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TUBULAR REABSORPTION OF PROTEIN IN RATS WITH EXPERIMENTAL PROTEINURIA

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There is evidence that in the rat the glomerular filtrate contains a small amount of plasma protein (Walker, Bott, Oliver & MacDowell, 1941), and that some of this protein is reabsorbed by the cells of the proximal convoluted tubules (see review by Rather, 1952). The observation by Dock (1942) and by Gilson (1949) that, when plasma proteins in the rat were labelled with the dye T-1824 (Evans Blue), a selective accumulation of dye occurred in the cells of the proximal convoluted tubules has led to a method which allows quantitative information about the rate of tubular reabsorption of protein to be obtained. Sellers, Griggs, Marmorston & Goodman (1954) have given a value for the total rate of reabsorption of protein; they suggest that some 90% of filtered protein is reabsorbed by the tubules, the rest appearing as a small but constant proteinuria. These results were confirmed by Mendel (1959) who also showed that, in the rat, both albumin and globulin are reabsorbed by the tubules.

There is, however, a lack of quantitative data relating filtered protein to reabsorbed protein in the presence of increased protein loads. This is a matter of interest not merely in the normally functioning kidney but more particularly in nephrotic states. In the present work a nephrotic state was induced in rats, by injection of nephrotoxic serum (Heymann & Lund, 1948), the effect of which was to produce glomerular lesions without any demonstrable tubular defect. The animals thus treated manifested gross proteinuria and there was, as will be shown, an increased glomerular filtration of protein. The object was to investigate the quantitative relationship between increased protein loads and the tubular reabsorption of protein.

METHODS

The method for determining the rates of albumin and globulin reabsorption is based on the observation of Rawson (1943) that T-1824 in low concentration attaches itself to plasma albumin, and then, as the concentration of the dye is increased, to plasma globulin. It has been shown (Mendel, 1959) that it is possible, by the use of three increasing doses of T-1824,

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to obtain labelling of albumin alone, albumin plus α and β globulin, and finally γ globulin in addition. The rate of reabsorption of the three fractions can be calculated from the concentration of T-1824 attached to them and the T-1824 taken up by the kidneys.

Nephrotoxic serum. An anti-rat kidney serum was produced after the method of Heymann & Lund (1948). Saline-perfused, whole-rat-kidney homogenate was given in 0·5 g doses intra-peritoneally to 2 kg rabbits twice weekly for 3 weeks. The course of injections was repeated three times with weekly intervals between them. The rabbits were exsanguinated 6 days after the last injection and the serum was stored by deep freezing.

Experimental proteinuria. Wistar strain male rats of average weight 200 g (range 195–205 g) and fed on a diet containing 14 % protein (diet 41) were used. Experimental proteinuria was induced by the intravenous injection of 0·3 ml. of nephrotoxic serum through an indwelling polyethylene cannula in an external jugular vein. A marked increase in proteinuria usually occurred within 24 hr. In some animals a second intravenous injection was required on the next day. Histological examination of the kidneys on the third day after the onset of proteinuria showed either normal histology or, at the most, some thickening of the basement membranes in the glomeruli. The cells of the proximal convoluted tubules appeared to be normal.

Operative procedure. The methods used in the present work have been described previously in detail (Mendel, 1959). All experiments were carried out on the third day after the onset of proteinuria. An injection of T-1824 was given intravenously, and after a 3 hr period, during which the animal was deprived of food and water, the carotid artery was cannulated under ether anaesthesia and blood collected into a dry centrifuge tube. The kidneys were washed free of blood by perfusion with 0·9 % sodium chloride solution and removed for analysis.

Estimation of renal T-1824. The perfused kidneys were decapsulated and the renal pelves cut away; they were then homogenized in 6 ml. distilled water in a standard 'universal container' which fitted a tissue homogenizer (M.S.E.). In most experiments (thirty) the supernatant above the settled homogenate was colourless, indicating adequate perfusion. In eight experiments there was T-1824 colouring of the supernatant because of ineffective perfusion; this was usually associated with heavy proteinuria and may have been due to difficulty in washing out dye-stained protein in the capsular space and tubules. These experiments were rejected. T-1824 was estimated in the way previously described (Mendel, 1959).

