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Urinary Protein Components. III. Classification of Proteinuria Based on the Protein Clearance Results

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Six stages or grades of the kidney protein selectivity are described, according to number and kind of proteins appeared in the urine, the net clearances, the curve profiles, the slope and the glomerular sieving coefficients. Respective data of stages IV, V and VI are in strong contradiction to the one single site protein tubular absorption hypothesis. A four mechanisms scheme is proposed depending upon the glomerular sieving and tubular absorption combined activities.

New technical developments for the identification quantification and analysis of the different urinary protein components (11), have given new significance to the proteinuria, as well as to those diseases in which the proteinuria is a major finding (13). The appearance of different urinary protein components and the correlation between the protein clearance rates and the pathological parameters are reported (2). Different attempts to explain the type of respond to the various treatments, specially steroids (17) and the so call immunosupressors are described. Kidney protein selectivity appears to be related to the clinical-pathological picture (1) and therefore the protein renal handling (7). It is strongly being suggested that the protein components sieved through the kidney glomeruli are reabsorbed through the tubule cells, by using one single site or mechanism for all the proteins, independently of their structure or molecular size (16). However, experimental data seems to indicate that the tubular protein reabsorption possibly occurs through multiple sites (3), rather that one single site.

The purpose of this paper is to show evidente on the classification of proteinuria and the role played by the tubular multiple sites reabsorption hypothesis. Its correlation to the pathological findings will be published some where else.

Materials and Methods

GENERAL PROCEDURES. Protein clearances were determined on 64 patients hav-

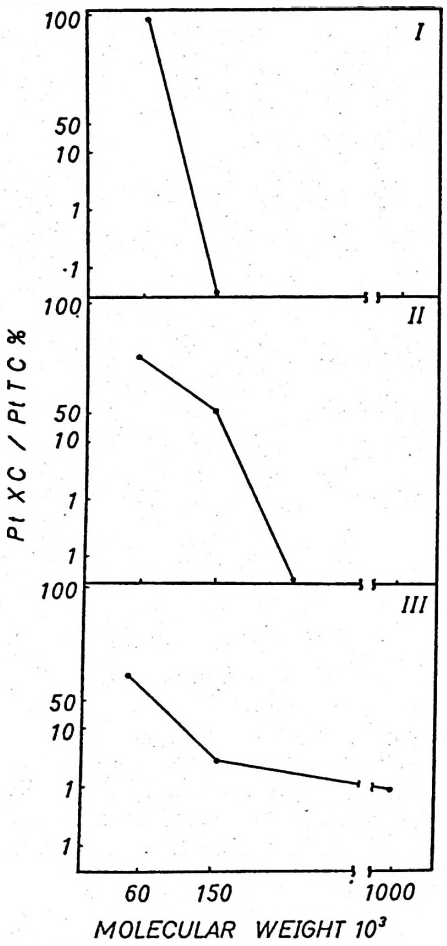

Fig. 1. *Typical proteinuria profile plotts.* Stages I, II and III. Experimental and plotting procedures were performed as described in Methods.

ing different renal diseases. Protein of various sizes ranging from 60,000 up to 800,000 molecular weight were determined by a three points scale. They respectively correspond to albumin, γ-globulins and α_2-macroglobulin. Collection of 24 hours samples was done following standard methods (5). Aliquots of the urine were concentrated by vacuum dialysis and the different protein components identified by immunoelectrophoresis (19) by using antiwhole blood plasma antiserum, obtained from Beringhwerke, Frezenius (Germany) and Hyland (U.S.A.) firms. Albumin, γ-globulins and α_2-macroglobulin quantifications were performed by the double immunodiffusion procedure (9) and by using quantitatively tested specific antiserums (12). Glomerular filtration rates were determined by the creatinine clearances (4). Total protein was tested by the biuret procedure (18). Standard electrophoresis at *p*H 8.6 in barbitone buffer (15) on a Beckman microzone device were run.

Semilogaritmic plotts in which the percentage of the specific protein/total protein clearances ratio toward the molecular weight of the specific protein constituents were used (Fig. 1). Plotting of both parameters showed a somehow lineal correlation.

SELECTIVITY PATTERN. Six stages or grades of selectivity are established depending upon five parameters: 1) The number and kind of the individual protein elements, present in the urine. 2) The net clearances of either albumin, γ-globulins or α_2-macroglobulin. 3) The curve profiles. 4) The slope of the curves, θ and 5) The glomerular sieving coefficients or φ (10).

Slope values were determined by taking into account the presence or absence of inflection at the γ-globulins point. Slopes were considered as left when the high point on the curve was located at the left side of the plotts and the curves are drawn from left to right. Curves with the higher point placed on the right side will determine right slope values.

