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Research Article

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Quantitative Immunological Determination of 12 Plasma Proteins Excreted in Human Urine Collected before and after Exercise

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ABSTRACT Urine was collected from 6 healthy male adults at rest and from 20 male adults after a marathon race (25 miles). The concentrated urines were quantitatively analyzed, by single radial immunodiffusion, for their content in 12 different plasma proteins: tryptophan-rich prealbumin, albumin, α_1-acid glycoprotein, α_1-antitrypsin, ceruloplasmin, haptoglobin, Gc-globulin, transferrin, hemopexin, β_2-glycoprotein I, γA-globulin, and γG-globulin.

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Strenuous exercise increased (up to 50-fold) the excretion of plasma proteins which represent 82% of the total proteins found in urine, instead of 57% in urine collected from humans at rest. There was particularly a significant rise of tryptophan-rich albumin, albumin, α_1-acid glycoprotein, transferrin, γA-globulin, and γG-globulin (0.26, 127, 11.8, 3.3, 1.2, and 2.0 μg respectively, out of a total excretion of 167 μg of plasma proteins per min). The ratio of γG-globulin to γA-globulin was 16:1. After exercise, the renal clearance of proteins increased from 2 to 40 times, but, as for the urine of normal subjects at rest, no direct relationship between molecular weight and renal clearance could be observed.

INTRODUCTION

In earlier studies, the amount of protein excreted in the urine in 24 hr by healthy humans at rest was estimated between 30 and 70 mg (1–5), although values of 100–400 mg have also been observed (6–8). Poortmans has reported that strenuous exercise causes an increase of protein excretion up to a hundred times the content present before exercise (9, 10).

Immunological investigations have established the presence of several plasma proteins in normal urine, collected from individuals both at rest and after exercise (9–13). In the latter case, immunoprecipitation techniques showed an increase in the number of plasma proteins (9) as well as in the number of proteins not present in plasma (10).

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The quantitative determination of individual plasma proteins in normal urine is of major interest for an understanding of the mechanism of proteinuria itself. An explanation based on the presence of proteins in normal urine has been presented by Hardwicke and Squire (14). It consists of the glomerular filtration of most of the plasma proteins, associated with a nonselective process of tubular reabsorption. Another mechanism, suggested by the results obtained in the present work, would also consider a glomerular filtration, but combined with a selective tubular reabsorption and with a secretion from the nephron. Little is known, however, of the fate of proteins in the kidney.

The concept of selective permeability introduced by Hardwicke and Squire (14) has been supported by the results of paper electrophoresis (15), of gel filtration (16, 17), and of semiquantitative immunodiffusion (18), but the absolute value of the content of each individual plasma protein in normal urine is not avaiable. Only albumin has been quantitatively determined in human urine (19, 20), with the results being expressed in terms of milligrams of protein excreted per 24 hr.

In the present study, several plasma proteins found in the urine collected from healthy humans both after rest, and after exercise, were quantitatively estimated in order to show whether or not exercise proteinurina is similar to the physiological proteinuria of subjects at rest. The results showed that normal physiological proteinuria is related only in part to the glomerular filtration according to the molecular weight of the macromolecules. In addition, not all the results are explained by the hypothesis of nonselective tubular reabsorption, and other mechanisms are proposed.

METHODS

Proteins of urine collected from healthy humans at rest. Urine samples were collected during 48 hr from 6 healthy male adults. None of the samples showed any protein content when examined with the boiling acetic acid test. Sodium merthiolate (0.01 g/100 ml) was added as a preservative, and the samples were kept overnight at 4°C. Then the urines were filtered to remove the uromucoid fraction, and the protein content of the filtrates was determined with the Amidoschwartz microtechnique (21). The urine volume was measured for estimation of the urinary output. Each filtrate was concentrated 1000–1500 times by ultrafiltration under reduced pressure (22) with inflated Visking membranes, $^{8}/_{32}$ inch in diameter. The final concentration of protein in the concentrated urine was determined with the Lowry method (23).

Proteins of urine collected from healthy humans after strenuous exercise. The urine from 20 individual athletes was collected within 1 hr after a marathon race (Boston Marathon 1966: 26 miles). Each was treated in the manner previously described for urine collected from humans at rest.

Single radial immunodiffusion technique. Quantitative determination of the following proteins in concentrated urines was performed according to the original technique of Mancini et al. (24): tryptophan-rich prealbumin, albumin, α_1-acid glycoprotein, α_1-antitrypsin, haptoglobin, Gc-globulin, hemopexin, and β_2-glycoprotein I. Ceruloplasmin, transferrin, γA-globulin, and γG-globulin were determined with Hyland immunodiffusion plates.