Estimation of serum T-1824. The method used to determine the concentration of T-1824 attached to individual serum proteins was also described in detail in the previous paper (Mendel, 1959). A concentration of anhydrous Na_2SO_4 of 23·5 g/100 ml. was used to precipitate total globulin, and 17·75 g/100 ml. to precipitate γ globulin. 0·2 ml. of serum was added to 3 ml. volumes of 0·9 % NaCl and of 23·5 and 17·75 % solutions of anhydrous Na_2SO_4 in centrifuge tubes. After mixing, 1·5 ml. of ether was added to the tubes containing Na_2SO_4; these were stoppered, mixed and centrifuged. The T-1824 content of the supernatants was determined after suitable dilution with distilled water.

Serum proteins. Samples of sera from individual rats were fractionated with anhydrous Na_2SO_4 as described above, and the protein content of the supernatant determined by the micro-Kjeldahl method.

Urinary proteins. 24 hr volumes of urine, measured to within 0·1 ml., were obtained from rats in metabolism cages with free food and water. Total protein was determined by the biuret method of Kingsley (1939) on the protein precipitated with 10 % trichloroacetic acid. The qualitative determination of urinary protein was made by paper electrophoresis after the method of Flynn & de Mayo (1951). Whatman No. 1 paper was used in 5×36 cm strips. A barbitone buffer of pH 8·6 and ionic strength 0·075 M with a constant current of 1·0 mA per strip for 18 hr gave good separation. The strips were stained with Azocarmine B and evaluated by scanning and planimetry.

Inulin clearance. The method of Dicker (1949) was used to determine the inulin clearance. Inulin in blood and urine was measured by the method of Roe, Epstein & Goldstein (1949).

Throughout this paper, mean values are given together with the standard error of the mean.

RESULTS

Serum albumin and proteinuria. The effect of proteinuria on the serum albumin was determined in ten rats with a wide range of experimental proteinuria. Table 1 shows the proteinuria before treatment with nephrotoxic serum, and the proteinuria and serum albumin on the third day after the onset of proteinuria following nephrotoxic serum. It can be seen that rats with proteinuria greater than 8 mg/hr had serum albumins below 2 g/100 ml.; in ten normal rats the mean serum albumin was $3{\cdot}1 \pm 0{\cdot}06$ g/100 ml.

TABLE 1. The relationship of proteinuria to serum albumin in ten rats. The values after treatment were obtained on the third day after the onset of increased protein excretion

Before treatment. Proteinuria (mg/hr)	After treatment	
	Proteinuria (mg/hr)	Serum albumin (g/100 ml.)
0·8	2·7	2·8
0·4	3·0	2·5
0·4	4·2	2·4
0·2	5·7	2·7
0·6	6·3	2·0
0·3	6·6	2·6
0·6	8·0	2·0
0·5	11·0	1·5
0·2	13·2	1·6
0·7	17·0	1·0

Dosage of T-1824 and its attachment to serum proteins. In experiments with normal rats doses of T-1824 which proved suitable for the progressive labelling of serum proteins were 2 mg/100 g rat, to label albumin; 5 mg/100 g rat, to label albumin plus α and β globulin, and 10 mg/100 g rat, to label all the serum proteins (Mendel, 1959). Albumin binds the greatest proportion of the dye. In rats with experimental proteinuria it seemed likely that the lowering of serum albumin might cause such a reduction of T-1824 binding power that the dosage of T-1824 previously used might not be suitable; doses of 2 mg/100 g rat might cause a significant labelling of α and β globulin and 5 mg/100 g rat might similarly cause labelling of γ globulin. Preliminary experiments were designed to investigate this possibility.

Rats with proteinuria greater than 8 mg/hr usually had oedema and ascites, which made the measurement of T-1824 disappearance curves extremely difficult. Consequently, experiments to determine the most suitable doses of T-1824 were carried out on eight rats with a mean

proteinuria of $7{\cdot}0 \pm 0{\cdot}17$ mg/hr. Doses of T-1824 suitable under these conditions were regarded as suitable in the presence of less-marked proteinuria.

