.

INTRODUCTION

It has been shown that the isolated microsome fraction from rat liver, when incubated under suitable conditions with ^{14}C amino acids, can synthesize rat serum albumin (2, 3). The microsomes involved in this synthesis consist of rough and smooth surfaced endoplasmic reticulum and free ribosomes (26). When treated with detergents, the membrane components become solubilized. The remaining ribonucleoprotein particles[1] have been shown on incubation to incorporate amino acids from an incubation mixture into protein (15, 18, 19, 30, 32). Since the morphological structure of the ribonucleoprotein particles is simpler than that of the microsomes, these particles were used in order to study the incorporation of ^{14}C amino acid into rat serum albumin under more closely defined conditions. The albumin, when precipitated by the addition of its antiserum, was highly radioactive (9, 10, 19–22). However, it was found that the labeled albumin, after purification by zone electrophoresis on cellulose acetate, lost nearly all its radioactivity (10). This was in contrast to what had been found when albumin was labeled in a cell-free system containing whole microsomes (2, 3). It has been suggested that a factor present in the microsomes might be important for the synthesis of rat serum albumin (1).

In the present investigation, some character-

[1] The term *ribonucleoprotein particles* has been used to indicate that ribosomes when treated with detergents are no longer identical with native ribosomes with respect to certain aspects of their protein synthetic activity.

istic properties of the immunologically reactive, labeled albumin derived from a cell-free isotope incorporation system containing ribonucleoprotein particles were studied. For this purpose, advantage has been taken of a method worked out by Perlmann *et al.* (23, 24, 27–29). This technique combines immunoelectrophoresis in agar gel with autoradiography.

Some aspects of this work have been presented previously (8).

MATERIALS AND METHODS

CHEMICALS: The disodium salt of ATP,[2] the sodium salt of GTP, creatine phosphate, ribonuclease, and tris were obtained from the Sigma Chemical Co., St. Louis, Missouri. Pyruvate kinase, creatine phosphokinase, and the silver barium salt of PEP were obtained from Boehringer und Soehne, Mannheim, Germany. Solutions of the free acid were prepared before use and adjusted to pH 7 (indicator paper) with 1 N KOH. The sodium salt of deoxycholic acid was obtained from Merck A.-G., Darmstadt, Germany, the nonionic detergent Lubrol W from Imperial Chemical Industries Ltd., Manchester, England, and β-mercaptoethanol from the Eastman Kodak Co., Rochester, New York.

RADIOACTIVE AMINO ACIDS: Uniformly labeled ^{14}C-L-leucine and ^{14}C-L-valine (90 mc/mmole each) were obtained from the Institut Pasteur, Paris, France.

ANIMALS: Sprague-Dawley rats (150 to 180 gm body weight) were fasted for approximately 18 hours before being killed by a blow on the head followed by decapitation. Non-fasted 5- to 7-day-old chickens were killed by decapitation.

RAT SERUM ALBUMIN: Rat serum was fractionated on DEAE-cellulose columns (31). The albumin-containing fraction eluted with 0.1 M phosphate buffer (pH 5.8) was dialyzed against distilled water and freeze-dried. The dried proteins were extracted with trichloroacetic acid–ethanol (5), as described by Campbell *et al.* (2). The antiserum obtained after immunization of rabbits showed one precipitation line when tested by immunoelectrophoresis with rat serum. After several days at 37°C two additional weak lines became visible.

CHICK SERUM ALBUMIN: This was isolated by column chromatography on DEAE-cellulose (31). After elution with 0.4 M buffer (pH 5.8, 4°C) it was rechromatographed on DEAE-cellulose in order to obtain a purer preparation. Antiserum to this purified preparation, when tested against chick serum, usually gave one strong line corresponding to albumin, and one or two weak lines. After testing against cell sap from chick liver, only the precipitate of albumin became visible.

PREPARATION OF ANTISERA AGAINST RAT SERUM ALBUMIN AND CHICK SERUM ALBUMIN: The albumins were emulsified with equal parts of Freund's complete adjuvant (4). Generally, each rabbit received three intramuscular injections (20 mg albumin per injection in 2 ml) of this emulsion, given at intervals of 10 days. The amount of antibody in the rabbit serum was determined by the quantitative precipitin method (14).

ISOLATION OF RIBONUCLEOPROTEIN PARTICLES: Ribonucleoprotein particles were isolated according to the method of Rendi and Hultin (30), but a medium containing 0.01 M $MgCl_2$, 0.025 M KCl, 0.035 M tris buffer (pH 7.8), and 0.25 M sucrose (medium A) was used in homogenizing the liver.

PREPARATION OF CELL SAP: Cell sap from rat liver and from chick liver was prepared as described earlier (10).

METHOD OF INCUBATION: Ribonucleoprotein particles from 30 to 40 gm of liver were suspended in 4 ml of medium A by gentle homogenization in a Potter type homogenizer. The incubation flasks contained the particle suspension, 2 ml of cell sap, 4 to 6 μc of ^{14}C-L-leucine or ^{14}C-L-valine, 20 μmoles of ATP, 100 μmoles of PEP or 200 μmoles of creatine phosphate, 400 μg of pyruvate kinase or 500 μg of creatine phosphokinase, and 2 μmoles of GTP in a total volume of 7.5 ml. This mixture was incubated for 2.5 hours at 35°C. The flasks were slightly covered during incubation to minimize evaporation.