Results

STAGE I OR HIGHLY SELECTIVE. Proteins present in the urine ranged from 60,000 up to 150,000 molecular weight. The albumin net clearances were increased as compared to normal people (12). Urinary proteins had low molecular weights (Table I). The γ-globulins clearances, if present, were lower than the albumin clearances. The left slope of this group

has a θ value ranging from 63° to 81° and mean value of 76° (Fig. 1).

STAGE II OR MODERATE SELECTIVITY. Molecular weight of the urinary protein content ranged from 60,000 up to 600,000. The net clearances of the albumin and γ-globulins were increased, however the heavy protein clearances were lower than those of the light components. The α_2-macroglobulin was never found (Table I). The mean value of the slope was θ left 64°, ranging from left 60° to 71° (Fig. 1).

STAGE III OR LOW SELECTIVITY. In this group the protein constituents cleared through the urine reached 1,000,000 m.w. and even higher (Table I). All the especific clearances were increased but the clearances of the light constituents were larger than those corresponding to heavy components (Table I). In this group the slope values still are left, being 47° the θ mean values. Extreme values ranged from 40° to 56° (Fig. 1).

STAGE IV OR NON-SELECTIVE. Four major findings define this stage or grade: 1) Net increase of all the protein clearances. 2) Larger γ-globulins clearance as compared to those of the albumin and α_2-macroglobulin. 3) Presence of urinary proteins possessing broad molecular weights. 4) The γ-globulin inflection point leads to slope values, that respectively possess right and left signs (Table I). The θ mean values are right 36° and left 53° being the range; right 21-48° and left 47-57° (Fig. 2).

STAGE V OR ANA-SELECTIVE. This group has similar characteristics to grade IV, however the decrease of the clearance of the middle molecular weight components (γ-globulins) related to the other proteins is the defining factor. The γ-globulins inflection point of this group is conditioning the appearance of slope values with left and right signs (Table I). The θ mean values are left 57° and right 16°. However, they range from left 72-60° and right 62-2° (Fig. 2).

STAGE VI OR INVERSE SELECTIVITY. Loss of heavy protein constituents is the predominant factor in this stage. The net clearance of the α_2-macroglobulin is larger than those of the γ-globulins and these are larger, as compared to the albumin clearance (Table I). Therefore the protein components are inversely cleared to their molecular weight. The θ mean values of the slopes are; right 27° (Fig. 2).

Table I. *Proteinuria and specific protein clearance components from 68 kidney patients.* Mean values experimental procedure and proteinuria classification were performed as described in «Methods».

STAGE	PROTEINURIA	ALBUMIN		γ-GLOBULINS		α_2-MACROGLOBULINE		SLOPE θ °	
	g/24 h.	Clearance ml/mn/1,73 m² 10^{-3}	φ^a 10^{-4}	Clearance ml/mn/1,73 m² 10^{-3}	$\varphi\gamma$ 10^{-4}	Clearance ml/mn/1,73 m² 10^{-3}	$\varphi\alpha_2M$ 10^{-4}	Sign *	Value
I	0.60	6.1	0.34	—	—	—	—	l	76
II	2.35	30.8	0.25	5.25	0.69	—	—	l	64
III	3.25	55.8	12.50	27.10	6.35	8.90	1.50	l	47
IV	4.70	50.0	4.20	61.00	3.80	4.60	0.72	r-l	36-53
V	5.76	146.3	13.30	13.20	1.38	35.00	2.81	l-r	57-16
VI	2.12	9.0	0.50	14.00	0.70	86.00	4.80	r	27

* l = left; r = right.

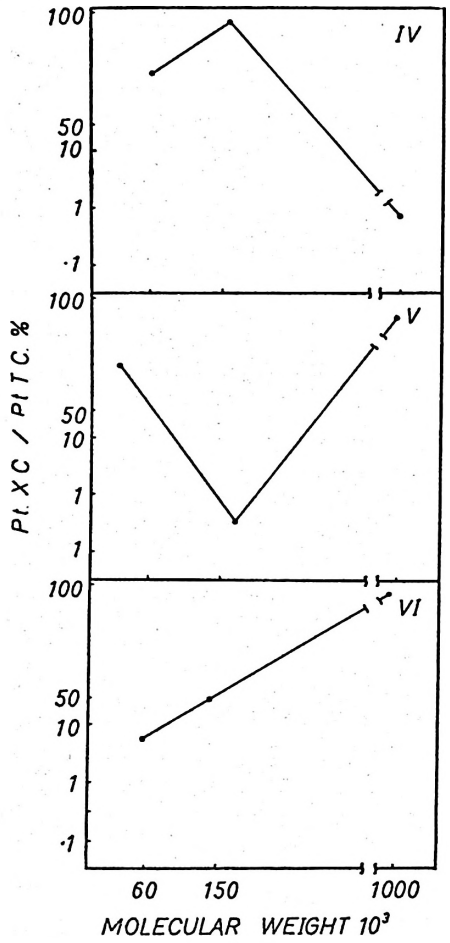

Fig. 2. *Typical non-selective proteinuria.* Stages IV, V and VI profiles.