T-1824 in the dosage of 1 mg/100 g rat, as compared with 2 mg/100 g in normal rats, was the smallest dose of dye which allowed measurable amounts of T-1824 to be recovered from the kidneys. With this dose of dye over 90 % of the total dye was attached to albumin in blood samples taken 5 min after injection. Doses of T-1824 of 5 mg/100 g rat were found to label γ globulin, the total content of dye in the serum being greater than that in the supernatant after fractionation with 17·75 % Na_2SO_4. Doses of T-1824 of 4 mg/100 g rat, however, did not cause significant labelling of γ globulin in samples of blood taken 5 min after injection, and were therefore used to label albumin plus α and β globulin. The dose of dye used to label all the serum proteins was kept at 10 mg/100 g rat.

Time concentration curves of T-1824 in serum. The rate of disappearance from the serum of the T-1824 attached to individual protein fractions was estimated in twenty-five rats with each dose of T-1824 (Figs. 1 and 2). The values at each interval of time are the means of five individual results, obtained with rats chosen so that the average proteinuria at each time interval was between 3 and 5 mg/hr. There was a similar proteinuria in the group of rats in which the renal uptake of T-1824 was determined.

Figure 1*A* shows the values obtained with doses of T-1824 of 1 mg/100 g rat, and Fig. 1*B* the values with 4 mg/100 g rat. The concentration of T-1824 attached to α and β globulin with the dose of T-1824 of 1 mg/100 g rat was less than 1 mg/100 ml. at any time interval and is not represented in the figure. Figure 2*A* shows the fall in concentration of T-1824 attached to albumin and α and β globulin with the dose of T-1824 of 10 mg/100 g rat, and Fig. 2*B* the fall of concentration of T-1824 attached to γ globulin.

Renal uptake of T-1824

The renal content of T-1824 was estimated in thirty rats, on the third day after the onset of increased proteinuria, and 3 hr after the intravenous injection of T-1824. Groups of ten rats were used with each dose of T-1824 and were chosen so that the mean proteinuria for each group was about 4 mg/hr. Table 2 shows the individual results for renal T-1824, the mean proteinuria and the mean serum albumin level in each group.

The mean values of renal T-1824 shown in Table 2 were greater than those found in normal rats used as controls. Thus the mean value of renal T-1824 with doses of T-1824 of 1 mg/100 g rat was of the same order as the mean value of $30 \pm 2{\cdot}4$ μg T-1824 obtained in ten normal rats with doses of T-1824 of 2 mg/100 g rat. The mean value with doses of T-1824

of 4 mg/100 g rat was significantly greater ($P < 0{\cdot}01$) than the $88 \pm 4{\cdot}2$ μg T-1824 obtained in ten normal rats with doses of T-1824 of 5 mg/100 g rat, and the mean value with doses of T-1824 of 10 mg/100 g

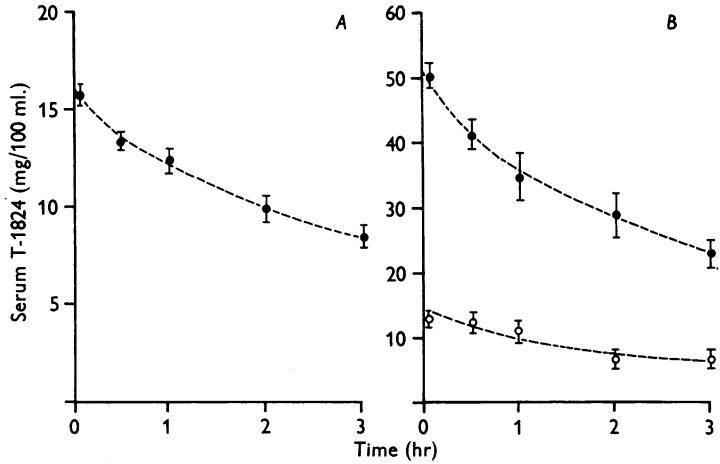

Fig. 1. The rate of fall of concentration of serum T-1824 following intravenous injection of T-1824 in rats with experimental proteinuria. Dose of T-1824 in *A*, 1 mg/100 g rat; in *B*, 4 mg/100 g. The values at each interval of time are the means of experiments in five rats. Vertical lines show S.E. of means. ● T-1824 attached to albumin; ○ T-1824 attached to α and β globulin.