ISOLATION OF PROTEINS AFTER INCUBATION: After addition of 2 ml of chick liver cell sap, the incubation mixture was diluted to 11 ml with medium A and centrifuged for 60 minutes at 105,000 *g*. The supernatant (soluble incubation mixture) was decanted and the particles were subjected to ultrasonic vibrations after suspension in 2 ml of distilled water. The procedure was that described by Campbell *et al.* (2) as modified by Campbell and Kernot (3). After sonication, distilled water was added together with sufficient 18 per cent NaCl to bring the NaCl concentration to 0.3 per cent and the final volume to 11 ml. The suspension was centrifuged at 105,000 *g* for 60 minutes. The supernatant (ultrasonic extract) was decanted.

IMMUNOLOGICAL ESTIMATIONS: The soluble incubation mixture and the ultrasonic extract were combined or treated separately. One half of the solutions was treated with 20 μg/ml ribonuclease for

[2] Abbreviations: tris, tris(hydroxymethyl)aminomethane; ATP, adenosine triphosphate; DEAE-cellulose, N,N-diethylaminoethyl cellulose; GTP, guanosine triphosphate; PEP, phosphoenolpyruvate; RNA, ribonucleic acid.

2 hours at 20°C. The untreated half was also kept at 20°C for 2 hours. In most of the experiments the fractions were dialyzed overnight against distilled water and freeze-dried. The freeze-dried samples were suspended in 0.2 ml of distilled water. Antigen and antiserum were reacted by means of double diffusion on agar plates according to Ouchterlony (25) and by means of immunoelectrophoresis in agar gel (11).

After 2 to 3 days at 37°C all agar plates were washed for 3 days in 0.9 per cent NaCl, for 3 days in running water, and for 2 additional days in distilled water in order to remove radioactive contaminations and non-precipitated protein (23). The agar plates were photographed and the agar was then dried to a thin film prior to autoradiography.

ZONE ELECTROPHORESIS ON CELLULOSE ACETATE: Zone electrophoresis was run according to Kohn (16, 17) as described in detail by Campbell *et al.* (2). For determination of radioactivity the strip was counted by continuous scanning of radiochromatograms. A Geiger-Müller tube (1.9 mg/cm^2) with a slide width of 6.5 mm was used. The automatic feeder moved the chromatogram 6.5 mm. After determination of radioactivity the strip was stained with amidoblack. The color intensity was measured as deflection in a recording Chromoscan (Joyce, Loebl and Co. Ltd., Team Valley, England). If zone electrophoresis was combined with the double diffusion method in agar gel, the strip after electrophoresis was put upside down on an agar gel plate parallel to the antiserum basins. The basins were filled with antiserum at the same time. After 3 hours at room temperature the strip was removed and the agar plates were incubated at 37°C as described in the previous section.

COLUMN CHROMATOGRAPHY ON DEAE-CELLULOSE: After incubation, the soluble fractions were dialyzed overnight against 0.0175 M phosphate buffer, pH 6.3. A DEAE-cellulose column (6 × 1 cm) was equilibrated with the same buffer. The fractions were pipetted onto the column. A stepwise elution as for serum proteins was used (31). The initial elution was made with 0.0175 M phosphate buffer, pH 6.3, followed for 3 to 4 hours by 0.0175 M phosphate buffer in 0.01 M β-mercaptoethanol in order to convert most of the polymeric and dimeric albumin into a monomeric form (12). This treatment was successively followed by 0.04 M phosphate buffer, pH 5.9, by 0.1 M buffer, pH 5.8, by 0.4 M buffer, pH 5.8, and finally by 1 M buffer, pH 5.2. Readings were made at 254 mμ with an automatic Uvicord recorder, or at 280 mμ in a Beckman model DU spectrophotometer. The buffer solutions were changed when the extinction of the preceding eluate was zero. The eluates were collected, dialyzed against distilled water, and freeze-dried. Before the immunological tests the proteins were dissolved in water.

MEASUREMENT OF RADIOACTIVITY: The residue from the particles after ultrasonication and the other protein-containing fractions, if not used for immunological tests, were treated with 5 per cent (*w/v*) trichloroacetic acid and the precipitates extracted according to a modified Schneider procedure (*cf.* Zamecnik *et al.* (33)). The extracted proteins were placed on 0.3 cm^2 aluminium planchettes and counted at infinite thickness with a thin mica end-window (1.9 mg/cm^2) Geiger-Müller counter. A standard planchette of 1 cm^2 containing 0.1 μc ^{14}C per gram gave approximately 100 counts per minute under these conditions.

AUTORADIOGRAPHY: The agar plates were covered with x-ray film (Ilford Ilfex or Gevaert Osray) and a second glass plate as described in detail by Morgan *et al.* (23).

