Serum albumin has many characteristics which make it an attractive subject for the study of protein biosynthesis. It is abundant in serum and is produced by the liver at a rapid rate. Its high stability and solubility make for easy isolation. Structurally it appears to consist of a single long chain of about 550 amino acid residues without prosthetic groups.

Some data on the formation of chicken serum albumin in vivo and in liver slices have been presented in previous reports (1-4). More detailed studies are being carried out on the rat, because the cytochemical and anatomical information available for rat liver allows one better to correlate findings on protein biosynthesis with concepts of cell structure.

This report presents some properties of rat serum albumin, and gives the quantity of serum albumin detected in various rat liver cell fractions (including nuclei, mitochondria, lysosomes, and “rough-surface” and “smooth-surface” microsomes) and in the soluble fraction from the intact liver and from isolated liver cells. The succeeding article concerns the time relations of albumin synthesis and secretion.

EXPERIMENTAL PROCEDURE

Animals—Male Wistar rats, 100 to 200 g, were used unless otherwise indicated. They were fed Animal Foundation Laboratory Diet ad libitum, except that rats were fasted 16 hours before preparation of microsome subfractions and cell membranes.

Preparation of Rat Serum Albumin—Citrated plasma was obtained by heart puncture from 100 rats weighing over 400 g each. Globulins were removed by adding an equal volume of saturated ammonium sulfate, and a crude albumin fraction precipitated by lowering the pH to 5.0 with 1 N acetic acid. The crude albumin was dialyzed, centrifuged, and lyophilized. This crude albumin was purified by starch electrophoresis at pH 7.5, \( \mu = 0.05 \) sodium phosphate, then at pH 4.7, \( \mu = 0.05 \) sodium acetate, and finally again at pH 7.5. The solution was applied, 10 ml per run, as a 1.5-cm strip in a bed (1 x 19 x 40 cm) of potato starch, and subjected to a voltage gradient of 5 volts per cm at 3° for 24 hours at pH 7.5, or for 44 hours at pH 4.7. After each run the central 1.5 cm of the albumin band was cut out, the albumin eluted with water, dialyzed, and lyophilized. By taking only the central portion of the albumin band after each run, a product was obtained which appeared to be homogeneous, although the yield was only 900 mg of albumin from 1,100 ml of blood.

For tests of recovery and adsorption, rat albumin was iodinated with \( \text{I}^{131} \) at pH 9.4 to an average level of less than 1 iodine atom per molecule (2). \( \text{I}^{131} \) was measured at constant volume in a well-type scintillation detector with a Packard two-channel pulse height analyzer.

Preparation of Antisera—Rat serum albumin or chicken serum albumin (4) was used for injection into rabbits with alum adjuvant, the \( \gamma \)-globulin fraction isolated as described (2), and diluted in 0.04 M sodium phosphate buffer, pH 6.8, so that 1 ml precipitated about 0.2 mg of albumin.

Cell Fractionations—The initial fractionation scheme was essentially that of de Duve et al. (5). Fairly rigorous homogenization was found necessary to break over 90% of the liver cells, especially when the procedure was applied to isolated whole cells. The liver was perfused with cold 0.15 M NaCl via the vena cava, blotted, cut, and homogenized with 3 volumes of 0.25 M sucrose-0.001 M EDTA for 5 passes at about 1,200 r.p.m. with a Teflon pestle. The homogenate was centrifuged at 1,000 x g for 10 minutes, and the sedimented nuclear fraction rehomogenized and centrifuged twice with 2 volumes of sucrose-EDTA. The combined supernatant and washings were centrifuged at 3,300 x g for 10 minutes, and the resulting mitochondrial fraction washed with 1 volume of sucrose. A small, brown, lysosome-rich fraction was sedimented at 12,000 x g for 10 minutes and washed once; the light-pink “fluff” on top of the brown lysosome pellet was removed by gentle rinsing. This fluff, which resembles microsomes in chemical composition (6, 7), was also found to approximate microsomes in serum albumin content, and was included with the microsome fraction. Finally, the microsomes were sedimented at 105,000 x g for 30 minutes.