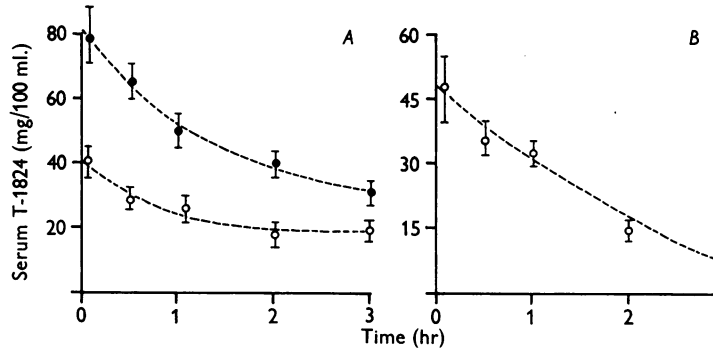

Fig. 2. The rate of fall of concentration of serum T-1824 in twenty-five rats following an intravenous injection of 10 mg T-1824/100 g rat. The values at each interval of time are the means of experiments in five rats: *A*, amount of T-1824 attached to albumin and α and β globulin; convention as in Fig. 1. *B*, T-1824 attached to γ globulin.

rat was significantly greater ($P < 0{\cdot}01$) than the 333 ± 20 μg T-1824 obtained in ten normal rats with similar doses of T-1824.

It has been shown previously (Mendel, 1959) that, when doses of T-1824 of 10 mg/100 g rat were given to normal rats, an amount of renal T-1824

could be recovered 2 min after injection which could not be explained on the basis of tubular reabsorption of protein, but appeared to be due to free T-1824 entering the cells of the proximal tubules as such. In six experiments on rats with experimental proteinuria, the kidneys were perfused within 2 min of giving an intravenous injection of T-1824 of 10 mg/100 g rat. The renal T-1824 recovered was 116 ± 6 μg, a value significantly greater ($P < 0{\cdot}01$) than the 33 ± 2 μg T-1824 recovered from six normal control rats. Similar experiments with doses of T-1824 of 1 and 4 mg/100 g rat did not show any initial rapid uptake of dye by the kidneys.

TABLE 2. Renal content of T-1824 (μg) in individual rats 3 hr after the intravenous injection of the doses of T-1824 shown, and the mean proteinuria and the mean serum albumin levels for each group

Dose of T-1824	1 mg/100 g rat	4 mg/100 g rat	10 mg/100 g rat
	23	211	730
	33	200	730
	28	182	475
	57	168	645
	19	160	484
	41	121	925
	33	150	606
	26	230	378
	46	149	960
	28	190	500
Means $\pm$ S.E.	$33 \pm 3{\cdot}6$	176 ± 10	643 ± 61
Mean proteinuria (mg/hr)	$4{\cdot}6 \pm 0{\cdot}8$	$3{\cdot}9 \pm 0{\cdot}6$	$3{\cdot}8 \pm 0{\cdot}6$
Mean serum albumin (g/100 ml.)	$2{\cdot}5 \pm 0{\cdot}1$	$2{\cdot}5 \pm 0{\cdot}1$	$2{\cdot}4 \pm 0{\cdot}1$

Calculation of tubular reabsorption of protein

The rate of tubular reabsorption of protein has been calculated from the serum proteins, the renal T-1824 and the mean value for T-1824 attached to individual serum proteins during the 3 hr period, which has been calculated from the area under the curves in Figs. 1 and 2. The mean serum protein values in the thirty rats in which the renal uptake of T-1824 was determined were: albumin $2{\cdot}5 \pm 0{\cdot}08$, α and β globulin $1{\cdot}7 \pm 0{\cdot}07$ and γ globulin $1{\cdot}4 \pm 0{\cdot}09$ g/100 ml. The other data are shown in Table 3.

Albumin. The mean value for renal T-1824 of $33 \pm 3{\cdot}6$ μg, obtained with the dose of T-1824 of 1 mg/100 g rat, is considered for the purpose of calculation to represent only albumin reabsorption, the attachment of T-1824 to α and β globulin being very small. From Table 3 it can be shown that there was $11 \pm 0{\cdot}5$ mg of T-1824 attached to $2{\cdot}5 \pm 0{\cdot}08$ g of serum albumin. The amount of albumin attached to the $33 \pm 3{\cdot}6$ μg of T-1824 recovered from the kidneys was therefore $7{\cdot}5 \pm 0{\cdot}8$ mg; this is albumin reabsorbed in 3 hr, representing a rate of albumin reabsorption of $2{\cdot}5 \pm 0{\cdot}3$ mg/hr.