RESULTS

Immunoelectrophoresis of the Soluble Fractions

Ribonucleoprotein particles from rat liver were incubated together with ^{14}C amino acid, ATP, GTP, PEP, or creatine phosphate in combination with the corresponding phosphokinases and rat liver cell sap. After incubation, chick liver cell sap was added to determine whether or not the radioactivity obtained in the immunologic precipitates of rat proteins was due to unspecific contamination of the antigen-antibody precipitates with radioactive substances. The soluble incubation mixture was then separated by centrifugation. The pellet was treated ultrasonically and the ultrasonic extract isolated by centrifugation. The soluble incubation mixture and the ultrasonic extract were combined. Since it was known from previous experiments (10) that albumin could be associated with RNA, half of this solution was treated with ribonuclease. The untreated and treated aliquots were then dialyzed, freeze-dried, and studied after immunoelectrophoresis on agar gel. If they were not dialyzed, the high salt-sucrose concentration of the freeze-dried samples seemed to interfere with the separation of the proteins. Therefore, dialysis of the samples before electrophoresis was done routinely. Fig. 1 *a* is a photograph of an immunoelectrophoretic experiment. Antiserum to rat albumin was added to the middle basins and antiserum to chick albumin to the outer basins. One precipitation line was obtained with each antiserum.

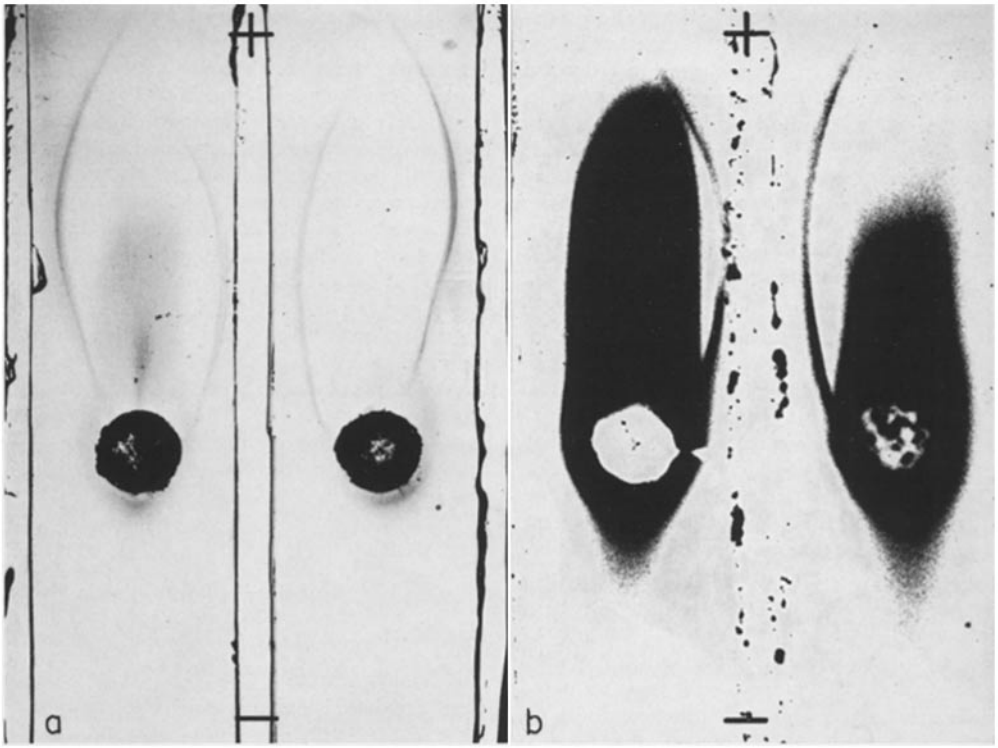

FIGURE 1

Fig. 1 *a*, photograph, Fig. 1 *b*, autoradiograph of immunoelectrophoretic plate, made with soluble incubation mixture plus ultrasonic extract. The incorporating system with ^{14}C amino acid contained, in a final volume of 7.5 ml: ribonucleoprotein particles from 30 gm of liver, 2 ml of cell sap from rat liver, 20 μmoles of ATP, 2 μmoles of GTP, 100 μmoles of PEP, 400 μg of pyruvate kinase, and 5 μc of ^{14}C-L-leucine. After incubation, prior to fractionation, cell sap from chick liver was added. Wells to the left contained untreated extract; to the right, extract treated with ribonuclease. Antiserum to rat serum albumin was added to the middle basin. Antiserum to chick serum albumin was added to the outer basins. Exposure time of autoradiograph, 7 days. Electrophoresis for 21 hours, 0.65 ma/cm cross-section, agar layer 2 mm thick.

Treatment with ribonuclease altered the electrophoretic mobility of the material which had accumulated near the starting point of the electrophoresis and which had not reacted with the antisera. However, the albumin itself seemed not to be significantly influenced by this treatment. The autoradiograph of the experiment illustrated in Fig. 1 *b* shows that the precipitate of the rat albumin is radioactive. After treatment with ribonuclease the radioactivity of the rat albumin precipitate became more clearly visible. The precipitate of the chick albumin remained unlabeled.