α_1-Antitrypsin and hemopexin used as standard were isolated from pooled normal human sera according to the procedure of Heide and Haupt (25). The following antisera were purchased: tryptophan-rich prealbumin (Behringwerke No. 846), α_1-acid glycoprotein (Mann No. R2648), α_1-antitrypsin (Behringwerke No. 882), haptoglobin (Mann No. R1790), Gc-globulin (Behringwerke No. 848), β_2-glycoprotein I (Behringwerke No. 845). The human albumin antiserum was prepared by immunization of a rabbit for a period of 3 wk.

The size of the ring-shaped precipitates was measured after the plates had been washed and stained according to the method of Mancini et al. (24).

RESULTS

Urine from normal subjects at rest. The total amount of protein excreted in urine is about 0.046 mg/ml or 0.035 mg/min (Table I). The amounts of each of the 12 plasma proteins investigated are presented in Table II. Whereas 98.5% of the proteins of plasma have been identified (if the lipoproteins and macroglobulins that are not present in normal urines are taken into consideration), only 57.1% of the proteins of serum could be identified in urine.

The ratios of the content of each individual protein to the total protein content show major differences between normal urine and normal serum (Table II). Albumin remains the major component of normal urine, although its relative content is lower than in normal serum. Determination with an immunological method showed the proportion of albumin to globulins to be 1.30:1 in normal serum, whereas it was 0.67:1 in normal urine. The relative content of the following plasma proteins was lower in normal urine than in normal serum: tryptophan-rich prealbumin, α_1-antitrypsin, Gc-globulin, transferrin, and γG-globulin. In contrast, the following proteins were present in

TABLE I

Total Protein Content Excreted in Urine Collected at Rest (6 Subjects) and after Exercise (20 Subjects)

	At rest			After exercise		
	Proteins excreted		Urinary output	Proteins excreted		Urinary output
	mg/ml	*mg/min*	*ml/min*	*mg/ml*	*mg/min*	*ml/min*
Mean	0.046	0.035	0.803	0.587	0.213	0.395
SD	0.009	0.007	0.164	0.267	0.124	0.171
Range	0.030–0.056	0.028–0.048	0.576–1.00	0.222–1.14	0.083–0.312	0.129–0.715

higher proportion in normal urine than in normal serum: α_1-acid glycoprotein, β_2-glycoprotein I, and γA-globulin. The ratio of γG-globulin to γA-globulin was 4.9:1 in normal urine, a value much lower than the 12.9:1 found for serum.

Of the 12 plasma proteins studied, albumin and γG-globulin were found to be the proteins excreted in the highest amounts during 24 hr in the urine of normal individuals at rest (Table II).

Urine collected from normal subjects after exercise. The urine collected from normal individuals after exercise contains more proteins than the urine collected from individuals after rest. A mean value of 0.587 mg/ml or 0.213 mg/min was observed (Table I). The rise of the protein concentration, expressed in milligrams per milliliter, was not compensated by the decrease of the urinary output. Consequently, the protein clearance was changed after exercise. The qualitative determination of the 12 proteins showed which proteins were mainly responsible for the increased proteinuria after exercise (Table III).

The proteins of plasma origin which are found in urine collected after exercise represent 82% of the total urinary proteins. This indicates that the increase of protein excretion in urine collected after exercise reflects mainly an increase of the plasma proteins.

The proportion of each protein to the total protein content in urine collected after exercise is reported in Table III. After exercise, the urinary excretion of some of the plasma proteins shows a marked increase when compared to the excretion at rest. The greatest increase is shown by the α_1-acid glycoprotein, followed by transferrin, tryptophan-rich prealbumin, Gc-globulin, albumin, and

TABLE II

Excretion of Plasma Protein in the Urine of Normal Subjects at Rest (6 Subjects)

