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Albumin was highly purified from a commercially available rat albumin preparation (Fraction V) using disc electrophoresis. The purified albumin had the same antigenicity as Fraction V. A monospecific anti-rat albumin rabbit serum was obtained. The antiserum was used in a double antibody quantitative method for determining rat albumin.

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— BRIEF NOTE —

**PREPARATION OF ANTI-RAT ALBUMIN ANTISERUM
AND ITS APPLICATION TO RADIOIMMUNOASSAY**

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Abstract. Albumin was highly purified from a commercially available rat albumin preparation (Fraction V) using disc electrophoresis. The purified albumin had the same antigenicity as Fraction V. A monospecific anti-rat albumin rabbit serum was obtained. The antiserum was used in a double antibody quantitative method for determining rat albumin.

Key words: rat albumin, anti-rat albumin antiserum,
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A specific marker for differentiated hepatic parenchymal cells is needed to study the survival of hepatic cells in culture. Albumin is a secretory protein synthesized exclusively by hepatic parenchymal cells (1). Quantitative detection of albumin secreted into cultured medium can be achieved by radioimmunoassay without killing cultured cells. Albumin secretion by isolated liver cells has already been demonstrated by radioimmunoprecipitation of newly synthesized and labeled albumin (2). Previous techniques including radial immunodiffusion (3) are, however, generally less sensitive than radioimmunoassay. This report describes methods of rat albumin purification, preparation of monospecific antiserum against rat albumin, and an application of this albumin to a double antibody method of radioimmunoassay.

Albumin was highly purified from rat albumin Fraction V (Miles Lab., Inc., Kankakee) by disc electrophoresis. Albumin Fraction V, which consisted of three main protein components (Fig. 1), was dissolved in 5 mM phosphate-buffered saline (PBS), pH 7.5, at a concentration of 5 mg/ml. Forty μ l aliquots of this solution were applied to 7% polyacrylamide gel columns with bromophenol blue (BPB) as a marker and subjected to electrophoresis for 60 min at

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4°C and 4 mA/tube. Immediately after each electrophoretic run, the albumin portion of electrophoresed gels was cut out following the blue bands of BPB-bound albumin. Minced gels were suspended in PBS, shaken for 1 h at room temperature and kept in a refrigerator overnight. The extract was concentrated by ultrafiltration and dialyzed against large quantities of PBS. Disc electrophoresis (Fig. 1) and immunoelectrophoresis of purified albumin thus obtained revealed a single protein with antigenicity identical to rat albumin Fraction V.

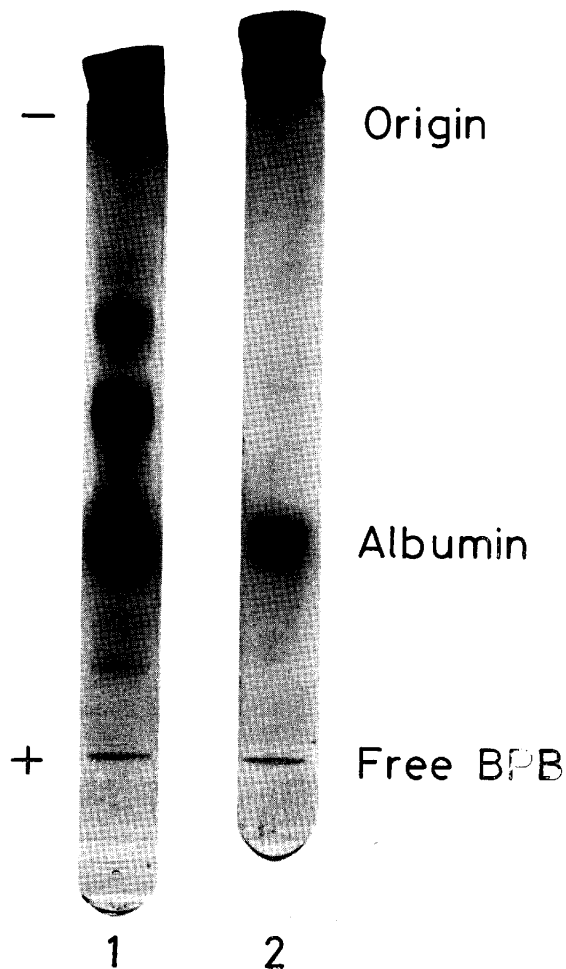


Fig. 1. Disc electrophoretic patterns of rat albumin Fraction V and purified albumin. Rat albumin Fraction V (50 μ l at 5 mg/ml) and purified albumin (50 μ l at 1.26 mg/ml) were applied to columns Nos. 1 and 2, respectively, and subjected to electrophoresis as described in the text. Proteins were stained with Amido Schwarz.