The distribution of the albumin and its radioactivity between the soluble incubation mixture and the ultrasonic extract is shown in Fig. 2 and Fig. 3. In Fig. 2 the photographs of an immunoelectrophoretic experiment are shown. The antigen-antibody precipitates of the soluble incubation mixture untreated and treated with ribonuclease are similar to those obtained in Fig. 1 *a*. The immunological precipitate of rat albumin from the ultrasonic extracts (Fig. 2 *b*) is stronger than that of chick albumin. This is due to the fact that significant amounts of cell sap proteins together with rat serum albumin-like proteins are closely associated with the ribonucleoprotein particles (6, 7, 10). This albumin is removed by treatment with ultrasonic vibrations. In addition, contaminating albumin,

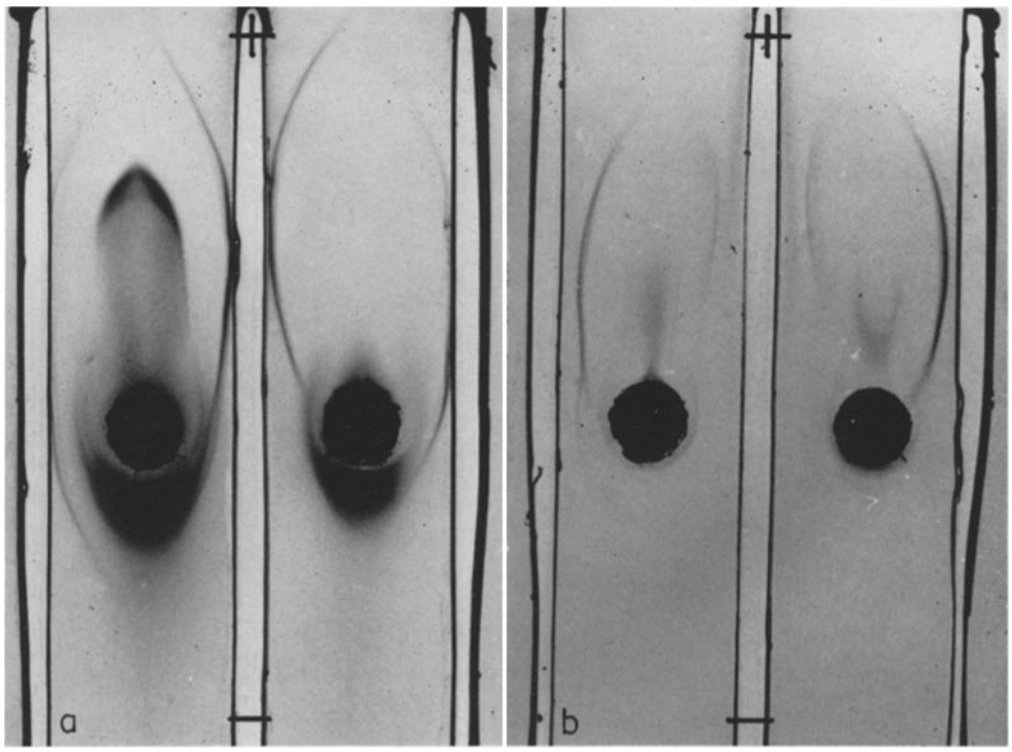

FIGURE 2

Photographs of immunoelectrophoretic plates after incubation with ^{14}C amino acid as described in Fig. 1, but with 5 μc of ^{14}C-L-valine. Prior to isolation of the soluble fractions, cell sap from chick liver was added. The soluble fractions were dialyzed separately before electrophoresis. Electrophoretic runs as described in Fig. 1. Fig. 2 *a*, soluble incubation mixture, left well untreated, right well treated with ribonuclease. Outer basins, antiserum to rat serum albumin; middle basin, antiserum to chick serum albumin. Fig. 2 *b*, as Fig. 2 *a*, but the soluble incubation mixture was replaced by ultrasonic extract.

present in the rat or chick cell saps added to the particles before or after the amino acid incorporation experiments, was similarly removed.

The corresponding autoradiographs (Fig. 3) show a very weak labeling of the rat albumin precipitate of the soluble incubation mixture (Fig. 3 *a*) as compared with that of the ultrasonic extract (Fig. 3 *b*). This suggests that nearly all radioactivity of the combined soluble fractions as illustrated in Fig. 1 must be derived from the ultrasonic extracts.

Additional Purification of Albumin

As a further control, it was thought desirable to include a purification step before the immunoelectrophoretic experiment. As can be seen from the above experiments, the immunological precipitates of the chick albumin remained nonradioactive. Therefore, in the following experiments no chick cell sap was added. The soluble incubation mixture and the ultrasonic extract were combined, dialyzed overnight against 0.0175 M phosphate buffer, pH 6.3, and chromatographed on DEAE-cellulose columns. The column had been equilibrated with the same buffer. A stepwise elution as for serum proteins was used (31). Since the albumin was of primary interest in these studies, no attention was paid to the proteins remaining on the column after elution with 1 M buffer.