SIEVING COEFFICIENT. Special emphasis may be put forward on the glomerular sieving coefficient analysis. Those stages, in which no selectivity is the predominant factor, the sieving coefficient is increased (Table I).

Discussion

Results described in this paper are an attempt to classify the proteinuria, based on the protein clearances data. The net clearances of the albumin, γ-globulins and α_2-macroglobulin coupled to the identification of the various protein components, existing in the urine, are the main sources of information.

Kidney selectivity toward plasma proteins implies a proportional correlation between the specific clearance and their molecular weight. Obviously, a proteinuria can be considered as selective, when the net clearance of the low molecular weight proteins exceed to the heavy constituents. Classification parameters can not be based on the slope values alone or either the regression lines, because they are isolated data which significance is difficult to assess.

A classification possessing six stages or grades, perhaps may help to give more significance to the proteinuria clearance information.

The data showed in the IV, V and VI stages are in contradiction to the one single site tubular reabsorption, hypothesis. This contradictory results may be summarized by the decrease and increase of the clearances of the middle molecular weight components as compared to the net clearances of the other constituents. Additionally, the inversion of the net clearances/molecular weight ratio (grade VI) is other source of contradiction. It is quite difficult to understand how any pathological process may lead to the dissociation of one single site mechanism of reabsorption. Therefore in order to change the affinity of the site toward the various substrates (proteins) different systems or sites must be involved. The glomerule sieving process is the resulting event of the ultrafiltration through a mesh of multiple layers of different pore sizes (8). Discussion of this mechanism is beyond the scope of this paper. The contradictory results related to the one single site reabsorption hypothesis may be explained, if the above mentioned glomerular device and the tubular multiple sites reabsorption are working together. The tubular reabsorption occurring at multiple sites, does not imply places of reabsorption but ways or mechanisms (6). However morphologi-

cal experiments show evidence on the level or reabsorption place and the protein being reabsorbed (14).

Proteinuria patterns in which the presence of α_2-macroglobulin is an important fact or finding, will mean that the pathological process is affecting the glomerular sieving mechanism and the tubular multiple reabsorption system. Both, glomerular and tubular functions have to be considered, if any meaningful correlation between the protein clearances and the clinical-pathological picture, want to be established. Four major stages or phases of the glomerular-tubular behaviour may be established, if the results corresponding to the six grades or stages of proteinuria are explained. In all the groups, the glomerular leak toward most plasma proteins is the common factor existing in the different selectivity patterns. Additionally, the tubular behaviour is the determining factor of the proteinuria. The tubular function in the different grades or groups may be described as follows:

1) Groups I and II, good function. 2) Group III: even overload, beyond the normal handling capability. 3) Groups IV and V: tubular overload and malfunction of some of the reabsorption sites. 4) Group VI: no tubular (function) overload, while the corresponding reabsorption sites are altered (Fig. 3).

In determining the tubular overload (partial or complete) the glomerular sieving coefficient plays an important role. An increase of the specific sieving coefficient implies functional overload of the specific tubular site. Though the correlation of the proteinuria with the clinical-pathological findings will be published later on, it can be put forward that no clinical-pathological correlation with the proteinuria was found. However prognosis significance do really exist.

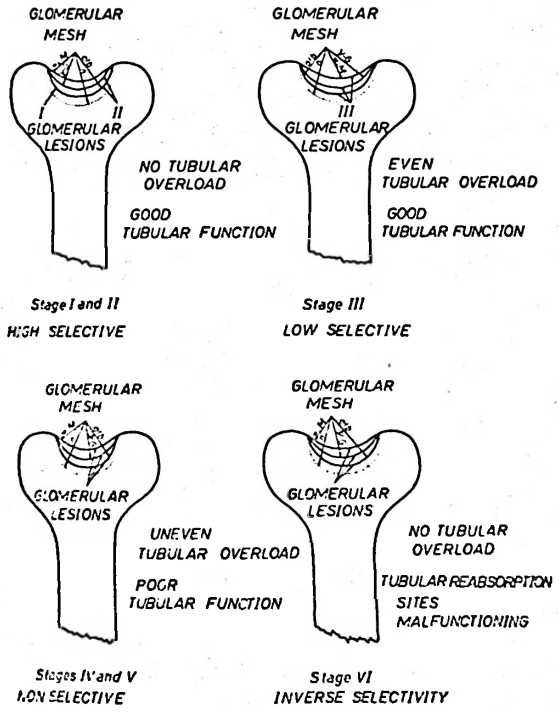

Fig. 3. *Proposal mechanism of proteinuria.* The different mechanism are based on two parameters hypothesis: 1) Glomerule: multiple layers (at least 2 or 3) of different pore sizes. 2) Tubule: multiple reabsorption sites.

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