Studies on the Synthesis of Serum Albumin by Ribonucleoprotein Particles Isolated from Rat Liver

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One of the objectives of the work in this Laboratory on the synthesis of serum albumin has been to determine the simplest morphological structure that can synthesize a specific protein. This should help our understanding of the mechanism of protein synthesis and should provide evidence of the role of the various morphological constituents of the cell in the biosynthesis of protein.

Experiments suggesting that the isolated microsome fraction from rat liver can synthesize serum albumin have been reported by Campbell, Greengard & Kernot (1960); Campbell & Kernot (1962). Ribonucleoprotein particles, isolated from the microsome fraction by removing the membrane component with various detergents, incorporate amino acid into protein (Korner, 1959, 1961*a*; Rendi & Hultin, 1960; Takanami, 1960; Kirsch, Siekevitz & Palade, 1960). This suggested that the synthesis of serum albumin could be studied in isolated particles. Korner (1960, 1961*b*) reported that ^{14}C -labelled amino acids on incubation were incorporated into serum albumin by isolated particles after treatment of the microsomal fraction with deoxycholate.

The ribonucleoprotein particles used in the metabolic experiments described below were prepared by the method of Rendi & Hultin (1960). This method provides particles with high activity for the incorporation of amino acids. There is evidence that these particles which are prepared with deoxycholate, Lubrol and a concentrated solution of potassium chloride are almost completely free of other constituents of the cytoplasm (Decken, 1961).

In albumin synthesis ribonucleoprotein particles differ in an important respect from the particles of the microsome fraction: the amount of serum albumin associated with the ribonucleoprotein particles is very small. Nevertheless, it seemed worth while to investigate the problem of albumin synthesis in such particles with the methods previously described for microsomes. Some aspects of this work have been reported by Decken & Campbell (1961*a*).

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MATERIALS AND METHODS

Chemicals. The dipotassium salt of ATP, the sodium salt of GTP and ribonuclease were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Pyruvate kinase and the silver barium salt of phosphoenolpyruvate were obtained from C. F. Boehringer und Soehne, Mannheim, Germany. Solutions of the free acid were prepared before use and adjusted to pH 7 (indicator paper) with n-KOH . The disodium salt of phosphoenolpyruvate was kindly given by Dr V. M. Clarke. Tris and GSH were obtained from British Drug Houses Ltd., Poole. Ovalbumin was obtained from Armour Laboratories, Eastbourne. The sodium salt of deoxycholic acid was obtained from Merck A.-G., Darmstadt, Germany. The non-ionic detergent Lubrol W was obtained from Imperial Chemical Industries Ltd., Manchester.

Radioactive amino acids. Uniformly labelled $\text{L-}[^{14}\text{C}]$ -leucine (90 mc/m-mole) was obtained from the Institut Pasteur, Paris, France.

Animals. Rats (150–200 g. body wt.) were Wistar albino strain, highly inbred at this Institute. The rats were starved for approx. 18 hr. before being killed by a blow on the head followed by decapitation.

Rat serum albumin. This was prepared as described by Campbell *et al.* (1960).

Antisera against rat serum albumin and ovalbumin. Rabbits were injected with alum-precipitated albumin (Campbell, 1955). The amount of antibody in the rabbit serum was estimated by serial dilution to be about 10 mg. of protein/ml. of serum.

Immunological examinations. The extracts from liver-ribonucleoprotein particles were tested for their albumin content either by the agar-diffusion technique (Ouchterlony, 1953) or by immunoelectrophoresis (Grabar & Williams, 1955).

Isolation of ribonucleoprotein particles. Ribonucleoprotein particles were isolated by the method of Rendi & Hultin (1960). The liver (8 g.) was homogenized in 13 ml. of a medium containing 10mM- MgCl_2 , 25 mM-KCl, 35 mM-tris buffer, pH 7.8, and 0.15M-sucrose (medium A). The tissue suspension was centrifuged for 10 min. at 12 000*g*. To the supernatant obtained 4 ml. of 2.5M-KCl in 10mM- MgCl_2 , 2 ml. of 10% deoxycholate and 1 ml. of 10% Lubrol W were added with stirring to give a final volume of 20 ml. The tubes of the no. 30 rotor of the Spinco preparative centrifuge (model L) were loaded with 22 ml. of a medium containing 10mM- MgCl_2 , 0.6M-KCl, 35 mM-tris buffer, pH 7.8, and 0.30M-sucrose. On this medium 10 ml. of the detergent-treated supernatant was layered. The tubes were centrifuged for 90 min. at 78 000*g*. The supernatant was sucked off with a pipette attached to a vacuum pump, the

