

# Micropuncture Studies of Proximal Tubule Albumin Concentrations in Normal and Nephrotic Rats

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**ABSTRACT** The concentration of serum albumin in proximal tubule fluid of normal rats and animals with aminonucleoside nephrosis was studied using renal micropuncture techniques. Albumin was quantitated by an ultramicrodisc electrophoresis method capable of measuring  $3 \times 10^{-11}$  g of albumin, in 10 nl volumes. With this sensitivity, only small samples of tubule fluid were required for analysis. Collection times could be kept short, therefore decreasing the opportunity for sample contamination with extraneous serum albumin. The measured mean concentration of albumin in proximal tubule fluid (1 mg/100 ml in females and 0.7 mg/100 ml in males) was somewhat lower than values reported by others, but even these values are apt to have been artifactually high as a result of animal preparation and trace contamination of samples during micropuncture. Rats injected with aminonucleoside of puromycin 4 days earlier, showed a significant increase in tubule-fluid albumin concentration coincident with a fall in serum albumin concentration and a 43-fold increase in urine albumin concentration. Tubular absorption of albumin was small relative to that of water. Although albumin filtration was significantly increased over that in normal animals, the glomerular basement membrane still served as a highly efficient barrier to albumin transfer.

## INTRODUCTION

According to current theory, the kidney plays an important role in the metabolism of serum albumin. The concentration of albumin in the glomerular filtrate of a number of animal species has been estimated to be in the order of 20 mg/100 ml (1-4), a small fraction being

excreted while the remainder is absorbed by the renal tubule epithelial cells. If this were true of the rat, for instance, the animal would filter and absorb an amount of albumin equivalent to its total albumin pool each day (5). Renal micropuncture studies in rats and dogs have revealed albumin concentrations in proximal tubule fluid in the order of 2-10 mg/100 ml or more (6-10). A concentration of even 5 mg/100 ml still places the kidney in a position of filtering and absorbing albumin at a rate approximating the known catabolic rate of this protein in the rat (11). Since it has been shown that nephrectomy does not alter the rate of albumin catabolism in normal rats (11), one would assume that this large amount of protein is reabsorbed into the circulation intact. There is an ever present risk that samples collected by renal micropuncture might be contaminated with extraneous serum albumin. The limitations of sensitivity of the methods used to date have required relatively prolonged collection periods to obtain the volumes of fluid required for albumin quantitation. Because of the possibility of increased albumin contamination of tubule fluid samples during protracted collection periods, this study has utilized a micromodification of disc gel electrophoresis which is sufficiently sensitive to permit a reassessment of proximal tubule-albumin concentrations in normal and nephrotic rats without resorting to unduly long collection periods.

## METHODS

*Analytic techniques.* Albumin was quantitated using an ultramicromodification of disc gel electrophoresis. Details of the procedure have been published elsewhere (12). Electrophoresis columns were prepared in 3 cm long, 140  $\mu$  i.d. constant bore capillary tubes (Drummond Scientific Co, Broomall, Pa.) that had been cleaned in concentrated nitric acid, rinsed, and siliconized. After exhaustive testing with small pore gels of various compositions, a 22% solution of recrystallized (13) acrylamide (Eastman Kodak Co, Rochester, N. Y.) with 0.5% hydantoin was adopted. As suggested by Neuhoff (14), the addition of hydantoin increased

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porosity, stabilized gelation, and provided greater consistency of pore size. Gels of this composition gave far more consistent results in albumin quantitation than the usual 7% acrylamide separative gel used for macrodisc electrophoresis of plasma. Apart from this change and the omission of sucrose from the large pore gel, the gels and running buffers were made up as described by Davis (15).

A precisely measured 2 cm length of small pore gel solution, pH 8.8, was aspirated into each capillary tube, degassed for 15 sec and allowed to gel in a water saturated atmosphere for 16–24 hr. Thereafter, a 3 mm length of large pore gel solution was layered onto the small pore gel under microscopic visualization, taking care not to disturb the junction between the two gels. 20 min later, when gelation was complete, the protein sample was applied over the large pore gel with a 5 cm long, 30–40  $\mu$  o.d. capillary. The height of the sample was measured precisely with an eyepiece micrometer, and its volume derived. A second anticonvectant stacking gel was not mixed with the tubule fluid sample. To avoid loss of the sample by diffusion into the upper buffer reservoir, the end of each capillary was sealed with large pore gel before electrophoresis.