α and β globulin. The value for renal T-1824 of 176 ± 10 μg represents albumin plus α and β globulin reabsorption (Table 3). Before the rate of α and β globulin reabsorption can be calculated it is necessary to calculate the renal T-1824 accounted for by albumin. The mean value for T-1824 attached to 2·5 ± 0·08 g of albumin under these conditions was 34 ± 3·3 mg, so that 1 mg of albumin was attached to 13·6 ± 1·3 μg of dye. Since the amount of albumin reabsorbed during the 3 hr period was 7·5 ± 0·8 mg, the amount of T-1824 accounted for by albumin was 102 ± 14 μg. The renal T-1824 accounted for by α and β globulin reabsorption was the remaining dye, i.e. 74 ± 17 μg, and as 9 ± 0·9 mg of T-1824 was attached to 1·7 ± 0·07 g of α and β globulin, the protein represented by 74 ± 17 μg of dye was 13·9 ± 3·2 mg, giving a rate of α and β globulin reabsorption of 4·6 ± 1·1 mg/hr.

TABLE 3. Renal T-1824 3 hr after intravenous injection of T-1824, and the mean concentration of T-1824 attached to serum protein fractions during the 3 hr after intravenous injection of T-1824

Dose of T-1824 (mg/100 g rat)	Renal T-1824 (μg)	Mean T-1824 attached to serum proteins (mg/100 ml.)		
		Albumin	α+β globulin	γ globulin
1	33 ± 3·6	11 ± 0·5	—	—
4	176 ± 10	34 ± 3·3	9 ± 0·9	—
10	643 ± 61	50 ± 5·5	23 ± 3·2	34 ± 2·1

γ globulin. The value of 643 ± 61 μg of renal T-1824 (Table 3) represents reabsorption of all the serum protein fractions. The amount of T-1824 accounted for by albumin and α and β globulin reabsorption has been calculated as explained above, from the serum protein values, the mean concentration of T-1824 attached to them, and the rate of reabsorption. Thus albumin accounted for 150 ± 23 μg T-1824, and α and β globulin 188 ± 49 μg T-1824. In addition 116 ± 6 μg of renal T-1824 has been accounted for by free dye. The amount of T-1824 accounted for by γ globulin was thus 189 ± 82 μg, and as 34 ± 2·1 mg of T-1824 was attached to 1·4 ± 0·09 g of γ globulin, 189 ± 82 μg of T-1824 was attached to 7·7 ± 3·4 mg of γ globulin, representing a rate of γ globulin reabsorption of 2·5 ± 1·1 mg/hr.

Proteinuria and tubular reabsorption of protein

Proteinuria. Electrophoresis of urinary protein was carried out on twenty individual urines obtained from rats in which renal T-1824 was subsequently measured. Urines were collected for a period of 24 hr immediately before giving the dye. Figure 3 shows the results of electrophoresis of serum and urine obtained from a rat 3 days after the onset of increased proteinuria. It can be seen that albumin, and α_1, β and γ

globulin appeared in the urine. The mean rate of proteinuria in the twenty rats was $4{\cdot}2 \pm 0{\cdot}5$ mg/hr. The mean rates of excretion of individual serum proteins were: albumin $1{\cdot}8 \pm 0{\cdot}2$ mg/hr, α globulin $1{\cdot}0 \pm 0{\cdot}22$ mg/hr, β globulin $0{\cdot}9 \pm 0{\cdot}12$ mg/hr and γ globulin $0{\cdot}5 \pm 0{\cdot}07$ mg/hr.