It can be seen (Table I) that the radioactivity was unequally distributed between the protein peaks obtained. The treatment with buffered β-mercaptoethanol did not release measurable

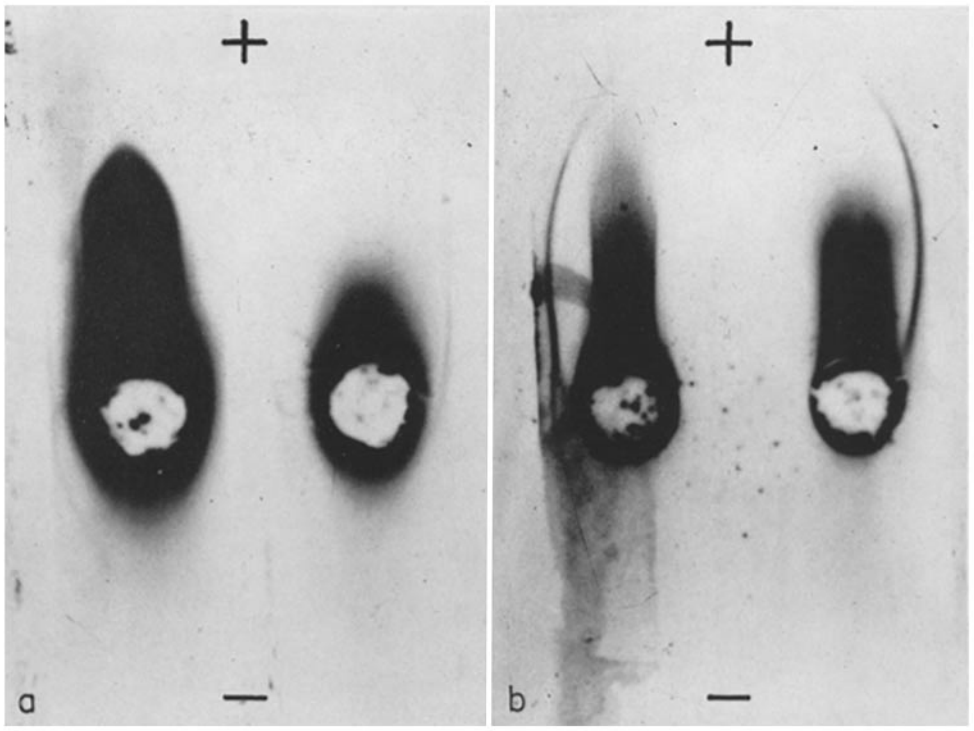

FIGURE 3

Autoradiographs of immunoelectrophoretic plates corresponding to the photographs shown in Fig. 2. Exposure time of autoradiographs, 7 days.

amounts of protein from the column. The particulate fraction had a specific radioactivity of 2400 CPM/mg protein, giving total counts of 58,000. Corresponding chromatographic fractions from several experiments were dialyzed against water, freeze-dried, and examined by immunoelectrophoresis. As illustrated in Fig. 4, antiserum to rat albumin was added to the outer basins and antiserum to whole rat serum to the middle basin of the agar plate. Both antisera gave one precipitation arc with the eluates of 0.1 M buffer and 0.4 M buffer (Fig. 4 *a*), although both eluates contained proteins other than albumin. The eluates of the other buffer concentrations gave no precipitation lines. The autoradiograph shown in Fig. 4 *b* illustrates the radioactivity of the antigen precipitates.

Comparative Electrophoresis in Agar Gel and on Cellulose Acetate

It has been shown (13) that several types of albumin-like antigens might exist in a cell-free system. The electrophoretic mobility of albumin derived from cell sap and ribonucleoprotein extracts of perfused rat liver was therefore studied by immunoelectrophoresis and compared with that of rat serum albumin. Before electrophoresis, the albumins were isolated by column chromatography on DEAE-cellulose after treatment with β-mercaptoethanol (see under Methods). The columns were run in parallel. Most of the albumin was eluted with 0.1 M phosphate buffer. A small amount of albumin which gave a very weak and diffuse precipitate with the antiserum was eluted with 0.4 M buffer. All fractions were run on electrophoresis in agar gel simultaneously with the 0.1 M and 0.4 M eluates of the rat serum. After electrophoresis for 21 hours and addition of antiserum, no clearcut differences in electrophoretic mobility between the precipitated albumins could be seen.