Electrophoresis was performed in an apparatus described previously (12), using a Kepko (Kepko, Inc, Flushing, N. Y.) 1500 v high voltage power supply at a constant potential of 25 v/cm. After electrophoresis, the gel columns were extruded from their capillary tubes, fixed for 20 min in 7% acetic acid, washed, and stained in an aqueous solution of coomassie blue. Excess dye was eluted with water until the gel between the discs was colorless. Each column was placed on a 1 mm thick, optically plane "CO quartz" slide (Esco Products, Oak Ridge, N. J.) and oriented parallel with the edges of the slide. The optical density of the coomassie blue-stained discs was determined directly on a specially built Canalco® ultramicro-densitometer (Canal Industries, Bethesda, Md.). Up to 12 samples were subjected to electrophoresis simultaneously. Samples of standard pooled rat serum diluted in saline to contain 1.03 and 3.1 mg/100 ml albumin respectively, were included with each run. The remaining positions were occupied by samples of tubule fluid, plasma, and urine from each animal. Plasma samples were diluted 1:1000 in 140 mM NaCl, and urine diluted to contain an appropriate concentration for measurement between standards.

Experiments were performed on male and female Sprague-Dawley rats (COBS, Charles River Breeding Laboratories, Inc, North Wilmington, Mass.) weighing 190–250 g. The animals were kept in individual metabolic cages for at least 1 day before experimentation with free access to Purina lab chow and water. Anesthesia was induced by the intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The animals were prepared for micropuncture studies as described previously (16). In brief, the kidney was exposed through an abdomino-flank incision, freed as gently as possible from the peri-renal fat, placed in a lucite holder, and covered with warm mineral oil. Great care was taken to avoid distorting the renal pedicle. The kidney was not decapsulated. 140 mM NaCl (1 ml) was injected into a femoral vein and infused continuously at a rate of 0.05 ml/min.

Micropuncture was performed with 8–10  $\mu$  o.d. pipets ground so that their tips were sharp enough to slide easily into the tubule lumens and cause minimal injury. Great care was taken to avoid distorting the tubules or touching their inner walls. Samples collected from tubules obviously injured in any way were not used for protein analysis. Unless

these precautions were followed scrupulously, falsely high protein values might be obtained (see Discussion). A droplet of Sudan black-stained mineral oil, 2–3 tubule diameters in length, was injected into the tubule lumen and fluid obtained at such a rate that the oil droplet remained stationary. Collections were made over the shortest possible time to obtain adequate volumes of fluid for analysis, never exceeding 3 min. At the end of each collection, the pipet tip was sealed with mineral oil and rinsed thoroughly with distilled water. The tubule fluid samples were introduced into a capillary tube and immediately transferred to the prepared electrophoresis columns. Localization of tubule puncture site, when performed, was achieved by the intratubular injection of latex and subsequent microdissection of the nephron.

To see whether protein on the external surface of micropipets results in significant contamination of tubule fluid samples, pipets were filled with 20–28 nl volumes of 140 mM saline solution aspirated from a constant bore capillary and sealed with a small oil drop. Tubule punctures were performed in the customary fashion, the oil droplets expressed, and the pipets were allowed to remain in the proximal tubule for a period of 3 min without collecting tubule fluid. Following this, the pipets were removed, rinsed with saline, and their entire contents were transferred to electrophoresis columns for albumin assay.

Urine samples for albumin assay were obtained by gentle bladder compression or puncture at the completion of each series of micropuncture studies. To determine the effect of animal preparation on albumin excretion, a urine sample was obtained from nine normal rats by "mid-stream" collection before anesthetization. This was achieved by raising the tail sharply and catching the urine expelled by the rat in a clean test tube. Whenever this was unsuccessful, urine was obtained by bladder compression after the animal had been anesthetized but before laparotomy. The rats then were prepared in an identical manner to that used in micropuncture experiments, taking care to avoid distortion or compression of the renal vein and ureter, but micropuncture was not performed. Urine was expressed from the bladder 1 hr after the laparotomy was performed. The urinary albumin concentration before and after micropuncture preparation was measured as described above and expressed as a function of urine creatinine concentration. Urine creatinine was measured by an Auto-analyzer method.