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insides of the tubes were dried with filter paper, and each pellet was carefully washed with 3×2 ml. of medium A. Each tube contained the ribonucleoprotein particles from 4 g. wet wt. of liver.

Cell sap. Microsomes were prepared by a method which followed closely that of Zamecnik & Keller (1954). The liver was homogenized in 2.5 times its weight of medium A. The tissue suspension was centrifuged for 10 min. at 12 000g, and the supernatant centrifuged in the no. 40 rotor of the Spinco preparative centrifuge (model L) at 105 000g for 60 min. The supernatant is the cell sap. Only the clear part of the cell sap was used.

Preparation of pH 5 fraction. Clear cell sap (10 ml.), diluted with 3 vol. of medium A, was titrated to pH 5.2 as described by Hoagland, Keller & Zamecnik (1956). The precipitate was collected by centrifuging for 5 min. at 12 000g, suspended in 1 ml. of 0.4M-tris buffer, pH 7.8, by slight homogenization in a small all-glass Potter-type homogenizer and diluted to 5 ml. with medium A.

Method of incubation. The particles were suspended by gentle homogenization in a Potter-type homogenizer in a volume of medium A equivalent to one-tenth of the weight of liver from which they were derived. Each incubation tube contained 0.5 ml. of this particle suspension, 0.2 ml. of cell sap or pH 5 fraction, [^{14}C]leucine, with and without 2 μmoles of ATP, 10 μmoles of phosphoenolpyruvate, 50 μg . of pyruvate kinase and, in the presence of pH 5 fraction, 0.25 μmole of GTP in a total volume of 1 ml. Incubation was for 60 min. at 37°.

Isolation of proteins after incubation. The incubation mixture was diluted to 2.3 ml. with medium A and centrifuged for 60 min. at 90 000g. The supernatant was decanted and the particles were subjected to ultrasonic vibrations after suspension in 2 ml. of water. The procedure was that described by Campbell *et al.* (1960), as modified by Campbell & Kernot (1962). After ultrasonic treatment 0.25 ml. of water was added together with sufficient 18% NaCl to bring the NaCl concentration to 0.4%. The suspension was frozen, thawed and centrifuged again at 90 000g for 60 min. The supernatant was decanted.

Before isolation of serum albumin from the soluble fraction of the incubation medium and the ultrasonic extract of the particles 30 μg . of rat albumin was added to the ultrasonic extract. To each extract was then added 60 μg . of ovalbumin, which was removed by the addition of the ovalbumin antiserum and incubation at 37° [for details see Campbell *et al.* (1960)]. To the supernatants was then added an antiserum to rat albumin.

The residue from the particles after ultrasonic treatment and the supernatants from the albumin-antibody precipitates were treated with 5% (w/v) trichloroacetic acid and the precipitates extracted according to a modified Schneider procedure (Zamecnik, Lofffield, Stephenson & Steele, 1951).

Purification of albumin. After the incubation of particles on a large scale the albumin was purified by extraction with phosphoric acid and ethanol and finally by preparative electrophoresis on cellulose acetate as described by Campbell & Kernot (1962).

Measurement of radioactivity. (1) Trichloroacetic acid-precipitated proteins. The extracted proteins were placed on 0.28 cm.² Perspex disks and counted at infinite thickness with a thin mica end-window (2 mg./cm.²) Geiger-Müller counter. A standard planchet of 1 cm.² containing

1 μC of ^{14}C /g. gave approx. 1000 counts/min. under these conditions. (2) Albumin. The radioactivity was determined as described by Campbell & Stone (1957).

The proteins of the experiment whose results are presented in Table 3 were counted in the same way but with a thin-window gas-flow counter (Nuclear-Chicago Corp.). The standard planchet gave 1850 counts/min. under these conditions.