Plasma proteins*	Excretion in urine									Relative proportion to total protein in urine (in serum)‡		Clearance
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range			
	mg/100 ml			*μg/min*			*mg/24 hr*			%		
Tryptophan-rich prealbumin	0.0023	0.0008	0.002–0.004	0.018	0.007	0.012–0.033	0.026	0.010	0.017–0.047	0.07	(0.4)	0.06
Albumin	1.00	0.258	0.738–1.26	8.839	3.219	6.160–15.200	12.73	4.63	8.87–21.9	40.0	(57.1)	0.20
α_1-Acid glycoprotein	0.036	0.011	0.024–0.057	0.287	0.095	0.204–0.470	0.413	0.136	0.293–0.676	1.3	(1.0)	0.44
α_1-Antitrypsin	0.027	0.011	0.013–0.047	0.214	0.091	0.130–0.391	0.308	0.131	0.187–0.563	1.0	(5.1)	0.07
Ceruloplasmin	0.005	0.001	0.004–0.008	0.039	0.004	0.032–0.046	0.056	0.006	0.046–0.006	0.2	(0.4)	0.13
Haptoglobin	0.015	0.013	0–0.033	0.126	0.122	0–0.290	0.181	0.175	0.000–0.417	0.7	(1.4)	0.12
Gc-globulin	0.0023	0.0008	0.002–0.004	0.018	0.007	0.011–0.033	0.026	0.010	0.016–0.047	0.07	(1.0)	0.13
Transferrin	0.019	0.011	0.011–0.042	0.155	0.068	0.990–0.267	0.223	0.097	0.129–0.384	0.5	(3.5)	0.06
Hemopexin	0.017	0.004	0.012–0.024	0.140	0.039	0.099–0.199	0.201	0.056	0.142–0.287	0.7	(1.4)	0.14
β_2-Glycoprotein I	0.028	0.008	0.018–0.040	0.233	0.068	0.150–0.333	0.321	0.098	0.216–0.480	1.0	(0.2)	1.10
γA-globulin	0.045	0.014	0.031–0.072	0.351	0.066	0.258–0.416	0.505	0.095	0.371–0.600	1.8	(1.4)	0.36
γG-globulin	0.221	0.067	0.160–0.342	1.71	0.306	1.37–2.09	2.46	0.443	1.97–3.01	8.9	(18.1)	0.11

* The usual synonyms of the plasma proteins mentioned are: prealbumin S_1-protein and thyroxine-binding prealbumin, for tryptophan-rich prealbumin; orosomucoid and α_1-seromucoid for α_1-acid glycoprotein; 3,5S-α_1-glycoprotein for α_1-antitrypsin; β_{1B}-globulin, seromucoid β_{1B}, and hemo-binding β-globulin for hemopexin; β_{2X}-globulin for β_2-glycoprotein I; β_{2A}-globulin for γA-globulin; and γ-globulin, $7S\gamma$-globulin, and γ_2-globulin for γG-globulin.

‡ In parentheses, values for normal human serum (25).

TABLE III

Excretion of Plasma Proteins in the Urine of Normal Subjects after Exercise (20 Subjects)

Plasma proteins	Excretion in urine						Relative proportion to total protein in urine (in serum)*		Clearance
	Mean	SD	Range	Mean	SD	Range			
	mg/100 ml			*μg/min*			%		*μl/min*
Tryptophan-rich prealbumin	0.068	0.074	0.001–0.305	0.257	0.309	0.022–1.26	0.2	(0.4)	0.51
Albumin	37.1	23.4	3.34–80.8	126.7	89.918	22.9–405.4	59.2	(57.1)	3.70
α_1-Acid glycoprotein	3.434	3.125	0.211–10.8	11.83	10.217	1.50–38.6	6.7	(1.0)	21.0
α_1-Antitrypsin	0.587	0.707	0.033–3.28	2.04	2.020	0.266–7.62	1.2	(5.1)	0.77
Ceruloplasmin	0.104	0.087	0.008–0.423	0.389	0.438	0.05–2.12	0.2	(0.4)	1.30
Haptoglobin	0.237	0.203	0–0.625	0.788	0.694	0–2.89	0.5	(1.4)	0.92
Gc-globulin	0.102	0.100	0.002–0.384	0.367	0.376	0.013–0.593	0.2	(1.0)	0.58
Transferrin	1.0	0.689	0.036–2.32	3.31	2.188	0.26–8.49	1.9	(3.5)	1.70
Hemopexin	0.338	0.251	0.028–0.917	1.21	1.056	0.191–4.24	0.5	(1.4)	1.30
β_2-Glycoprotein I	0.091	0.093	0.016–0.371	0.345	0.401	0.054–1.54	0.2	(0.2)	1.80
γA-globulin	0.361	0.261	0.028–0.809	1.18	0.824	0.19–3.09	0.7	(1.4)	1.40
γG-globulin	5.87	4.39	0.645–17.5	20.09	18.58	3.62–88.2	11.2	(18.1)	1.50

* In parentheses, values for normal human serum (25).