Nuclear fractions prepared by this procedure were heavily contaminated by mitochondria, therefore the nuclear and mitochondrial fractions were combined before extraction of albumin so that the recovery of mitochondria might be complete. Nuclei almost free of contamination were prepared from separate aliquots of liver by the method of Hogeboom, Schneider, and Striebich (8) or by the modification of Wilczok and Chorasy (9). These samples were found to contain negligible albumin.

“Rough-surface” and “smooth-surface” microsomes were isolated by sedimentation through sucrose solutions of differing density as described by Rothschild (10). Liver was perfused with 0.15 M NaCl and homogenized with 4 volumes of 0.88 M sucrose. After the first pass the slurry was poured through a
stainless steel screen with 0.0006-μ openings to remove connective tissue. Nuclei, mitochondria, and unbroken cells were sedimented at 25,000 × g for 20 minutes. The supernatant was diluted with an equal volume of 1.76 M sucrose, and portions (1 to 2 ml) centrifuged at 105,000 × g (Spinico model L ultracentrifuge, No. 40 rotor) for 10 to 16 hours in a 12-ml tube with 8 ml of 1.23 M sucrose and 1 ml of 0.15 M sucrose layered carefully above. The smooth surface fraction was removed with a J-tube from the boundary above the 1.23 M sucrose, diluted with 3 volumes of water, and sedimented as a clear-brown, gelatinous pellet. The rough-surface fraction (the clear-red pellet from the 10- to 16-hour spin) was washed once by homogenizing with 0.25 M sucrose and centrifuging. Electron microscopy showed a preponderance of 150 A granules in the rough-surface preparation and almost complete absence of granules in the smooth-surface preparation.

Parenchymal cell membranes were isolated in low yield by the method of Neville (11). Under the phase microscope or electron microscope, they displayed the angular configurations and other features he reported.

Isolated Whole Cell Preparation—Liver was perfused with 0.15 M NaCl under pressure so that it distended, then cut, and the cells isolated as described by Kaltenbach (12), pressing gently through 0.0006-, 0.0005-, and, finally, 0.0006-μ screens with 0.15 M NaCl. The cells were washed free of debris by four or more centrifugations at 35 × g for 2 minutes. Yield was approximately 10% of the initial liver; microscopically, the cells appeared intact and almost free from contamination by debris.

Extraction of Albumin from Particulate Fractions—Sedimented fractions were suspended uniformly in water, aliquots taken for chemical analyses, and the remainder made to 0.5% sodium deoxycholate (Fisher No. 8-285) and 0.05 M Tris (pH 8.0), about 1 ml per g of tissue. After 3 to 16 hours at 38° they were centrifuged for 20 minutes at 25,000 × g, and the supernatants dialyzed at 3° against 0.1 M NaHCO₃, then against water, and finally against 0.02 M sodium phosphate buffer of pH 6.2 to precipitate the remaining deoxycholate. After centrifuging, the supernatants were made to 0.04 M phosphate, pH 6.8, for the precipitation of albumin.

Determination of Serum Albumin—To lessen coprecipitation of nonspecific proteins, of significance mainly in later studies with C₁⁴, all samples were first treated with chicken serum albumin and anti-chicken albumin, using 1.5 times as much chicken albumin as the rat albumin present. After 1 hour at 38° and 16 hours at 3°, the precipitates were discarded. Chicken albumin was used in preference to bovine albumin (2), because bovine and rat albumins cross-reacted slightly. Chicken albumin and rat albumin did not cross-react in tests in agar, and no rat albumin₁² was lost in the chicken albumin-antibody precipitate. Four or more aliquots of the supernatants, each containing 5 to 8 μg of rat albumin, were adjusted to 0.5 ml, and 0.1 ml of anti-rat albumin was added. After 1 hour at 38° and 16 hours at 3°, the specific precipitates were centrifuged down, washed 3 times with 0.2 ml of 0.04 M phosphate, pH 6.8, and the protein in the precipitate determined by the BSP-binding procedure of Glick et al. (13). To the precipitate was added 0.1 ml of 0.53 M NaOH, followed by 0.2 ml of 0.016% BSP-0.133 M citric acid-0.27 M HCl. After 15 minutes at 38°, the fine BSP-protein precipitate was removed, washed with 0.01 M NaOH, transferred to a measured volume of 0.1 M NaOH, and the absorbancy read at 580 μM. The BSP remaining in the supernatant was also determined.