Analysis. RNA was determined by the orcinol method of Meijbaum (1939).

RESULTS

Albumin content of ribonucleoprotein particles

Possible release of albumin when the ribonucleoprotein particles are disrupted with ribonuclease. Particles were prepared from 48 g. of liver. They were divided into two. One part was treated with ribonuclease as described by Roth (1960). The particles were suspended in 10 ml. of 0.1M-sodium acetate buffer, pH 7.4, containing 0.5 mg. of ribonuclease/ml. and incubated for 1 hr. at 37°. The other part was suspended in the same buffer in the absence of enzyme and kept at 2°. After incubation the particles were separated by centrifuging. The supernatants were cleared with ovalbumin and the amount of rat albumin was determined. This showed that the amount of albumin released by the enzyme was not significant with this method of estimation, in spite of a reduction of the proportion of RNA in the ribonucleoprotein from 53 to 17%. However, both samples of ribonucleoprotein particles might have contained some albumin.

Albumin content of extract of particles obtained by ultrasonic treatment. Ultrasonic treatment of ribonucleoprotein particles released ribonucleoprotein with a content of 24% of RNA, representing 1-3% of the total RNA and 3-6% of the total protein of the particles (Decken & Campbell, 1961b). The amount of albumin in such an extract was determined. Estimation of the precipitate caused by the addition of an antiserum to rat albumin indicated no more than trace amounts of albumin in the extracts. The presence of an immunologically active fragment of serum albumin was tested for by the method of Porter (1957). It was shown that there was some substance present in the extract which inhibited the precipitation of albumin with antiserum when the proportion of antigen to antibody was near the point of equivalence. With a constant amount of antiserum and of rat albumin, increasing amounts of extract led to a progressive decrease in the amount of precipitated albumin. This effect could have been due to the presence in the extract of either albumin itself or an immunologically active fragment. Therefore the immunological behaviour of the extract was compared with that of rat serum albumin on diffusion into antiserum in agar gel by the method

of Ouchterlony (1953). The antiserum to rat albumin was placed into the centre cup, rat albumin and extract in the outer wells. The extract derived from 24 g. of liver was dialysed against aq. 0.9% sodium chloride and freeze-dried before use. It gave a line that was confluent with normal albumin at 100 μ g./ml. From this it was estimated that the extract from the particles from 24 g. of liver contained approx. 30 μ g. of albumin. There was no sign on the Ouchterlony plate of lines other than that due to albumin. The extract was further examined by immunoelectrophoresis; the extract was not dialysed, as we wanted to retain any low-molecular-weight fragment that might be present. On immunoelectrophoresis on agar no evidence was found of a component other than albumin reacting with antiserum.

Albumin content of extract of particles obtained after incubation. When the particles from 48 g. of rat liver were incubated in the tris-buffered medium A for 1 hr. at 37° albumin was detected in the incubation medium after removal of particles. Immunoelectrophoresis of an extract obtained by ultrasonic treatment of the incubated particles failed to show the presence of albumin. It appears therefore that the albumin associated with the particles becomes soluble during incubation at 37°.

The ribonucleoprotein liberated from the particles by incubation as described above contained 12% of RNA and represented 8% of the total protein and 1–2% of the total RNA.

Possible synthesis of serum albumin by ribonucleoprotein particles incubated in the presence of cell sap

When ribonucleoprotein particles were incubated with [^{14} C]leucine in the presence of cell sap with and without ATP and phosphoenolpyruvate an energy-dependent incorporation of radioactivity into the various proteins was found. Typical results are shown in Table 1. Since the particles lose most of the serum albumin initially associated with them to the incubation medium, it was necessary after ultrasonic treatment of the particles to add a small amount of carrier albumin to the soluble extract. With the soluble part of the incubation medium this was not necessary as the cell sap present during the incubation contained plenty of albumin.