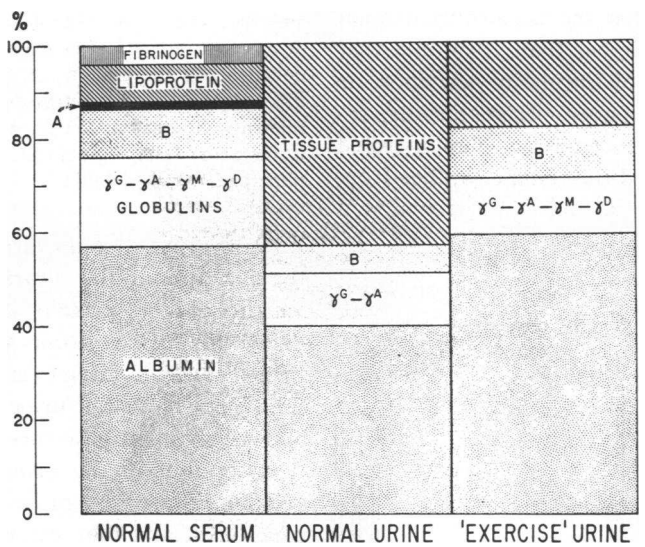

FIGURE 1 Repartition of plasma proteins in normal serum, normal urine, and "exercise" urine. The proportion of plasma proteins (A) not tested in urine was 1.5% and included easily precipitable α_1-glycoprotein, 4.6S postalbumin, tryptophan-poor α_1-glycoprotein, thyroxine-binding globulin, α_{1X}-glycoprotein, intertrypsin inhibitor, α_{2HS}-glycoprotein, Zn-α_2-glycoprotein, α_2-neuraminoglycoprotein, α_2-C'$_T$-globulin, β_{1A-1C}-globulins, β_{1E}-globulin, plasminogen, β_{2K}-globulin, 2S γ_2-globulin. The plasma proteins (B) tested in urine included: tryptophan-rich prealbumin, albumin, α_1-acid glycoprotein, α_1-antitrypsin, ceruloplasmin, haptoglobin, Gc-globulin, transferrin, hemopexin, β_2-glycoprotein I, γA-globulin, and γG-globulin. The term "tissue proteins" describes proteins that are not present in plasma or are present at a level below detection. The "exercise" urine shows the presence of subunits of γM-globulins, represented as γM in the graph.

γG-globulin. In contrast, exercise caused a decrease in the relative proportion of the following plasma proteins: β_2-glycoprotein I, haptoglobin, hemopexin, and γA-globulin. The albumin to globulin ratio reached a slightly higher value than that of its serum counterpart. Furthermore, the ratio of γG-globulin to γA-globulin in urine collected after exercise was 16:1, a value higher than that found for normal serum.

Comparison of the proteins of normal serum with the proteins of urines collected both at rest and after exercise. Fig. 1 shows the relative proportion of plasma proteins in plasma, and in both types of urine. Normal urine collected at rest contained an important fraction (43%) of proteins which were not of plasma origin. After exercise, this fraction was reduced to 18%.

The renal clearance of the 12 plasma proteins investigated may be determined (Tables II and III), if it is assumed that the hemoconcentration during exercise is not higher than 10%, and that the relative proportion of the major fractions, as shown by the peaks in paper electrophoresis, remains unchanged after exercise (unpublished data). Exercise increased the renal clearance from

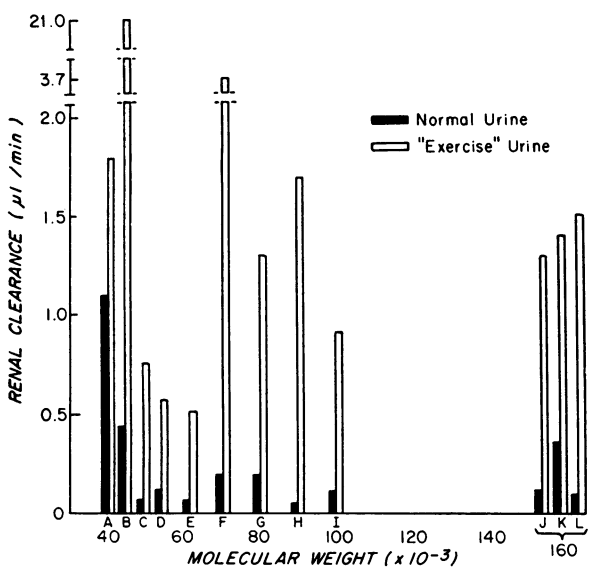

FIGURE 2 Comparison between the molecular weight of a protein and its renal clearance in normal urine and "exercise" urine. The letter under each couple of columns identifies the plasma protein tested: *A*, β_2-glycoprotein I; *B*, α_1-acid glycoprotein; *C*, α_1-antitrypsin; *D*, Gc-globulin, *E*, tryptophan-rich prealbumin; *F*, albumin; *G*, hemopexin; *H*, transferrin; *I*, haptoglobin; *J*, ceruloplasmin; *K*, γA-globulin; and *L*, γG-globulin.