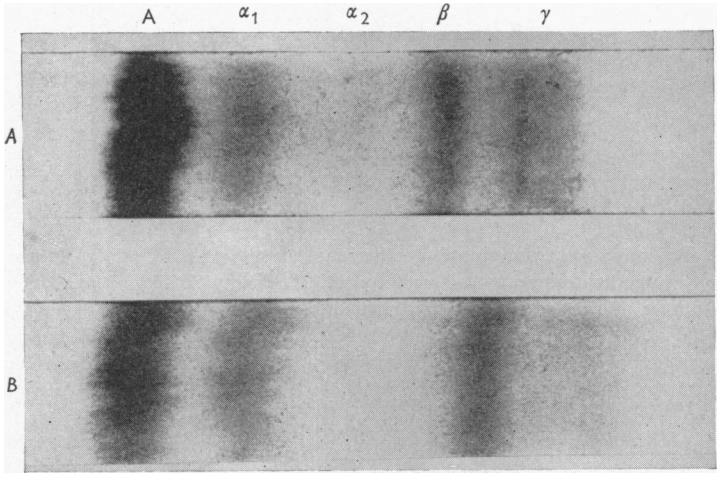

Fig. 3. Electrophoresis of serum and urine proteins of a rat with experimental proteinuria. *A*, serum; *B*, urine. Rate of proteinuria 4·2 mg/hr.

Proteinuria and renal T-1824. The relationship of the renal uptake of T-1824 to the rate of proteinuria in thirty rats is shown in Fig. 4. Figure 4*A* shows the renal uptake of T-1824-labelled albumin and the rate of urinary albumin excretion, Fig. 4*B* the renal uptake of T-1824-labelled albumin and α and β globulin and the rate of excretion of these fractions in the urine, and Fig. 4*C* the renal uptake of T-1824 with all the serum proteins labelled and the total rate of protein excretion. It can be seen that there was no clear relationship between the reabsorption of labelled protein and its rate of excretion. The correlation coefficient for the data shown in Fig. 4*A* was 0·6, which is not significant at 5% level for a sample of this size.

Proteinuria and G.F.R. The relationship of proteinuria and glomerular filtration rate, determined by inulin clearance, was determined in ten rats 3 days after the onset of increased proteinuria. The mean rate of proteinuria in the group was $4{\cdot}7 \pm 0{\cdot}7$ mg/hr and the mean inulin clearance $0{\cdot}49 \pm 0{\cdot}02$ ml./100 g rat/min, a value which did not differ significantly from the $0{\cdot}47 \pm 0{\cdot}02$ ml./100 g rat found in ten normal rats. Figure 5 shows proteinuria plotted against inulin clearance in the individual rats. It can be seen that these were not related.

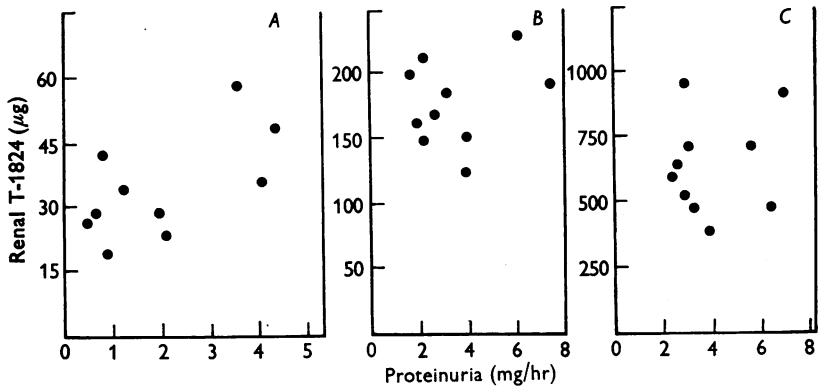

Fig. 4. The relation of proteinuria to the renal uptake of T-1824-labelled proteins in thirty rats with experimental proteinuria. *A*, dose of T-1824, 1 mg/100 g rat; renal uptake of labelled albumin and urine albumin excretion. *B*, dose of T-1824, 4 mg/100 g rat; renal uptake of labelled albumin plus α and β globulin, and urinary albumin plus α and β globulin excretion. *C*, dose of T-1824 10 mg/100 g rat; renal uptake of T-1824 with all serum protein labelled and total proteinuria.

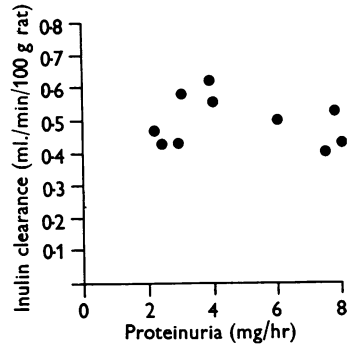

Fig. 5. The relation of proteinuria to inulin clearance in ten rats with experimental proteinuria.