The particulate albumin was radioactive after incubation in the presence of energy. The activity was considerably greater than that of the ovalbumin, suggesting that the radioactivity of the rat albumin was not due to contamination. The other soluble proteins released from the particles by ultrasonic treatment were also active, but the total activity was only 20% of that of the insoluble particulate proteins. The albumin recovered from the soluble part of the incubation medium was also

Table 1. Incorporation of [^{14} C]leucine into protein by ribonucleoprotein particles

Particles were prepared from 32 g. of liver from rats starved for 18 hr. The particles were suspended in medium and an amount corresponding to 5 g. (wet wt.) of liver was added to each tube. The tubes also contained, in a total volume of 1 ml., 10 μ moles of phosphoenolpyruvate, 2 μ moles of ATP, 50 μ g. of pyruvate kinase, 1 μ C of [^{14} C]leucine and 0.2 ml. of cell sap. Control tubes contained no phosphoenolpyruvate, ATP or kinase. After incubation for 60 min. at 37° the particles were separated by centrifuging at 90 000g for 60 min. The particles were treated ultrasonically and again centrifuged. The particulate protein was treated with trichloroacetic acid (5%, w/v) and extracted with solvents. To the soluble fraction from the particles was added 30 μ g. of rat serum albumin. The soluble fraction, together with that from the incubation medium, was then incubated with ovalbumin (60 μ g.) and antiovalbumin. After removal of the precipitate the rat albumin was precipitated by the addition of antiserum to rat serum albumin. After removal of this precipitate the remaining proteins were precipitated by the addition of trichloroacetic acid (5%, w/v) and the precipitate was extracted. Radioactivity of the trichloroacetic acid-precipitated proteins was determined at infinite thickness (0.28 cm.²). Total count of these proteins was calculated from the specific activity and the weight of protein obtained. The radioactivity of the albumin precipitates was determined at infinite thickness (counts/min./mg.). Total counts of the ovalbumin precipitates are given in parentheses. Results given are the averages of duplicates.

Particles		Without energy plus cell sap				With energy plus cell sap				With energy plus pH 5 fraction			
		Specific activity		Total count (counts/min.)		Specific activity		Total count (counts/min.)		Specific activity		Total count (counts/min.)	
		Infinite thickness	Infinite thickness			Infinite thickness	Infinite thickness			Infinite thickness	Infinite thickness		
Incubation medium	Insoluble	133	—	450		2083	—	8665		1960	—	6050	
	Soluble	67	—	311		400	—	1860		277	—	680	
	Non-albumin Albumin	—	1061	37 (16)		—	8088	297 (61)		—	3690	140 (81)	
	Soluble	4	—	30		171	—	1280		—	7450	69 (33)	
	Non-albumin	—	—	11 (10)		—	4560	80 (36)		—	—	—	
	Albumin	—	360			—				—	—	—	

radioactive. The difference between the ovalbumin and rat-albumin counts was less marked, in agreement with previous findings in a corresponding system of whole microsomes (Campbell *et al.* 1960). The apparent radioactivity of rat albumin may, however, be due to contamination.

Since some RNA was released from the particles by ultrasonic treatment it seemed possible that the radioactivity of the albumin precipitate was due to ^{14}C -labelled amino acid bound to RNA. The RNA content of the various precipitates was therefore determined. The precipitates of proteins derived from the particles were contaminated with about 7 $\mu\text{g.}$ of RNA/100 $\mu\text{g.}$ of ovalbumin and about 14 $\mu\text{g.}$ of RNA/100 $\mu\text{g.}$ of rat albumin. Accordingly the specific radioactivity of the rat-albumin precipitates was determined after their extraction with hot trichloroacetic acid. This treatment did not, however, significantly affect the results.

Possible synthesis of serum albumin by the incubation of ribonucleoprotein particles in the presence of added pH 5 fraction

As has been shown by Littlefield & Keller (1957), the cell sap can be replaced by pH 5 fraction in cell-free systems with either microsomes or tumour particles. Thus albumin synthesis by the particles was studied in the presence of pH 5 fraction. In the experiments shown in Table 1 the results are directly comparable with those from the particle-cell-sap system. To avoid contamination of the pH 5 fraction with cell sap the latter was diluted with medium before precipitation at pH 5.2. The specific activity of the particulate insoluble proteins was about the same as in the particle-cell-sap system. The particulate soluble proteins show a lower incorporation. This is partially due to the somewhat smaller amount of particles used, as indicated by the total counts of the insoluble proteins. The radioactivity of the albumin shows again a significant incorporation of ^{14}C leucine.