*Recovery and identification of albumin.* Prolonged collections of proximal tubule fluid were made so as to obtain volumes of 48–90 nl. Accurately measured 16–20 nl portions of these samples were introduced into electrophoresis columns for albumin measurement. A precisely measured 20–28 nl volume of a saline solution containing 7.1 mg/100 rat serum albumin (Fraction V, Pentex Biochemical, Kankakee, Ill.) was added to a similar volume of a second measured portion of each tubule fluid sample. The tubule fluid, albumin solution, a 12–16 nl portion of the mixed sample, and bracketing standard albumin solutions were subjected to electrophoresis simultaneously. On completion of electrophoresis, the gels were extruded rapidly from their capillary tubes. The position of the coomassie blue-stained protein disc in each gel was carefully measured with a filar eyepiece and its density measured densitometrically. The nominal concentration of albumin in the mixed solution of tubule fluid and standard albumin was compared with the measured concentration.

This procedure was also followed using five samples of normal rat urine diluted 1:5 in saline to contain 0.6 to 1.9 mg/100 ml albumin.

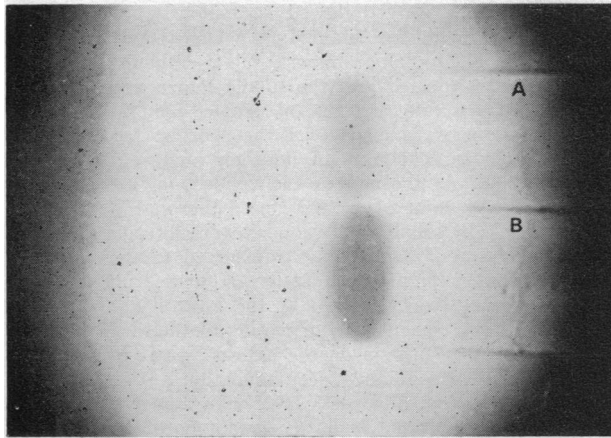


FIGURE 1 Disc gel electrophoresis of (A) a portion of proximal tubule fluid and (B) a second portion of the same sample mixed with a solution of powdered rat albumin in saline (Pentex Fraction V). Note the increased density of the albumin band in (B).

*Studies of aminonucleoside nephrosis.* Aminonucleoside of puromycin, 6-dimethyl amino-9[3' amino-3' deoxyribosyl] purine (Sigma Chemical Co, Saint Louis, Mo.) was injected intravenously as a single dose of 10 mg/100 g of body weight. Micropuncture studies were performed as described above on the 4th day after the aminonucleoside had been administered.

Statistical analyses were made according to the methods of Snedecor (17). Except where noted, all values reported in this paper represent the mean  $\pm$ SEM.

## RESULTS

Normal tubule fluid samples subjected to electrophoresis usually yielded only a single identifiable protein band. Under the conditions employed, this band had identical electrophoretic mobility with the albumin moiety of fresh rat serum albumin and with commercially obtained powdered rat-serum albumin (Pentex, Fraction V). See Fig. 1. The addition of accurately measured volumes of a 7.1 mg/100 ml powdered albumin solution in saline to each of three proximal-tubule fluid samples of known albumin content yielded a single narrow band after electrophoresis. These samples theoretically contained  $5.4\text{--}7.2 \times 10^{-10}$  g of albumin. The measured mean total albumin content of the three mixed samples was  $97 \pm 6\%$  (SE) (range 0.95–1.04) of the nominal content. The same procedure employed with five urine samples yielded a mean recovery of  $101 \pm 5\%$  (range 0.87–1.14) of the nominal albumin content.