Previous experiments (10) showed that ^{14}C-labeled albumin could be obtained from amino acid–incorporating systems which contained

ribonucleoprotein particles. When this albumin was run on zone electrophoresis on cellulose acetate, nearly all radioactivity was lost. On the other hand, it is evident that albumin obtained after column chromatography on DEAE-cellulose or after electrophoresis in agar gel is in all respects similar to rat serum albumin. To explain the differences between the previous experiments and the above results, electrophoresis on cellulose acetate was combined with the agar gel diffusion method (see under Methods). Rat serum albumin was run in parallel with the soluble fractions obtained after an isotope-incorporating experiment. None of the preparations was treated with reducing agents. The electrophoretic mobility of rat serum albumin was somewhat less than that of the albumin separated by this method from the soluble fractions. In the latter case, there was also an immunological precipitate, localized near the origin of the electrophoresis. These experiments indicated that there may be certain differences between rat serum albumin and the radioactive antigen-active albumin isolated from a cell-free incorporating system containing ribonucleoprotein particles. Therefore, rat serum albumin, labeled *in vivo* with ^{14}C-L-valine, was isolated on a DEAE-cellulose column. In parallel, the soluble incubation mixture and the ultrasonic extract were chromatographed. The 0.1 M column eluates were dialyzed and freeze-dried. Cellulose acetate strips were charged with 1 mg of protein and, after electrophoresis, the distribution of radioactivity was determined. The results are shown in the lower curves of Fig. 5. The upper curves show the corresponding protein concentrations after staining with amidoblack. It can be seen that most of the *in vitro*–labeled proteins stayed at the origin of the electrophoretic run and also that most of the radioactivity was found there. Some radioactivity was registered at the same distance from the origin as the rat serum albumin, and radioactivity was also found in proteins with a somewhat higher electrophoretic mobility than that of the rat serum albumin. If the proteins which stayed at the origin were eluted and run on agar gel, no immunological precipitates could be obtained. In other experiments, the cellulose acetate strips, after electrophoresis, were put on agar gel and the albumin was precipitated by its antiserum. A photograph of such an experiment is shown in Fig. 6. In contrast to what has been found with the original soluble fractions before fractionation on DEAE-cellulose columns, it can be seen that only one immunological precipitate was formed with the albumin of the cell-free incubation system. The concentration of the albumin was low. Moreover, the electrophoretic mobility of this albumin was in all experiments higher than that of rat serum albumin. The maximal concentration of protein was found at a distance from the origin of 10.5 cm, while rat serum albumin had its maximum concentration between 8.5 and 10 cm.

TABLE I

Distribution of Radioactivity in Proteins of Soluble Incubation Mixture Plus Ultrasonic Extract after Column Chromatography on DEAE-Cellulose

Phosphate buffer conc.	Radioactivity (CPM)	
	Per mg protein	Total
0.0175 M, pH 6.3	1,100	13,200
0.0175 M in 0.01 M β-mercaptoethanol, pH 6.3	nil	nil
0.04 M, pH 5.9	37	110
0.1 M, pH 5.8	183	1,280
0.4 M, pH 5.8	244	980
1 M, pH 5.2	302	910

Ribonucleoprotein particles from 32 gm liver were incubated with 2 ml of cell sap from rat liver, 4 μc of ^{14}C-L-leucine, 20 μmoles of ATP, 200 μmoles of creatine phosphate, 500 μg of creatine phosphokinase, and 2 μmoles of GTP. No chick cell sap was added after incubation. The soluble incubation mixture and the ultrasonic extract were combined, dialyzed against 0.0175 M phosphate buffer, pH 6.3, and adsorbed on DEAE-cellulose column. The eluates were collected, precipitated with 5 per cent (*w/v*) trichloroacetic acid, extracted, and plated for counting.

DISCUSSION

It has been shown in a number of publications (23, 24, 27–29) that the pattern of protein labeling in an amino acid–incorporating experiment can be studied by combining immunological reactions in agar gel with autoradiography. Most of these reports concern incorporating experiments with the 12,000 *g* supernatant of a rat liver homogenate or a microsomal–cell sap system. A few experiments were done using a ribonucleoprotein particle–cell sap system. A number of barely