The amount of albumin in the pH 5 fraction was low, so that with the amount of pH 5 fraction used in each incubation the quantity of albumin would be negligible. Hence in these experiments a small amount of carrier albumin was added to the soluble part of the incubation medium when the particles had been removed after incubation. Since the amount of carrier albumin added was less than that in the system containing cell sap the specific radioactivity of the soluble albumin was very much higher in the presence of pH 5 fraction than with cell sap. In all other respects the results are similar to those obtained by incubation in the presence of cell sap.

Large-scale incubation of particles. Although the above-mentioned results suggested that there had been an incorporation of ^{14}C leucine into serum

albumin during the incubation of the ribonucleoprotein particles, previous experience indicated that it was desirable to characterize the radioactive albumin further. The following experiments were therefore carried out.

The particles from 48 g. of rat liver were incubated for 60 min. with phosphoenolpyruvate, ATP, pyruvate kinase and cell sap in the proportions described in the Materials and Methods section, together with 10 μC of ^{14}C leucine. After ultrasonic treatment of the particles 1.5 mg. of albumin was added to the extract as carrier. The albumin was then purified, first by the phosphoric acid-ethanol extraction and then by zone electrophoresis on cellulose acetate as described by Campbell & Kernot (1962). The albumin was also purified from the soluble incubation medium. For the purification a greater amount of albumin is needed than already is present in the soluble incubation medium. Therefore, 1 mg. of albumin as carrier was added.

The radioactivity of the insoluble protein from the ultrasonically treated ribonucleoprotein particles was 3216 counts/min. at infinite thickness (0.28 cm.²). The specific radioactivity of the albumin isolated from the particles and the soluble incubation medium is given in Table 2 (a). As a result of electrophoresis approximately 80% of the radioactivity, based on the specific activity of the albumin, was lost.

A similar experiment with a large quantity of particles was carried out, in which the particles were incubated in the presence of pH 5 fraction. Albumin was isolated from the particle extract and the soluble part of the incubation medium in the same way as before except that 2 mg. of albumin was added as carrier in both cases. The radioactivity of the insoluble protein from the ultrasonically treated particles was 5160 counts/min. at infinite thickness (0.28 cm.²). A sample of the albumin from the ultrasonic extract had a specific

Table 2. *Specific radioactivity of serum albumin isolated after incubation of ribonucleoprotein particles*

Radioactivity was measured with a Nuclear-Chicago Corp. thin-window gas-flow counter as described in the Materials and Methods section.

	Specific activity of albumin (counts/min./mg.)	
	From particles	From incubation medium
(a) From particles incubated in the presence of cell sap		
Before electrophoresis	500	555
After electrophoresis	95	143
(b) From particles incubated in the presence of pH 5 fraction		
Before electrophoresis	350	2124
After electrophoresis	80	350

radioactivity of 5700 counts/min./mg. The radioactivity of the serum albumin after electrophoresis is shown in Table 2 (b). Again there was a considerable fall in the radioactivity of the albumin on electrophoresis.

Search for albumin-binding factors in liver extracts

Tomasi (1961) reported that extracts of human liver contain a factor that binds albumin. To check whether rat-liver extracts contain such a factor, agar plates were set up according to the method of Ouchterlony. The centre well contained cell sap prepared from rat liver by homogenizing 5 g. of liver in 10 ml. of aq. 0.9% sodium chloride. The outer wells contained rat serum albumin at concentrations of 1, 5 and 10% and also in one well an antiserum to rat albumin. A line appeared within 24 hr. between the antiserum and the centre well. After 6 days lines also appeared between the 5 and 10% serum albumin wells. No line appeared opposite the 1% solution. A similar experiment was carried out with cell sap prepared by homogenizing 1 g. of liver in 10 ml. of aq. 0.9% sodium chloride. In this case no lines appeared. When the cell sap was freeze-dried and was resuspended in one-tenth the original volume lines appeared in the gel after only 4 days.