*Contamination of samples by protein on the external surface of pipets.* Five proximal tubule punctures were performed in which pipets containing 20–28 nl 140 mM saline were inserted into the tubule lumen, but fluid was not collected. On electrophoresis, albumin was not detectable in any of these saline samples. Albumin which

may have contaminated the external surface of the pipet tip during the micropuncture procedure apparently was removed by washing of the pipet before transfer of its contents, indicating that albumin contamination of tubule fluid samples was not introduced in this way. Since tubule fluid was not aspirated, however, these experiments do not rule out the possibility that the micropuncture procedure may have produced albumin leakage into the proximal tubule which would be of importance in collection experiments.

Four normal samples of tubule fluid collected from proximal tubules of the same rat contained 0.1–1.3 mg/100 ml of albumin (mean 0.65 mg/100 ml).

*The effect of micropuncture procedures on albuminuria.* Total 24 hr albumin excretion of nine normal female rats was  $0.18 \pm 0.03$  mg/100 g body weight (concentration,  $3.8 \pm 0.8$  mg/100 ml). The urine albumin:creatinine ratio in the spontaneously-excreted urine samples collected under oil for 16–24 hr before micropuncture preparation was  $0.044 \pm 0.008$  (SE), a ratio not significantly different from that of  $0.039 \pm 0.008$  obtained by bladder compression just before experimentation ( $P > 0.30$ ). The albumin:creatinine concentration ratio of urine of 14 female rats prepared for micropuncture rose from  $0.031 \pm 0.005$  to  $0.174 \pm 0.037$  in the succeeding hour. The urinary albumin:creatinine ratio of three anesthetized rats that were not subjected to laparotomy did not increase in the 1-hr study period ( $P > 0.10$ ).

*Proximal tubule fluid albumin concentration in normal rats.* Values obtained in normal rats are shown in Table I. The mean albumin concentration in 71 proximal-tubule fluid samples of 41 female rats was  $1.0 \pm 0.1$

TABLE I  
Albumin Concentration in Proximal Tubule Fluid,  
Urine, and Plasma

	Normal		Amino-nucleoside*
	Male	Female	
Proximal tubule Albumin concentration, mg/100 ml	$0.7 \pm 0.2$ ‡ (0–3.1)¶ N = 23/8¶	$1.0 \pm 0.1$ (0–5.4) N = 71/21	$4.5 \pm 0.7$ § (0.02–15.5) N = 44/11
Urine albumin** Concentration, mg/100 ml	$26.1 \pm 7.8$ § (8.6–59.5)	$6.8 \pm 1.9$ (0–22.9)	$293.1 \pm 80.3$ § (30.6–778)
Plasma albumin Concentration, g/100 ml	$2.7 \pm 0.2$ (1.9–3.4)	$2.8 \pm 0.1$ (2.3–3.4)	$2.0 \pm 0.2$ § (1.1–2.7)

\* All studies performed on female rats.

‡ All values are given as mean  $\pm$ SEM.

§ Numbers in parenthesis represent the range of values.

¶ Number of samples derived from number of rats.

§ Signifies statistically significant difference from female control,  $P < 0.02$  or less.

\*\* Measured at the end of micropuncture experiments.

(SE) mg/100 ml. 25 samples had an albumin concentration of 0.1 mg/100 ml or less, and the remainder had values distributed between 0.12 and 5.4 mg/100 ml (Fig. 2). 23 proximal-tubule fluid samples obtained from eight male rats contained  $0.7 \pm 0.2$  mg/100 ml albumin, seven containing 0.1 mg/100 ml albumin or less. The difference between the mean values obtained in males and females was not statistically significant ( $P > 0.05$ ).

The mean urine albumin concentration of normal female rats was  $6.8 \pm 1.9$  (SE) mg/100 ml at the end of micropuncture, a value only 26% of that found in males ( $P < 0.005$ ). The urine albumin concentration of each rat did not correlate with the mean proximal tubule fluid albumin concentration of that animal ( $r = 0.65$ ,  $P < 0.05$  in females;  $r = 0.45$ ,  $P > 0.1$  in males). Serum albumin concentrations in the two groups were not different ( $P > 0.30$ ).

There was no correlation between proximal tubule puncture site and tubule fluid albumin concentration in 45 samples of 18 female rats ( $r = 0.05$ ,  $P > 0.1$ ) or in 16 samples of 7 male rats ( $r = 0.25$ ,  $P > 0.1$ ). See Fig. 3.