DISCUSSION

In the present work rats were used in which proteinuria had been induced by the use of a nephrotoxic serum. It is known that the primary lesion induced by such means is in the glomerulus (Ortega & Mellors, 1956) and that the kidneys may show minimal histological changes or severe degrees of glomerular and tubular damage. The more severely affected animals have oedema and ascites in varying degrees, and the syndrome has been likened to the equivalent pathological state in man (Heymann & Lund, 1948). It was unfortunately not possible in the present work to use animals with gross oedema and ascites. Nevertheless, it is reasonable

to assume that the animals used were suffering from a pathological process, less advanced, but part of the same syndrome.

The results indicate that there was an increased uptake of T-1824-labelled protein by the kidneys, the values for renal T-1824 being greater than those obtained in normal rats. An increase in the renal uptake of labelled protein has previously been observed by Sellers, Smith, Marmorston & Goodman (1952) using T-1824-labelled protein in rats with renin proteinuria, and by Spector (1954) using ^{131}I-labelled protein in rats with an experimental glomerular lesion of the type produced in the present work. The calculated rate of tubular reabsorption of protein was 9·6 mg/hr, consisting of 2·5 mg/hr albumin, 4·6 mg/hr α and β globulin and 2·5 mg/hr γ globulin. In the normal rat the rate of tubular reabsorption of protein has previously been reported to be of the order of 3·6 mg/hr and the rates of reabsorption of the individual serum proteins as albumin 1·1, α and β globulin 1·4 and γ globulin 1·1 mg/hr (Mendel, 1959). It can be seen that in the present experiments there was a more than twofold increase in tubular reabsorption of protein, and that the increase was shown by each of the labelled serum protein fractions.

The relationship of filtered protein to reabsorbed protein shows that with a G.F.R. of 0·49 ml./min/100 g rat, the total protein filtered for a 200 g rat/hr in 59 ml. filtrate was 13·6 mg (tubular reabsorption 9·6 mg, proteinuria 4 mg) and the concentration of protein in the glomerular filtrate thus about 23 mg/100 ml. Some 70 % of the filtered protein was reabsorbed by the tubules. The percentage reabsorption of the individual serum protein fractions, calculated from the rate of reabsorption and the rate of urinary loss, was albumin 58 %, α and β globulin 70 % and γ globulin 83 %.

In the nephrotic animals the uptake of labelled protein by the kidneys did not show any further increase with proteinuria increasing above about 4 mg/hr. It would seem therefore that under the conditions of these experiments tubular reabsorption of protein was maximal. It may well be that some undetected tubular damage was present and it is possible that a completely normal tubule is capable of greater reabsorption. Nevertheless, a rate of reabsorption of 9·6 mg/hr is important in the conservation of plasma protein. If the value of total serum T-1824 taken 5 min after intravenous injection is used for the calculation of plasma volume, values of 11·7, 12·5 and 11·8 ml. are obtained for the three doses of dye. With an average plasma volume of 12 ml. and a total serum protein value of 5·6 g/100 ml., the total circulating protein is of the order of 670 mg. With a rate of tubular reabsorption of protein of 9·6 mg/hr it follows that about one third of the total circulating protein could be reabsorbed by the tubules daily.

SUMMARY

1. Tubular reabsorption of protein has been measured, by use of the dye T-1824, in rats with experimental proteinuria produced by means of a nephrotoxic serum.

2. Renal uptake of T-1824-labelled protein was measured in rats with rates of proteinuria of up to 8 mg/hr and found to be increased.

3. The calculated rate of tubular reabsorption of protein in rats with proteinuria of about 4·0 mg/hr was 9·6 mg/hr. The rates of reabsorption of individual serum protein fractions were: albumin 2·5, α and β globulin 4·6 and γ globulin 2·5 mg/hr.

4. Tubular reabsorption of protein accounted for 70% of the total filtered load. The protein content of the glomerular filtrate was calculated to be about 23 mg/100 ml.

5. Renal uptake of T-1824 was not further increased with increased rates of proteinuria, suggesting that under the conditions of these experiments protein reabsorption was maximal.

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