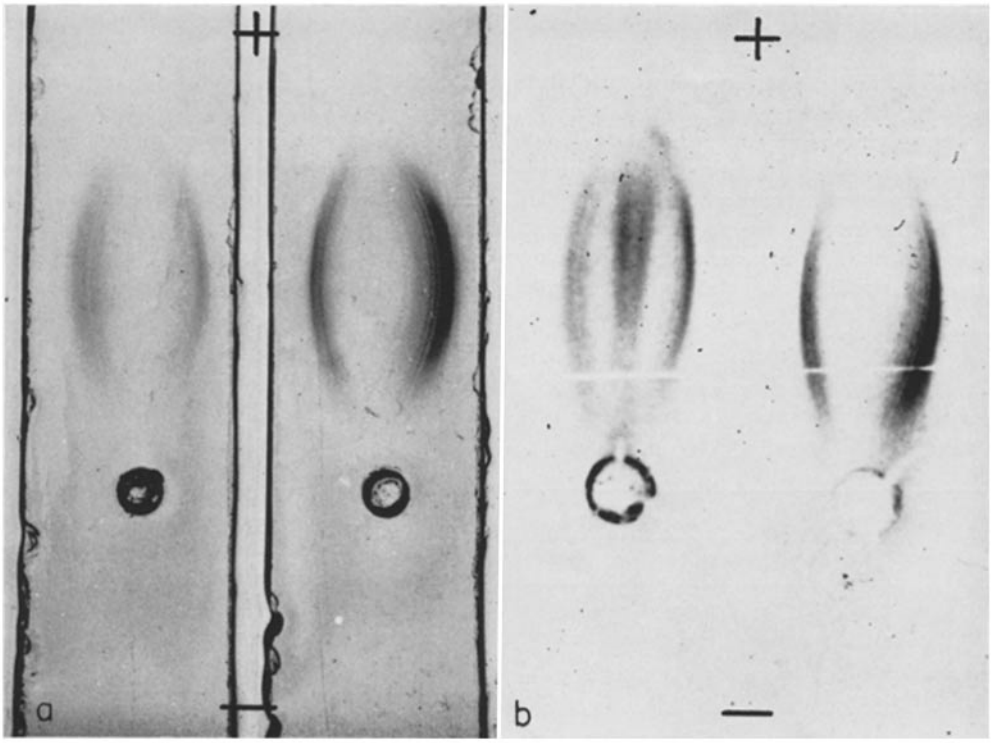

FIGURE 4

Fig. 4 *a*, photograph, Fig. 4 *b*, autoradiograph of immunoelectrophoretic plate made with eluates obtained after column chromatography of the soluble incubation mixture plus ultrasonic extract. Incubation with ^{14}C amino acid as described in Fig. 1. No cell sap from chick liver was added after incubation. Well to the right, eluate with 0.1 M phosphate buffer; well to the left, eluate with 0.4 M phosphate buffer. Outer basins, antiserum to rat serum albumin; middle basin, antiserum to whole rat serum. Exposure time of autoradiograph, 40 days. Electrophoretic runs as described in Fig. 1.

visible but strongly labeled precipitates formed by serum protein–like antigens could be demonstrated, suggesting that antigenic proteins may be at least partially synthesized by isolated ribonucleoprotein particles. The present data on the labeling of an albumin-like antigen on the ribonucleoprotein particles are consistent with these findings.

By treating ribonucleoprotein particles ultrasonically after an isotope-incorporating experiment, it is possible to isolate a nucleoprotein fraction with very high radioactivity (10). Since the ribonucleoprotein particles are the initial site of incorporation of amino acids into protein, it seems reasonable to assume that the specific radioactivity of this nucleoprotein fraction is high. The present experiments demonstrate that the fraction released by ultrasonics contains a protein which is precipitated by the antiserum to rat serum albumin. They also show that the radioactivity of these immunological precipitates is higher than that of the corresponding precipitates of the soluble incubation mixture. It should be emphasized, however, that only at low levels of radioactivity is there a proportionality between the intensity of blackening of the autoradiograms and specific radioactivities. There may also be differences in the protein concentrations of the immunological precipitates which could affect their apparent radioactivity.

After incubation, some of the proteins of the soluble fractions are associated with RNA (10). It was thought that treatment with ribonuclease would remove at least part of the RNA, and that owing to this treatment the electrophoretic mobility of the proteins would change. As far

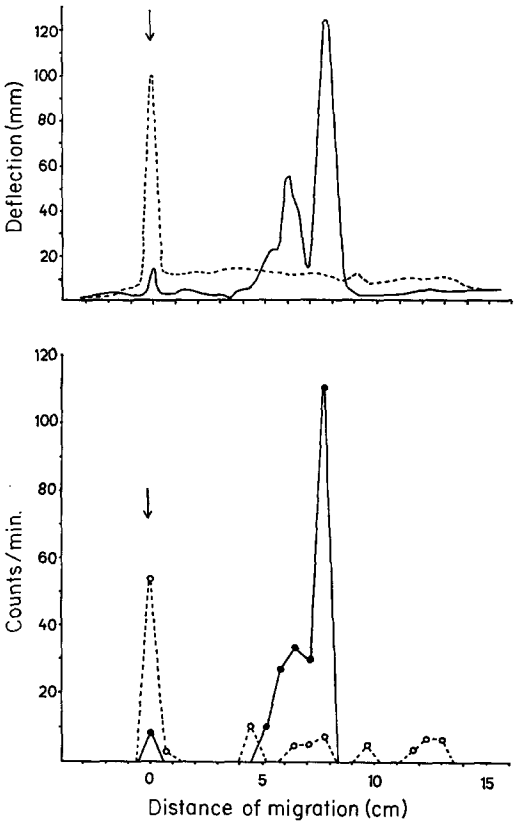

FIGURE 5

Zone electrophoresis on cellulose acetate of 0.1 M eluates from DEAE-cellulose columns. Electrophoretic runs 9 hours. The arrow indicates the origin of the electrophoresis. Solid circles, eluate of rat serum containing serum albumin, labeled *in vivo* with ^{14}C-L-valine. Open circles, eluate of soluble incubation mixture and ultrasonic extract of proteins labeled *in vitro* with ^{14}C-L-leucine in the presence of ribonucleoprotein particles as described in Fig. 1, except that no cell sap from chick liver was added after incubation.