To establish the specificity of the binding of the factor from rat liver and rat albumin, cell sap (from 5 g. of liver in 10 ml. of aq. 0.9% sodium chloride) was placed in the centre well and 10% rat albumin, ovalbumin, duck albumin and human albumin in the outer wells. Lines appeared between duck albumin and rat albumin and the centre well respectively. No lines appeared opposite human albumin or ovalbumin. The experiment was repeated with the proteins at a concentration of 5% and the same lines appeared after a longer time.

To check for the presence of a binding factor in liver extracts from other species involved in the present work, extracts were prepared from chick liver and duck liver. In each case cell sap was prepared from 5 g. of liver in 10 ml. of aq. 0.9% sodium chloride. These extracts were placed in the centre wells and the outer wells contained solutions with 10% of rat albumin, ovalbumin, chick albumin and duck albumin. In no case did a line appear between the wells with chick-liver extracts, but with duck-liver extract there was a reaction with both duck albumin and chick albumin.

DISCUSSION

Ever since Littlefield, Keller, Gross & Zamecnik (1955) showed that when a rat received an injection of ^{14}C -labelled amino acid the initial site of incorporation in the liver was the ribonucleoprotein particles of the microsome fraction, it has seemed

possible that such particles were the site of protein synthesis. However, the possibility could not be excluded that the synthesis of protein was completed elsewhere, possibly in the membranes of the endoplasmic reticulum. Against the concept of the membranes' playing a role in the actual synthesis was the synthesis of haemoglobin by particles from reticulocytes, which do not appear to have an endoplasmic reticulum (Schweet, Lamfrom & Allen, 1958). Siekevitz & Palade (1960*a, b*) have provided evidence that the ribonucleoprotein particles of the guinea-pig pancreas are the site of synthesis of chymotrypsinogen and probably ribonuclease. Two aspects of the present work must therefore be discussed: the association of serum albumin or fragments of it with the particles, and the possibility of the synthesis of serum albumin by the particles.

Even when care has been taken to isolate particles containing only very small amounts of substances other than ribonucleoproteins there is some serum albumin associated with them. The protein is not distinguishable from serum albumin on immunoelectrophoresis. Moreover, there is no indication of the presence of other substances reacting with an antiserum to serum albumin. The albumin appears to be released from the particles either by ultrasonic treatment or by incubation in a salt medium. The albumin might be present in the particles because they are the site of its synthesis or because of contamination of the particles with cell sap. In the experiments of Siekevitz & Palade (1960*a, b*) on the synthesis of chymotrypsinogen and ribonuclease there was a substantial release of the specific proteins when the ribonucleoprotein was disintegrated by incubation with ATP, ribonuclease or pyrophosphate. When such methods were used to disrupt the ribonucleoprotein particles from rat liver it could not be shown that the release of albumin was greater than that obtained when the particles were incubated in water (Campbell, 1961). Decken (1961) has shown that even when liver-ribonucleoprotein particles are carefully prepared they still contain amino acid-activating enzymes from the soluble cytoplasm. It appears likely therefore that the albumin associated with ribonucleoprotein particles is due to the presence of a small amount of soluble cytoplasm and that its presence cannot be adduced as evidence on the site of albumin synthesis.

The initial experiments on the possible synthesis of serum albumin by the ribonucleoprotein particles suggested that there was an incorporation of ^{14}C -labelled amino acid into serum albumin. This would have been consistent with the synthesis of the protein by the particles and would have been in accord with the results of Korner (1960) and Lingrel & Webster (1961). Since, however, the

particles contain, after incubation, so small an amount of albumin that it was necessary to add carrier albumin and that the difference in the activity of the clearing precipitate of ovalbumin and the activity of the rat albumin was not as great as it would have been in a microsomal system, the results had to be interpreted with caution. The experiments were therefore repeated under conditions in which it was possible to purify the radioactive serum albumin. When this was done the radioactivity of the albumin progressively fell as the purification proceeded. Even after extraction with phosphoric acid-ethanol and precipitation with specific antiserum the albumin remained radioactive. It was not until zone electrophoresis was applied that the activity fell to a small value. In general the specific radioactivity of the albumin fell by 80 % as a result of electrophoresis.