**Aminonucleoside studies.** Animals given 10 mg aminonucleoside intravenously, experienced a rise of BUN (blood urea nitrogen) concentration from  $14 \pm 1$  (SE) mg/100 ml in the control state to  $25 \pm 2$  mg/100 ml on the 4th day after injection ( $P < 0.01$ ). At that time, their urine albumin concentration was  $293 \pm 80$  mg/100 ml, a markedly higher concentration than the value of  $6.8 \pm 1.9$  mg/100 ml obtained in control rats after micropuncture ( $P < 0.001$ ). Premicropuncture albumin concentrations were not measured, and albumin:creatinine ratios were not determined. The serum albumin concen-

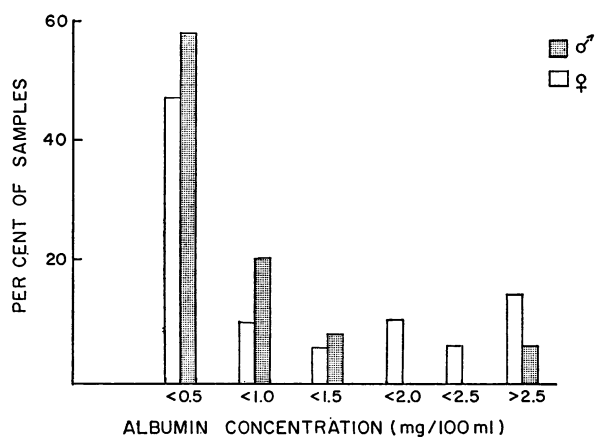


FIGURE 2 The distribution of proximal-tubule fluid samples of normal rats according to albumin concentration. The class intervals shown are incremental, e.g.,  $<1.0$  represents 0.51 to 0.99 mg/100 ml.

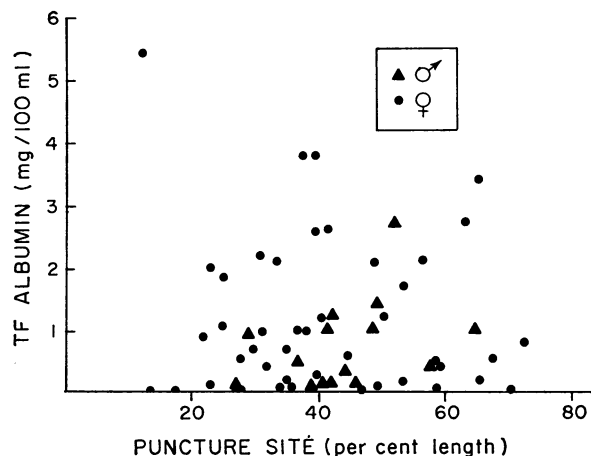


FIGURE 3 Proximal tubule fluid albumin concentrations of normal rats as a function of collection site. The regression coefficients for males and females were 0.05 and 0.25 respectively, and no correlation could be demonstrated ( $P > 0.1$ ).

tration of these 10 rats was depressed from the control value of  $2.8 \pm 0.1$ – $2.0 \pm 0.2$  g/100 ml ( $P < 0.01$ ).

These animals experienced a mean weight gain of  $23 \pm 6$  (SE) in the 4 day period after injection and were somewhat lethargic. Their limbs were not edematous at this time, but significant volumes of ascitic fluid were found in many animals when they were prepared for micropuncture.

The kidney surface viewed under the dissecting microscope in vivo at  $216 \times$  magnification was somewhat abnormal. The surface epithelium appeared pale and slightly granular and tubule lumens varied in size from small to normal. Proximal tubule fluid flow, judged from the rate of flow of injected oil droplets, generally was brisk. On insertion of micropipets, however, the epithelium was judged to be slightly friable; small volumes of tubule fluid leaked from the puncture site after removal of the pipet from a few nephrons, a finding which is rare in normal kidneys. All these changes were considerably more severe in animals examined on the 5th and subsequent days after aminonucleoside injection. Micropuncture studies were not performed after the 4th day, therefore, because of the markedly increased risk of contamination of collected samples with extraneous albumin.