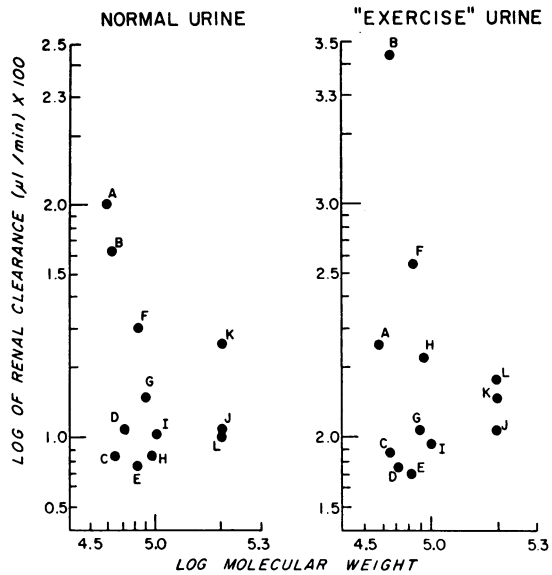

FIGURE 3 Relationship between the molecular weight and the renal clearance of plasma proteins excreted in urine after rest and after exercise. The letter besides each dot identifies the protein: *A*, β_2-glycoprotein I; *B*, α_1-acid glycoprotein; *C*, α_1-antitrypsin; *D*, Gc-globulin; *E*, tryptophan-rich prealbumin; *F*, albumin; *G*, hemopexin; *H*, transferrin; *I*, haptoglobin; *J*, ceruloplasmin; *K*, γA-globulin; and *L*, γG-globulin.

2 to 40 times, but no direct relationship between the increase of the clearance and the molecular weight of the protein excreted was observed, either for urines from subjects at rest or for urines collected after exercise (Fig. 2). The proteins present in the urine collected after exercise showed a higher clearance value than did the proteins present in the urines collected at rest. No relation, however, can be established between the clearances, and they cannot be related directly to the molecular weight of the proteins. Thus, when the renal clearance of the plasma proteins is plotted on double logarithmic graph paper against the corresponding molecular weight, the distribution does not follow a straight line, either for the products obtained from urines collected at rest, or for the products from the urines collected after exercise (Fig. 3).

DISCUSSION

The presence of plasma proteins in normal urine (10, 26–28) is confirmed by the present data; in addition, quantitative estimation of several of these proteins is given. Urine excreted by normal

subjects at rest contains 43% of proteins ("tissue proteins") which are not present in the plasma, or present only in trace amount. Among these proteins the presence of substances originating from the kidney, the urinary tract, and the seminal glands has been shown (10).

The estimation of the urinary excretion of plasma proteins by healthy subjects has been reported only for albumin, the following methods being used: paper electrophoresis (1, 2, 8, 29), chemical isolation (20, 30), and immunoprecipitation (19). The highest values were obtained by paper electrophoresis (17–31 mg/24 hr), and represent most probably an overestimation, since they take into account the urinary glycoproteins and glycopeptides, some of which have the same electrophoretic mobility as albumin. More accurate results were obtained by Berggård and Risinger (19) with an immunologic procedure, and a similar method has been employed in the present study; both sets of data are in agreement and show a mean excretion rate of 8–12 mg of albumin per 24 hr. In addition to albumin, γG- and γA-globulins represent the major part of the plasma proteins detected in normal urine, and they are excreted at a rate of 2.4 and 0.5 mg/hr, respectively. Previous studies have shown that the albumin, γG-globulin, and transferrin that are excreted in normal urine originate from the plasma (31) and that the ultrafiltration method used for the concentration of urine leaves intact the albumin (32), transferrin (10), and γG-globulin (33, 34) present in solution. Since the immunoglobins are present in urine as a complete molecule (33), as well as subunits (34–39), the content of γG- and γA-globulins might be overestimated because of the presence of free light and heavy chains, and Fab and Fc fragments. Furthermore, the presence in urine of a secretory type γA-globulin (11S) (40) might invalidate the results obtained for γA-globulin. The other plasma proteins that were investigated were excreted at a rate lower (< 0.4 mg/24 hr) than that of either albumin, γG-globulin, or transferrin.

