A Procedure for Concentration of Normal Urinary Albumin and Globulins.* (27047)

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Normal adult human urine contains an estimated 15-30 mg/day of albumin and an undetermined quantity of protein corresponding electrophoretically to serum globulins(1,2). Webb, Rose and Sehon(3) concentrated urinary albumin by electrophoresis on starch and elution of the appropriate region. Some physical and serological properties were studied and found to correspond to serum albumin. The presence of γ-globulins, predominantly in a fragmented state, has also been unequivocally shown(3,4) in a similar manner. Zone electrophoresis is generally an unsatisfactory procedure for separating γ-globulins in reasonable purity(4,5,6).

This paper describes a procedure for concentration and estimation of albumin and of globulins from normal urine, almost free of extrinsic carbohydrate material. "Globulins" refer here only to those proteins migrating toward the cathode at pH 4.5(7).

Materials and methods. Lyophilized fraction RS-1 of the total nondialyzable urinary solids (8) was the starting material. This is the veronal buffer-soluble, nonultrafiltrable fraction.

A 5% solution of RS-1 was precipitated by saturation with $(NH_4)_2SO_4$. After 2 hr standing in the refrigerator, the mixture was centrifuged (35000 × g × 45 min). Most of the uronic acid in RS-1 was in the clear, colorless supernate, which amounted to 4% of the starting weight of RS-1. The precipitate was redissolved in water, dialyzed free of $(NH_4)_2SO_4$, then relyophilized. If this preliminary precipitation was omitted, the subsequent separation was unsatisfactory.

Five hundred mg of the lyophilized material, corresponding to eight 24-hour urines (from male laboratory workers following their daily routine), was mixed with about 5 ml water and placed on a 21 x 170 mm DEAE-cellulose column (OH form). The column was eluted stepwise; successive eluants were 500 ml portions of water, 0.05 M NaH_2PO_4, and 0.05 M NaH_2PO_4—0.05 M NaCl. Flow rate was 25 ml/hr.

The Ouchterlony agar gel double diffusion technic was used for serological examinations (9).

Results and discussion. The colorless second eluate contained the serum globulins. The third eluate consisted almost entirely of albumin, and on lyophilization was a cream colored, fluffy powder.

Albumin. Yields were reproducible, amounting to 100 ± 10 mg, which represents an excretory rate of about 12 mg/day.

On analysis, a typical sample of the lyophilized material contained 15.4% N, 2.3% hexosamine and 3% hexose, but no hexuronic or sialic acids. Some contamination is also indicated by the electrophoretic pattern (Fig. 1). This contamination was estimated planimetrically at 5—15% in various preparations.

Zone electrophoresis on polyacrylamide gel ("disc electrophoresis" apparatus, Canal Industrial Corp., Bethesda) demonstrated a single sharp band, migrating like serum albumin. This technic has very high sensitivity and resolution, but mucosubstances may not necessarily be stained.

Analytical ultracentrifugation (10 mg in 0.9 ml M NaCl; 56,100 rpm; 134 min) of urinary albumin demonstrated a single gradient, moving in the double sector cell at the same rate as a simultaneously run serum albumin sample.

Serologically, the material gave a single line of identity with serum albumin against rabbit anti-human serum albumin (Sylvana Co., Milburn, N. J.) and also against rabbit anti-human serum (Fig. 2). This is a sensitive indication that the contaminants either are heter-

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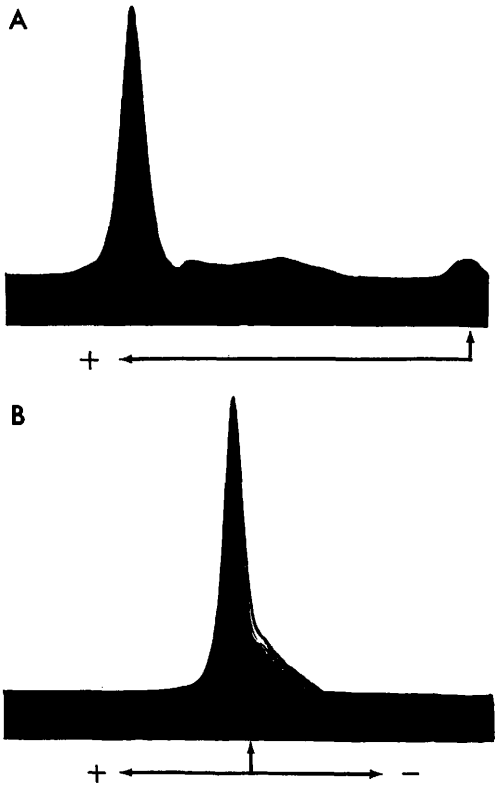

FIG. 1. Electrophoretic patterns of urinary albumin (A) at pH 8.6 (6 mg/ml, veronal, 0.1M, 5.6 v/cm, 1°C, asc. mobility -6.4 cm^2/v sec) and (B) at pH 4.5 (8 mg/ml, acetate, 0.1M, 5 v/cm, 1°C, desc. mobility -0.29 cm^2/v sec) after 4 hr. migration. Spinco model H instrument.