44 proximal-tubule fluid samples of 11 rats contained albumin at a mean concentration of  $4.5 \pm 0.7$  (SE) mg/100 ml (Table I). The albumin concentration of 11 samples obtained from the first one-half (21–43%) of proximal tubule length of 6 rats was  $2.5 \pm 0.8$  mg/100 ml while that of 8 samples obtained from the more distal one-half (46–65%) was  $5.6 \pm 1.6$  mg/100 ml ( $P$

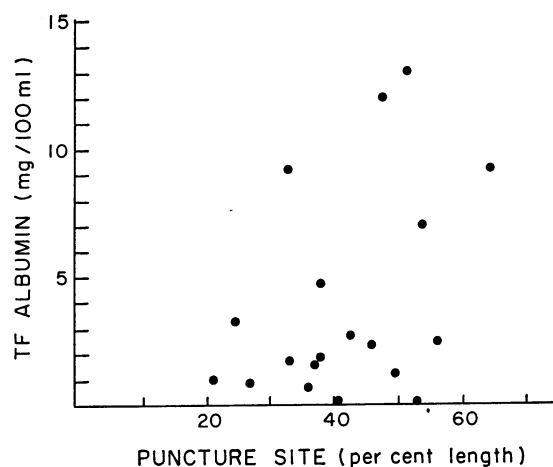


FIGURE 4 Proximal tubule fluid albumin concentrations of female rats injected with aminonucleoside of puromycin. The regression coefficient for these values was 0.39, and the correlation between albumin concentration and collection site was of borderline, if any, significance ( $0.1 > P > 0.05$ ).

$< 0.05$ ). Puncture site was not measured in the remainder. The regression coefficient for the entire series in which the site of collection was obtained was 0.39 ( $0.1 > P > 0.05$ ), however, a correlation which is not statistically significant. See Fig. 4. Tubule fluid albumin concentration did not correlate either with urine ( $r = 0.06$ ,  $P > 0.10$ ) or serum albumin ( $r = 0.24$ ,  $P > 0.10$ ) concentration.

## DISCUSSION

The normal glomerular basement membrane is considered to be poorly permeable to albumin. The albumin concentration of glomerular filtrate, measured indirectly, has been reported to be in the order of 20–30 mg/100 ml (1–4), a far smaller concentration than that present in plasma. Renal micropuncture studies have revealed even lower concentrations (7–10). Walker, Bott, Oliver, and MacDowell (6) measured the total protein concentration in proximal tubule fluid of rats by an acid flocculation technique almost 30 yr ago. 16 of their 41 samples of proximal tubule fluid contained more than 30 mg/100 ml of protein, their lower detection limit, values ranging up to 200 mg/100 ml. The majority of samples, however, contained too little protein to be detected by their technique. Dirks, Clapp, and Berliner (7) used the gel immunodiffusion technique of Soot-hill (18) to measure albumin and gamma globulin specifically in proximal tubule fluid of the dog. Their method had a stated detection limit of  $8 \times 10^{-9}$  g of albumin and was capable of quantifying albumin at concentrations down to 2 mg/100 ml in 400 nl tubule-

fluid samples. The mean albumin concentration in 20 of the 38 proximal-tubule samples analyzed was 5.9 mg/100 ml. Because of the limitations of sample size and methodologic sensitivity, albumin in the remaining 18 samples, 47% of all those assayed, was present in too small an amount to permit quantitation. More recently, Carone, Post, and Banks (8). Van Liew, Buentig, Stolte, and Boylan (9), and Leber and Marsh (10), who also used immunologic techniques, have reported proximal tubule albumin concentrations in the rat approximating those reported by Dirks and associates (7). In each study, however, relatively large samples were needed to obtain sufficient protein for quantitation. Collection times, therefore, were quite prolonged.