The question then arises whether the albumin after zone electrophoresis was significantly radioactive. Its radioactivity was such as to make accurate measurements difficult. It is possible that if the treatment had been repeated even the residual radioactivity would have been lost. It may be assumed that there is no significant incorporation of [^{14}C]leucine into serum albumin during the incubation of the ribonucleoprotein particles. It is necessary therefore to account for the apparent radioactivity of the serum albumin before purification by zone electrophoresis. It appears that, during the incubation of ribonucleoprotein particles, [^{14}C]leucine is incorporated into a protein that binds to albumin, is precipitated with it by the addition of antiserum, but is separated from albumin on electrophoresis. Such a protein in extracts of human liver has been described by Tomasi (1961). It is now shown that a similar protein appears to be present in extracts of rat liver. The protein in rat liver does not react with ovalbumin, which may account in part for the lower radioactivity of the ovalbumin precipitates used to clear the rat albumin. The present results are therefore consistent with the synthesis by the particles of an albumin-binding protein rather than of albumin itself. Previous results in which an incorporation of ^{14}C -labelled amino acids into serum albumin during the incubation of a liver-microsome fraction was demonstrated are not affected by the present findings; in experiments by Campbell *et al.* (1960) the albumin was purified by zone electrophoresis. An investigation of the distribution of [^{14}C]leucine among the peptides isolated from the radioactive albumin supported the idea that a synthesis of albumin had taken place.

Decken & Campbell (1961*c*) showed that the smooth-surfaced membranes of the endoplasmic reticulum contained in the microsome fraction from rat liver are not responsible for albumin syn-

thesis. These experiments suggested that it was the fraction rich in elements of the rough-surfaced reticulum that was responsible. When the microsome fraction is treated with detergents the ribonucleoprotein particles obtained will be derived from both the free particles and the particles formerly attached to the reticulum. The present results suggest either that the particles attached to the reticulum are not in themselves the site of albumin synthesis or that in the treatment with detergent they have lost some factor essential for this synthesis.

Our results are not in accord with those of Korner (1960, 1961*b*, 1962 [published in full after submission of the present paper]). He concluded that the ribonucleoprotein particles prepared by treatment with deoxycholate could synthesize albumin. The possibility exists that deoxycholate-treated particles retain some component of the reticulum and can perform the synthesis but that particles treated with deoxycholate, Lubrol and potassium chloride cannot. There is at present, however, no evidence to support this view. It seems more likely that the explanation of the difference in the results lies in the extent of the purification of the 'radioactive' serum albumin.

SUMMARY

1. The release of serum albumin from ribonucleoprotein particles prepared by treating the microsome fraction from rat liver with deoxycholate, Lubrol and potassium chloride was investigated. It is concluded that the small amount of albumin associated with such particles is due to their contamination with soluble cytoplasm.

2. No evidence of substances other than serum albumin reacting with an antiserum with rat serum albumin could be detected in extracts from the ribonucleoprotein particles.

3. When the particles were incubated with [^{14}C]leucine in the presence of phosphoenolpyruvate and either soluble cytoplasm or pH 5 fraction, there was an energy-dependent incorporation of amino acid into a substance precipitated with rat-albumin antiserum. This was found both for an extract of the particles obtained by treatment ultrasonically and for the soluble part of the incubation medium.

4. When the extracts obtained from the incubation of particles with [^{14}C]leucine were purified there was a progressive loss in the specific radioactivity of the serum albumin. The loss after zone electrophoresis was such as to lead to the conclusion that there was no significant incorporation of radioactive amino acid into serum albumin during the incubation of ribonucleoprotein particles prepared by the method described.

5. Evidence is provided for the presence in extracts of rat liver of a substance that can be bound to rat albumin. The specificity of this binding factor is examined. The results of the experiments on the synthesis of serum albumin are interpreted in the light of these findings.