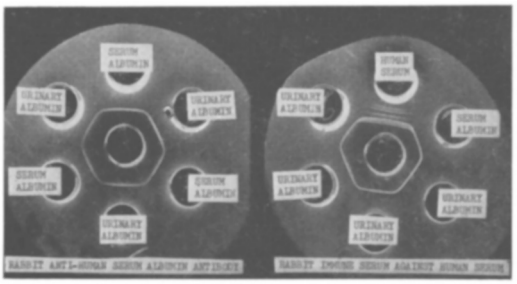

FIG. 2. Precipitin reaction on Ouchterlony plates. Left: Alternate wells of serum albumin and urinary albumin against rabbit anti-human serum albumin (center well). Right: Wells of human serum (top), serum albumin (top, right) and urinary albumin against rabbit anti-human serum (center well).

ogeneous, nonantigenic, or originate from the genitourinary tract.

The following experiments were intended to determine whether the recovery was quantitative. Albumin equivalent to about 3mg/24 hr urine was present in eluate 2, as estimated from its electrophoretic diagram (pH 8.6). Serologically detectable, but not electrophoretically detectable, quantities of albumin were also present in a fourth eluate, made with 0.05 M NaH_2PO_4—0.1 M NaCl, but not further elutions with 0.05 M NaH_2PO_4—0.25 M NaCl and with 0.1 M NaOH, which removed the rest of the RS-1 from the column (overall recovery by weight, 92% or more).

Two further main nondialyzable fractions remain(8): fraction R-1 (nonultrafiltrable, buffer insoluble), in which albumin could not be detected serologically, and the ultrafiltrate, which has been subdivided into several subfractions(10). A sample of ultrafiltrable subfraction UF-3 was found by immunoelectrophoresis and disc electrophoresis to contain some albumin. After concentration of the albumin by $(NH_4)_2SO_4$ precipitation(10), a quantity estimated to correspond to 0.3 mg/24 hr urine was seen by free electrophoresis, although none was demonstrable by conventional paper electrophoresis (serum albumin control) using Light Green SF dye for visualization; the albumin from RS-1 also stains very poorly with this dye. The other ultrafiltrate subfractions were negative, except for a barely serologically detectable trace in UF-2, presumably adsorbed on this insoluble fraction. Evidently the material isolated from RS-1 represents essentially all the normal urinary albumin.

The estimated average normal excretion of albumin thus totals about 16 mg/24 hr urine, three-fourths of which is isolated by the foregoing procedure.

Globulins. The lyophilized second eluate was suspended in 5 ml water, and dialyzed against a half-saturated $(NH_4)_2SO_4$ solution for 48 hours in the cold. After removal of the precipitate, the supernate was dialyzed against saturated $(NH_4)_2SO_4$. The resulting precipitate was removed and the supernate and 2 precipitates dialyzed, lyophilized, weighed, analyzed (Table I) and examined electrophoretically(2). As expected(11), all the globulins were in the first precipitate. The second precipitate contained some albumin, as mentioned above, and also material migrating in the α_2 to γ region at pH 8.6, but at pH 4.5 only material

TABLE I. Analyses of Eluate 2, in Per Cent of Lyophilized Dry Weight.

	Total eluate 2	Ppt. sat/2-$(NH_4)_2SO_4$	Ppt. sat-$(NH_4)_2SO_4$	Supernate
Mg recovered	197	57	99	26
Hexose*	13.3	8.2	14.9	24.1
N	14.41	15.92	13.35	8.04
Hexosamine†	7.4	2.7	5.1	6.7

* Anthrone method; standard was equal weights galactose and mannose.

† As glucosamine base.

migrating like albumin was seen. The supernate contained material which moved in the α_2 to γ region at pH 8.6, being a broad peak centered in the β region. Evidently, on separation as described, some further portion of RS-1 had become soluble in saturated $(NH_4)_2SO_4$.

The analyses (Table I) as compared with serum globulins(12,13), indicate some contamination with mucosubstances of the "serum globulin" fraction precipitated by half saturation with $(NH_4)_2SO_4$. However, all detectable material behaving like serum globulins at pH 4.5 was present in this fraction, and in 70-fold concentration as compared with the total nondialyzable solids(8). Five components, including some albumin, were seen by disc electrophoresis. Incomplete solubility of the fraction in water and solubility in dilute salt solutions confirms the presence of globulins. The results indicate that normal excretion of recognized serum globulins (including γ globulins) does not exceed about 6 mg/day.

Summary. A procedure for concentration of albumin and globulins from normal urine is described. The albumin appears to be identical with serum albumin, and excretory rate is about 16 mg/day, under conditions of normal activity. Excretion of globulins amounts to less than 6 mg/day.

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Alternate Pathways of Glucose Metabolism in *Azotobacter agilis.** (27048)

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Johnson, Sobek, and Clifton(1) found that more C_2-carbon from glucose-2-C^{14} than C_1-carbon from glucose-1-C^{14} was assimilated by *Azotobacter agilis*, suggesting that a pentose pathway, involving an immediate decarboxylation, might be of quantitative importance in the metabolism of this organism. The presence of an active glucose-6-phosphate dehydrogenase had been demonstrated by Mortenson and Wilson(2,3). Extracts used by these workers also oxidized 6-phosphogluconate (6-PG) rap-

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