In preliminary phases of the present study, the mean albumin concentration of 19 proximal-tubule fluid samples was  $8.3 \pm 2.4$  (SE) mg/100 ml, a value comparable to those reported by others (7–9). Albumin concentrations ranged from less than 0.1 mg/100 ml to 49.6 mg/100 ml, a 500-fold difference. This wide range of values and the variation of albumin concentration in tubules of a given kidney indicated that glomerular permeability or proximal tubule protein absorption was exceedingly variable, or that some source of error was present in these experiments. It was noted that the higher concentrations of albumin were obtained most frequently when large samples of tubule fluid, 100 nl or more, were collected, however carefully. Analysis of small portions of these large tubule fluid samples gave the same high values, however, indicating that the values obtained were not elevated as the result of flaws in the analytical method. In addition, small breaks in micropuncture technique often were associated with very high tubule fluid albumin concentrations. It seemed, therefore, that any error introduced most likely was related to contamination of the tubule fluid with extraneous plasma albumin. Pulsation and slight respiratory movement of the kidney around a pipet placed in a tubule lumen inevitably traumatize the nephron, and prolonged collection periods further increases the opportunity for tubular injury. Covert contamination with trace amounts of serum albumin could easily result and greatly inflate the tubule fluid albumin values. The contamination of a sample of protein free fluid with only 0.1% of its volume of plasma would result in an albumin concentration of 2.8 mg/100 ml. In the case of tubule fluid samples, such scant contamination would be unrecognizable. It seemed very likely, therefore, that many, if not all, of the higher values obtained in the preliminary study were the result of inapparent tubule injury. This entire series of experiments was discarded.

In subsequent experiments, micropuncture was performed with very sharp, small pipets so as to minimize

tubule injury. Any recognizable distortion of the tubule on inserting the micropipet or the slightest uncertainty as to any aspect of the collection procedure was considered adequate reason for discarding the sample without albumin assay. Collection periods were kept short, never exceeding 3 min. Far lower proximal tubule albumin concentrations were obtained when these precautions were taken, although no change was made in the analytic technique or animal preparation. The mean albumin concentration in proximal tubule fluid obtained from female rats was 1.0 mg/100 ml, while that of male rats was 0.7 mg/100 ml. The finding that the urine albumin:creatinine concentration ratio increases some 5-fold purely as a result of micropuncture preparation suggests that these values are significantly higher than those expected in intact animals. It seems quite possible that values were additionally inflated by serum contamination introduced in the process of micropuncture despite the rigorous precautions taken to prevent it. 35% of the tubule fluid samples derived from female rats and 29% of samples from males contained 0.1 mg/100 ml of albumin or less. The remaining values were not distributed according to a Poisson distribution as would be expected of a "normal" population of values, but instead were dispersed evenly up to 5.4 mg/100 ml. According to statistical theory (17), the modal and mean values of a "normal" population should be very similar. In this instance, the mode was far lower than the mean value, and, in view of the ease with which contamination can be produced, would appear more likely to represent the true mean of proximal tubule albumin concentration. The finding of comparable proximal tubule albumin concentrations in male and female rats whose urine albumin concentration was greatly different also might be explained on the basis of error. If the true proximal tubule albumin concentration were indeed 0.1 mg/100 ml or less, differences in the albumin concentration of glomerular filtrate of the two sexes could not be detected in an inflated mean value. In addition, no correlation was found between proximal tubule albumin concentration and the site of fluid collection either in these experiments or those reported earlier (7-10). While this finding might represent absorption of albumin by the proximal tubule epithelium at a rate equal to that of water, it seems more likely to be another indication of the addition of extraneous albumin in an amount which would be expected to be independent of the proximal tubule puncture site. It could be argued, on the other hand, that the methods used resulted in falsely low albumin concentrations in a large number of samples. This seems unlikely because control studies have shown that the method as used is capable of consistently measuring as little as  $3 \times 10^{-11}$  g or as much as

$10^9$  g of albumin within the limits of ionic strength, pH, and cation content expected for the tubule fluid or urine (12). Since standard, plasma, urine, and tubule fluid samples were all handled in an identical manner, sporadic protein absorption to glass would have been readily detected by inconsistencies in standard and plasma samples. Such inconsistencies were not found. In addition, control recovery experiments showed excellent agreement between calculated and measured values, and in no instance gave results that approached one-tenth of the nominal value.