The excretion of plasma proteins in urine is increased after strenuous exercise. This result is in agreement with the work previously reported (10). The present study shows a significant rise (up to 50-fold), particularly of α_1-acid glycoprotein, albumin, and transferrin. In fact, the excretion of most plasma proteins increases after exercise, since they represent 82% of the total proteins excreted in "exercise" urine.

The data for the renal clearance of the proteins excreted in urine before exercise are scattered over a wide range. No direct relationship could be found between the molecular weight of a protein and the value of its renal clearance. In normal urine of subjects at rest some low molecular weight proteins (e.g. tryptophan-rich prealbumin, Gc-globulin, and α_1-antitrypsin) have a lower clearance than do proteins having a higher molecular weight (e.g. albumin and hemopexin).

A rise in the renal clearance of proteins was observed after exercise, although the rate of this increase was not the same for all proteins. The observation made for the urine of normal subjects at rest, namely that no direct relationship between molecular weight and renal clearance could be observed, was also made for "exercise" proteinuria. For example, α_1-acid glycoprotein, which has a low molecular weight (44,000), had the highest clearance, whereas the increase of the renal clearance of the β_2-glycoprotein I (molecular weight 40,000), was only slight. Of special interest was the clearance of the immunoglobulins γA and γG.

When excreted in urine of normal subjects at rest, the γA-globulin showed a rate of clearance three times that of the γG. This phenomenon may be explained by the presence in urine of the secretory type γA-globulin, which possesses some antigenic sites common to plasma γA-globulin (40). After physical activity, a higher urinary excretion of plasma proteins causes the higher renal clearance of γG-globulin.

The glomerular permeability seems to be selective, depending on size and shape. The excretion of the low molecular weight proteins (molecular weight $< 40{,}000$), which are present in low concentration in plasma but in a much higher concentration in urine, can be explained only on the basis of a glomerular clearance higher than that of the usual proteins, such as albumin.

Thus the $3S\gamma_1$-globulin, which has a molecular weight of 25,000 and passes easily through the glomerular basement membrane, shows a plasma concentration of approximately 1 mg/liter (41), far below the actual sensitivity of the immunodiffusion test, whereas its concentration in normal human urine is much higher (42).

It is generally assumed that under pathological conditions the passage of proteins through the glomerulus is approximately proportional to their molecular weight (14, 16–18, 43, 44). However, when the values of renal clearance observed in proteinuria occurring after exercise were plotted against the molecular weight, the diagram was not completely superimposable on that obtained for normal physiological proteinuria at rest. In addition, Maclean and Petrie (17) have reported that the correlation between molecular weight and clearance of proteins is statistically not significant for patients excreting less than 1 g of urinary proteins per day. These observations suggest that the mechanism of severe pathological proteinuria is different from that of physiological proteinuria before and after exercise. In order to explain our present results, it is necessary to assume that properties other than molecular weight play a role in the mechanism of normal physiological proteinuria, for example preferential tubular reabsorption or penetration of proteins into the nephron at postglomerular sites.

Two other hypotheses may explain the increase of the renal clearance of plasma proteins by muscular activity. Firstly, the presence of higher molecular weight substances in "exercise urine" might result from an increase in the glomerular permeability. Secondly, it is possible that tubular reabsorption has reached its maximum value (Tm) for most of these plasma proteins, thus causing a rise in the amount and in the number of plasma proteins excreted after exercise. The previous (10) and present data give some support to this second hypothesis. The lack of a simple relationship between clearance and molecular weight of the protein excreted could be interpreted by a decrease in the molecular weight during passage through the kidney, as observed by Maiorca and Scarpioni in nephropathic patients (45). This explanation is not valid for the urine excreted by healthy subjects, since it has been shown that the plasma proteins isolated from urine had molecular weights identical with the corresponding ones isolated from plasma (20, 32, 33). Modification of the antigenic sites of the plasma proteins during passage through the urinary tract is not ruled out by our experiments, but this seems to be the least plausible explanation for the results presented in this study.

Thus, it appears that the proteinurias observed before and after exercise in normal subjects have a mechanism different from that of the pathological proteinurias, for which a simpler explanation has been given.

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