If rat albumin₁² was added to isolated cell fractions before extraction of albumin, 75 to 85% was recovered in the albumin-antibody precipitates. Albumin values were routinely corrected for 80% recovery.

Determination of albumin in five specimens of rat serum gave an average of 3.6 g per 100 ml by the immunological method compared to 3.8 g per 100 ml by the method of Howe (14).

Chemical Analyses—Nitrogen was assayed by nesslerization after Kjeldahl digestion with CuSO₄. RNA was determined by the orcinol reaction (15) and total nucleic acid by the absorbancy at 260 μM of perchloric acid extracts. The standard was yeast RNA. Lipid phosphorus was determined on 1:1 alcohol-ether extracts (15). Blood remaining in liver after perfusion was estimated by determining hemoglobin in the homogenates by the benzidine reaction (16). Results were corrected by subtracting a blank value equivalent to 0.016 ml of blood per g of liver found for blood-free liver cell preparations. The quantity of liver in homogenates was assayed by the turbidity test with cationic detergent (benzalkonium chloride) in 0.05 M NaOH described by Jacox (17), with a standard curve prepared with whole, unperfused liver.

RESULTS AND DISCUSSION

Properties of Rat Serum Albumin—The rat serum albumin preparation migrated as a single band on paper or on cellulose acetate at pH 4.7 or 8.0. It gave a single precipitin line in agar at various concentrations against rabbit anti-rat serum. It showed less than 1% of the “fast alpha” globulin component reported by Jacox in the test for turbidity with cationic detergent (17). A single NH₄₃-terminal glutamic acid residue was detected by the fluorodinitrobenzene technique (18) and a single COOH-terminal alanine residue was found by hydrazinolysis (18).

Some of the physical and chemical properties of rat serum albumin are recorded in Table I. Data from other workers are included where indicated. Rat albumin is similar to the albumins of other species, although crude preparations show greater heterogeneity on electrophoresis between pH 4 and 5 (17, 20). The albumins of two other rodent species, the rabbit (22) and the guinea pig, also have NH₄₃-terminal glutamic acid or glutamine in place of the NH₃-terminal aspartic acid shown by the albumins of other mammals or of birds (18).

Level of Serum Albumin in Cell Fractions—In Table II are given the composition and serum albumin content of various types of liver cell fractions. The results for nitrogen, RNA, and lipid phosphorus are in fair agreement with reported values (23, 24). Rough-surface microsomes, high in ribosomes, are particularly high in RNA, whereas the smooth-surface microsomes are low in RNA and high in phospholipid.

The intracellular distribution of rat serum albumin is similar to that of chicken serum albumin (2), except that in the rat the level in microsomes is higher. No albumin is found in purified nuclei of either species when extracted by several differ-
ent means, although loss of albumin from nuclei during isolation cannot be ruled out.

The lysosome fraction of de Duve (25) is very low in albumin, which might be predicted from the postulated role of these particles as containers of hydrolytic enzymes.

Most of the particulate-bound albumin is in the microsomal fraction, which is reportedly derived from the endoplasmic reticulum of the cytoplasm (23). The value given is higher than that reported earlier (26), due mainly to the use of deoxycholate rather than acidity to extract microsomes. When microsomes are separated into rough-surface (granular) and smooth-surface (agranular) types, little albumin is found with the rough-surface microsomes, although 75% of the microsomal RNA is recovered with this fraction. The concentration of serum albumin in smooth-surface microsomes is 3 times that of the whole microsome fraction, and 6 times that of the rough-surface type. The recovery of smooth-surface microsomes is incomplete, and it is possible that most of the microsomal albumin is in the smooth-surface reticulum within the cell. It should be pointed out that the smooth-surface fraction may also include the microvilli of the cell membrane into which the agranular reticulum appears to merge (23).