An anti-rat albumin antiserum was obtained from rabbits immunized with the purified albumin. Three male rabbits were injected four times at an interval of two weeks by subcutaneous injections of 3.4 mg albumin emulsified with an equal volume of Freund's complete adjuvant. The animals were bled 8 weeks after the first injection, and serum was separated. The obtained antiserum was monospecific for rat albumin (Fig. 2) and did not crossreact with bovine albumin.

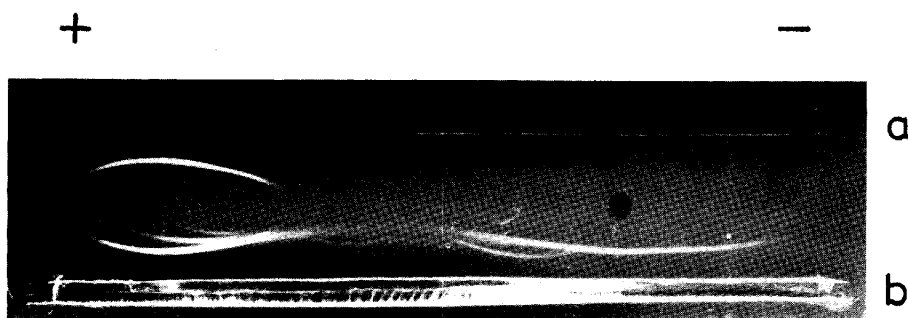


Fig. 2. Specificity test of anti-rat albumin rabbit serum by immunoelectrophoresis. Fifteen μ l aliquots of rat serum (1 : 32) were placed in a center well. The sample was subjected to electrophoresis on 1.5% agarose (12.5 mM barbital buffer, pH 8.6) for 90 min at room temperature, 6 mA/plate and a constant voltage of 60 V. After the electrophoretic run, anti-rat albumin rabbit serum (1 : 32) (Trough a) and anti-rat whole serum rabbit serum (1 : 32) (Trough b) were poured into the indicated troughs. The double diffusion was performed at room temperature for 24 h.

Purified albumin was labeled with ^{125}I by the chloramine T method (4), and separated from free radioactive iodine by gel filtration (Fig. 3). One mCi

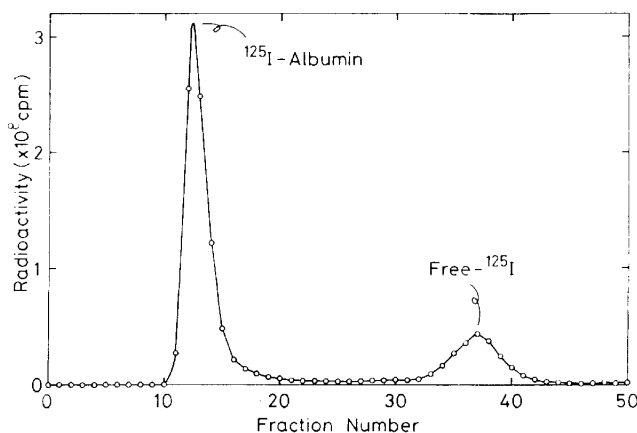


Fig. 3. Isolation of ^{125}I -albumin by column chromatography on Sephadex G-25.

of Na ^{125}I (Radiochemical Centre, specific radioactivity 11–17 mCi/ μg I, 10 μl) was reacted with 100 μg of purified albumin (20 μl at 5 mg/ml) at 4°C using chloramine T (25 μl at 3.5 mg/ml) in phosphate buffer, pH 7.8 (15 μl). After gentle mixing for 25 sec, sodium metabisulfite (100 μl at 3.5 mg/ml) and potassium iodide (200 μl at 10 mg/ml) were added. The reaction mixture was transferred to a Sephadex G-25 column equilibrated previously with PBS containing 1% bovine serum albumin (BSA), then eluted with PBS at a flow rate of 60 ml/h, 1 ml fractions being collected in tubes containing 0.1 ml of 10% BSA in PBS. Three fractions (Fraction Nos. 12, 13 and 14) were pooled, frozen at –20°C and diluted to 400 times with 1% BSA in PBS before use.