Upper curves: deflection after staining with amidoblack. A deflection of 150 mm was obtained with 0.25 mg protein per cm^2. *Lower curves:* radioactivity of the eluates.

as the albumin is concerned, no striking effect could be observed. Some of the other proteins, however, seemed to be influenced by this treatment. Either the character of the nucleoproteins had changed with respect to their electric charge, or radioactive amino acids, loosely bound to RNA, had been removed. The treatment seemed to be advantageous for the identification of radioactive immunological precipitates.

Possible differences between the albumin-like antigen and rat serum albumin have been studied by comparative electrophoresis in agar gel. Jungblut *et al.* (13), using other methods, were able to separate four types of albumin from rat liver microsomes, all of which reacted with antiserum to rat serum albumin. The albumin eluted from the DEAE-cellulose columns in the present experiments appeared in two fractions. However, it was not possible to show significant differences between their electrophoretic mobilities in agar gel.

Campbell *et al.* (2) showed that rat liver microsomes incorporate amino acids *in vitro* into albumin which had the same electrophoretic properties on cellulose acetate strips as had rat serum albumin. This was, however, not the case when the microsomes were replaced by ribonucleoprotein particles (10). From the present investigation it is evident that albumin derived from a particulate system has electrophoretic mobility on cellulose acetate strips which differs from that of rat serum albumin. Furthermore, it could be shown by combining electrophoresis on cellulose acetate with the double diffusion method in agar gel that this albumin was precipitated by antiserum to rat serum albumin (*cf.* Fig. 6). If tested by the double diffusion method of Ouchterlony (25) after serial dilution of the antigen, this albumin showed the same immunological properties as rat serum albumin. That this antigen-active albumin showed a significant radioactivity could be demonstrated by immunoelectrophoresis in agar gel combined with autoradiography (Fig. 4).

Zone electrophoresis on cellulose acetate is known to be a sensitive method for the separation of proteins (16, 17). It must be considered possible that the differences in mobility observed following electrophoresis on cellulose acetate are due to a disparity in the actual amount of albumin in the two samples although the original amount of protein applied was the same in both cases. If the albumin isolated from the particulate system is not identical with rat serum albumin, the above results may indicate that a precursor to serum albumin or a serum albumin derivative is labeled *in vitro* by ribonucleoprotein particles. It has been suggested (1) that microsomal membranes may play an important role in the synthesis of rat serum albumin. The results of the present investigation would be in favor of this hypothesis, especially since whole rat liver microsomes are able to synthesize rat serum albumin (2).

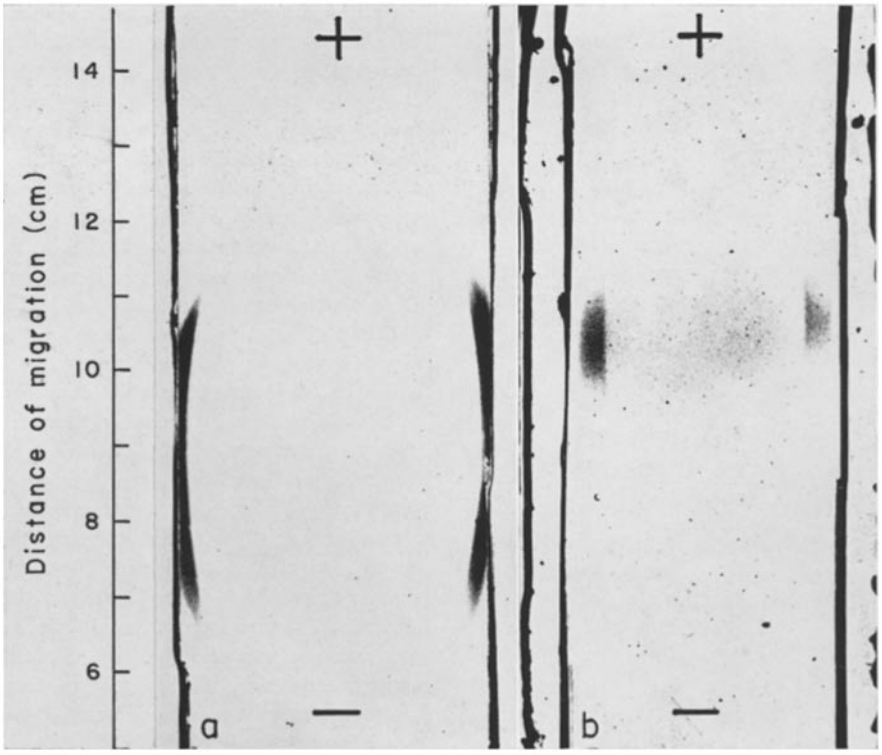

FIGURE 6

Zone electrophoresis on cellulose acetate combined with the method of double diffusion in agar gel of 0.1 M eluates from DEAE-cellulose columns. Electrophoretic runs 10 hours. After electrophoresis the strip was put on agar gel, and antiserum to rat serum albumin was added to the basins. The origin of electrophoresis was at 0 cm (not shown in the figure). Immunological precipitates (*a*) of eluate of rat serum, containing albumin, (*b*) of eluate of soluble incubation mixture and ultrasonic extract.

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