Precise knowledge of the albumin concentration in glomerular filtrate is essential to a proper appreciation of the role played by the kidney in albumin homeostasis. If the 1 mg/100 ml tubule fluid value found in female rats in this study truly represents the albumin concentration in glomerular filtrate, some 5% of the total albumin pool (5) would be presented for tubular absorption each day. This is not an unreasonable amount. On the other hand, a filtered albumin concentration of 0.02 mg/100 ml would be sufficient to account for the normal amount of albuminuria and still presuppose some small degree of tubular absorption. In view of the considerations outlined above, it is suggested that the true concentration of albumin filtered at the glomerulus probably lies somewhere between the two limiting values, perhaps close to the modal value of 0.1 mg/100 found in this study. In that case, the role of tubular absorption in normal albumin catabolism would be extremely small.

Aminonucleoside of puromycin has been used frequently as a means of producing the nephrotic syndrome in rats (19). Given as a single dose of 10 mg/100 g body weight intravenously, this agent produces proteinuria, azotemia, and tubular degenerative changes which increase with the passage of time. Such alterations appear consistently but are still relatively mild on the 4th day after injection, the time used in this study. Considerably greater degrees of abnormality are present on the 5th and subsequent days after aminonucleoside injection,<sup>1</sup> but micropuncture studies were not done after the fourth day because of the increased possibility of protein leakage across markedly abnormal appearing and friable tubular epithelium.

While the urine albumin concentration of aminonucleoside injected rats increased some 43-fold, the mean proximal tubule fluid albumin concentration was only four and one-half times higher than that measured in control animals. This finding might be construed as an indication that aminonucleoside nephrosis results primarily from inhibition of tubular protein absorption

<sup>1</sup> Unpublished observations.

with only a small change in glomerular permeability. GFR and urine albumin:creatinine ratios were not measured in these studies so that one cannot make exact comparisons of albumin filtration and excretion between the control and aminonucleoside groups. According to the BUN values, however, the GFR of aminonucleoside treated rats probably was significantly reduced, making the increase in urine albumin concentration even more remarkable. Differences in urine concentration ( $U_{0.5m}$ ) could not account for the markedly increased urine albumin concentration of the nephrotic rats. There can be little doubt, therefore, that a marked increase in urinary albumin excretion occurred, and that it was associated with only a modest increase in the measured mean proximal tubule fluid albumin concentration. The fact that albumin concentrations in the second half of the proximal tubule length were significantly higher than those in fluid collected from the first half of tubule length of albuminuric rats but not of control animals supports the conclusion that proximal tubular albumin absorption was scant. Unfortunately, it is not possible to say with any degree of certainty what the normal tubule fluid albumin absorption profile is. If, however, the true control proximal tubule fluid albumin concentration were 0.1 mg/100 ml, the modal value, rather than the 1 mg/100 ml mean value which might be factitiously high, the discrepancy between tubule fluid and urine albumin concentrations of the two groups would largely disappear and increased albumin filtration would then be the prime event in aminonucleoside nephrosis.

The contribution of contaminating albumin to the proximal tubule values of aminonucleoside injected animals is apt to have been small relative to that seen in normal animals. The addition of an amount of albumin that would raise the concentration in proximal tubule fluid from 0.1 to 1 mg/100 ml, a 10-fold difference, would increase the measured concentration by only 20% when added to a sample of the same size containing 4.5 mg/100 ml. Although urine and plasma creatinine concentrations were not measured in this study, the U/P creatinine concentration of five rats studied for other purposes 4 days after injection with the same dose of aminonucleoside was  $140 \pm 97$  (SE). Using this figure, the measured mean proximal tubule albumin concentration of 4.5 mg/100 ml, and an assumed mean proximal tubule inulin TF/P value of 2, one may calculate that the urine would contain some 315 mg/100 ml of albumin unless albumin were absorbed in significant amounts by the renal tubular epithelium. This value is remarkably close to the measured concentration. While such a calculation is necessarily inexact, it serves at least as a further index that tubular absorption of albumin in these nephrotic rats probably was slight rela-

tive to that of water. It also shows that contamination of samples during micropuncture did not grossly inflate the true tubule fluid albumin concentration. More definitive studies will be required to better quantify the role of tubule protein absorption both in normal rats and in this model of the nephrotic syndrome. It is noteworthy, however, that despite the observed increase in glomerular filtration of albumin, the glomerular basement membrane of the nephrotic animals with a low serum albumin concentration, ascites and significant albuminuria still served as a highly effective barrier to albumin filtration.

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