Quantitative determination of albumin content was carried out by a double antibody method using anti-rat albumin rabbit serum and anti-rabbit γ -globulin goat serum (Dinabot Radiochemical Lab., Tokyo, Japan) (5). The first reaction mixture consisted of 0.1 ml of samples to be tested or standard albumin, 0.1 ml of a 1 : 400 dilution of ^{125}I -albumin (about 30,000 cpm), 0.1 ml of a 1 : 2,500 dilution of anti-rat albumin rabbit serum (1st antibody), 0.1 ml of a 1 : 100 dilution of normal rabbit serum and 0.5 ml of PBS. This mixture was incubated at 4°C for 24 h. At the end of this period, 0.1 ml of a 1 : 30 dilution of anti-rabbit γ -globulin goat serum (2nd antibody) was added to the first reaction mixture. After the incubation at 4°C for 24 h, the final reaction mixture was centrifuged for 40 min at 4°C and 3,000 rpm, the supernatant decanted, and the radioactivity of the precipitate measured in a well-type scintillation counter

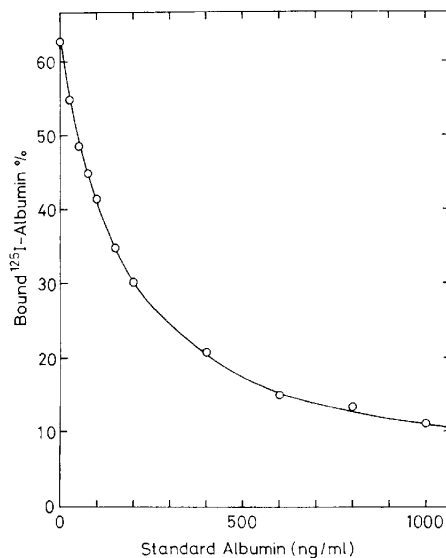


Fig. 4. Calibration curve of rat albumin by radioimmunoassay.

(Aloka Inc.). A calibration curve for determining rat albumin content was obtained as shown in Fig. 4. The curve was not affected quantitatively even by the addition of 0.1 ml aliquots of 20% bovine serum in the culture medium to the assay system. Albumin contents up to 500 ng/ml could be accurately measured by this technique (Fig. 5). Therefore, this reproducible method appears promising for future cytological studies of liver cells in tissue culture laboratories.

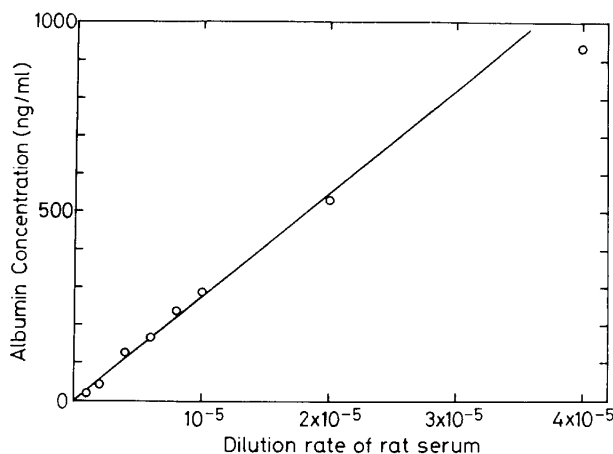


Fig. 5. Quantitation of albumin content in various dilutions of rat serum by radioimmunoassay using the double antibody method. Rat serum was diluted as indicated with 1% BSA in PBS. The results are expressed as the mean of 3 experiments.

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