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REFERENCES

- Campbell, P. N. (1955). *Biochem. J.* **61**, 496.
 Campbell, P. N. (1961). *Symp. Protein Biosynthesis*, p. 19. Ed. by Harris, R. J. C. London: Academic Press Inc.
 Campbell, P. N., Greengard, O. & Kernot, B. A. (1960). *Biochem. J.* **74**, 107.
 Campbell, P. N. & Kernot, B. A. (1962). *Biochem. J.* **82**, 262.
 Campbell, P. N. & Stone, N. E. (1957). *Biochem. J.* **66**, 19.
 Decken, A. von der (1961). *Exp. Cell Res.* **23**, 517.
 Decken, A. von der & Campbell, P. N. (1961a). *Biochem. J.* **80**, 39f.
 Decken, A. von der & Campbell, P. N. (1961b). *Abstr. 5th int. Congr. Biochem., Moscow*, p. 181.
 Decken, A. von der & Campbell, P. N. (1961c). *Biochem. J.* **80**, 8p.
 Grabar, P. & Williams, C. A., jun. (1955). *Biochim. biophys. Acta*, **17**, 67.
 Hoagland, M. B., Keller, E. B. & Zamecnik, P. C. (1956). *J. biol. Chem.* **218**, 345.
 Kirsch, J. F., Siekevitz, P. & Palade, G. E. (1960). *J. biol. Chem.* **235**, 1419.
 Korner, A. (1959). *Biochim. biophys. Acta*, **35**, 554.
 Korner, A. (1960). *Biochem. J.* **76**, 59p.
 Korner, A. (1961a). *Biochem. J.* **81**, 168.
 Korner, A. (1961b). *Mem. Soc. Endocrinol.* **11**, 60.
 Korner, A. (1962). *Biochem. J.* **83**, 69.
 Lingrel, J. B. & Webster, G. C. (1961). *Biochem. biophys. Res. Commun.* **5**, 57.
 Littlefield, J. W. & Keller, E. B. (1957). *J. biol. Chem.* **224**, 13.
 Littlefield, J. W., Keller, E. B., Gross, J. & Zamecnik, P. C. (1955). *J. biol. Chem.* **217**, 111.
 Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
 Ouchterlony, O. (1953). *Acta path. microbiol. scand.* **32**, 231.
 Porter, R. R. (1957). *Biochem. J.* **66**, 677.
 Rendi, R. & Hultin, T. (1960). *Exp. Cell Res.* **19**, 253.
 Roth, J. S. (1960). *J. biophys. biophys. Cytol.* **7**, 443.
 Schweet, R. S., Lamfrom, H. & Allen, E. (1958). *Proc. nat. Acad. Sci., Wash.*, **44**, 1029.
 Siekevitz, P. & Palade, G. E. (1960a). *J. biophys. biophys. Cytol.* **7**, 619.
 Siekevitz, P. & Palade, G. E. (1960b). *J. biophys. biophys. Cytol.* **7**, 631.
 Takanami, M. (1960). *Biochim. biophys. Acta*, **39**, 318.
 Tomasi, T. B. (1961). *J. Immunol.* **86**, 427.
 Zamecnik, P. C. & Keller, E. B. (1954). *J. biol. Chem.* **209**, 337.
 Zamecnik, P. C., Loftfield, R. B., Stephenson, M. L. & Steele, J. M. (1951). *Cancer Res.* **11**, 592.

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The Purification and Properties of a Proteolytic Enzyme, Rabbit Cathepsin E, and Further Studies on Rabbit Cathepsin D

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A proteolytic enzyme was prepared from rabbit spleen by Lapresle & Webb (1960) and was shown to be different from cathepsins A, B and C by Webb & Lapresle (1960). This enzyme will be called rabbit cathepsin D, since it is analogous to the cathepsin D isolated from bovine spleen by Press, Porter & Cebra (1960).

Further studies reported here demonstrated the existence in rabbit spleen of traces of another proteolytic enzyme. The investigation of the proteolytic activity of different rabbit-leucocyte preparations has shown that this second enzyme is present in larger amount in polymorphonuclear cells than in lymphocytes (J. Štefanovič, T. Webb & C. Lapresle, unpublished work). Bone marrow being

much richer in polymorphonuclear cells than the spleen, rabbit bone marrow was used as the starting material for the preparation of this second enzyme. This enzyme was purified and shown to be different from cathepsins A, B, C and D; it has been named cathepsin E.

The purification and properties of cathepsin E as well as further studies on rabbit cathepsin D are described in this paper.

MATERIALS

Diethylaminoethylcellulose and carboxymethylcellulose. Diethylaminoethylcellulose (DEAE-cellulose) and carboxymethylcellulose (Selectacel) were obtained from Brown