The concentration of albumin in the isolated cell membrane fraction was very low. To test whether the exposure to low osmotic pressure in the preparation of membranes had caused the loss of albumin, smooth-surface microsomes were similarly treated. Suspension in water at 0° for 1 hour caused a loss of about one-half of the albumin of the smooth-surface microsomes. Even with correction for this amount of loss, the albumin in membranes would be very low. The exact nature of the structures obtained by the membrane isolation technique is not clear; should it consist mainly of bile canaliculi, for instance, albumin bound to membranes on the sinusoidal surfaces would have escaped detection.

Appreciable albumin was found in the supernatant from the cell fractionation. About one-half of this amount can be attributed to albumin in the blood remaining after perfusion. To determine whether the balance of the soluble albumin is intracellular or in the extracellular space, the supernatant fraction from homogenates of isolated whole liver cells was examined. The washing procedure included in the preparation of these cells should remove any extracellular albumin.

Only 55 µg per g of albumin were detected in the supernatant from such cells (Table II). Isolated cells required longer homogenization than whole liver, however, and hence even this small amount of soluble albumin may have arisen to some extent from rupture of cell particles. On the other hand, some loss of intra-cellular soluble albumin probably occurred during washing of the cells, so that the reported figure may be in error in either direction. It does agree with the value of 60 µg per g which can be derived from the data of Gordon and Humphrey (27) on the distribution of injected rat serum albumin-I⁹¹.

The total particulate-bound albumin found was 0.53 mg per g. Marsh and Drabkin (28) found 0.50 mg per g, and a figure of 0.53 can be calculated from the results of Gordon and Humphrey (27).

Tests for Adsorption of Soluble Albumin to Particles—The possibility of adsorption or exchange of soluble albumin by particles during the isolation procedure was tested by addition of rat albumin-I⁹¹ to homogenates. The labeled albumin was completely recovered in the supernatant and washes (Table III). Buseh et al. (29) obtained similar results with C¹⁴-labeled rat albumin, although they observed considerable binding of labeled serum globulins.

Elution of Albumin from Particles—Some data on the amount of serum albumin extracted from microsomes and mitochondria by various treatments are given in Table IV. A more extensive study of the binding of albumin to chicken liver microsomes has been reported (1). As with chicken liver microsomes, deoxycholate was the most effective agent in releasing albumin from rat liver microsomes, implying that the microsomal albumin is
TABLE III
Recovery of rat serum albumin-I\(^{131}\) in cell fractionation procedure

A liver homogenate was prepared as described in the "Experimental Procedure" section and 1 \(\mu\)g of rat serum albumin-I\(^{131}\) (2 \(\mu\)c) added to an aliquot of 1 g before isolation of cell fractions.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Sediment</th>
<th>Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Nuclei</td>
<td>0.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Supernatant</td>
<td></td>
<td>99.1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>99.7</td>
</tr>
</tbody>
</table>

TABLE IV
Elution of serum albumin from particles

Cell fractions were prepared as described in the "Experimental Procedure" section and eluted using Tween 20 or freezing and thawing in lieu of deoxycholate. pH 2.4 was obtained with 0.05 M phosphoric acid; after 15 minutes at 0\(^{\circ}\) the solution was neutralized with 1 N NaOH.

<table>
<thead>
<tr>
<th>Particles</th>
<th>Treatment</th>
<th>Albumin eluted ((\mu)g/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes</td>
<td>Deoxycholate</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td>pH 2.4</td>
<td>170-300</td>
</tr>
<tr>
<td></td>
<td>Freeze 3X in water</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Deoxycholate</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Tween 20</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Freeze 3X in water</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>pH 2.4</td>
<td>15</td>
</tr>
<tr>
<td>Mitochondria</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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Theodore Peters, Jr.


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