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Urinary Protein Components
I. Basis and General Methodology

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Protein clearances of blood components is becoming one of the most accured procedures to test glomerule and tube behaviour. Molecular protein components lighter than 150,000 M.W. usually, are cleared.

A three points scale ranging from 60 to 900,000 M.W. is explored. Quantitative immuno-diffusion and electroforesis are used. Net clearance and sieving coefficient are, among others, the main patterns to be determined.

A deep and broad knowledge of the different urinary protein components has been reached, by the outstanding progress of the immunologic technics (1), the application of new type of materials to protein electrophoresis (12) and the use of new ways of chromatography fractionation on sephadex and agarose gels (5). This field is shaped by the above mentioned factors, coupled to the information explosion, and the spreading of the publications on various journals. In spite of these grievances and drawbacks, the stored information have opened, to the renal area, new ways of development and progress. Significance of these studies may be comparable to the introduction of the needle byopsy in the renal field.

Urinary proteins may have different sources of origin:

1) Blood. 2) Glomerular membranes. 3) Surfaces of tubular cells. 4) Low urinary tract.

The purpose of this paper, is to show evidence on the general methodology for the plasma proteins, appeared in the urine. Methods and procedures related to the glomerular and renal tubular proteins are on progress and will be published some whereelse.

Materials and Methods

Materials of general type. Biological fluids were concentrated by using Sartorius collodion membranes, applied to a low pressure apparatus. Microzone electrophoresis was performed on a Beckman brand, set up. Cellulose acetate membranes for immunodiffusion and electrophoretic technics were bought from Beckman (California, USA) and Sartorius (West

Germany) firms. Diffusion chambers were purchased from Atom (Barcelona). Other reagents used, were highest grade commercial products.

Antihuman Serum Antibodies. Special characteristics and purchasing place for each immuno-serum were as follows: Rabbit antibodies towards trypsin α_1-glycoprotein, acid α_1-glycoprotein, haptoglobin, transferrin, IgG-IgA-IgM immunoglobulins were obtained from Behringwerke (Germany). Rabbit antibodies anti-whole serum protein components, anti-ceruloplasmin, anti-α_2-macroglobulin were bought from Fresenius (Hamburg, Germany). The α_2-macroglobulin immunoplates were furnished by Hyland (California, USA).

Specimens collection and preliminary procedures. Blood samples and 24 hours urine collections were performed following standard procedures (7). Creatinine clearance (6) and total protein (previously, the urine was dialysed) in these samples were determined (8). Prior to the conventional and immunoelectrophoresis runs, a volume concentration to 0.7-0.9 ml from 10 ml urine aliquots were done.

Conventional and immuno-electrophoresis technics. Microzone electrophoresis at *p*H 8.2 (barbital buffer) and 150 volts, for 50 minutes, on cellulose acetate were run. After staining with Ponceau red and transparence with methyl alcohol, densitometry readings (Analytrol) were performed. Ten times spotting, in samples containing low level of urinary proteins, were done. By making 3-4 chanals, on 35 % methanol preserved, cellulose acetate membranes; immunoelectrophoresis begins. Afterwards, sample application and electrophoresis runs are carried out, as above described. When run is finished and without removing the membrane: 10-15 μl of antiserum towards human plasma protein are placed and spreading them through the whole chanal length. Then, a 24 hours diffusion is folloved by a 2 hours, 0.9 % sodium chloride thorough washing. Staining, is followed by 24 hours, nigrosin restaining and transparence. Working nigrosin solution is made up with 6 ml of stock solution in 5 % acetic acid (Stock solution contains: 100 mg of nigrosin in 100 ml, 2 % acetic acid).

Quantitative α_2-macroglobulin determinations. It is based on the immunodiffusion technic (4) by using the immunoplate (Hyland). On this plate an excess of α_2-macroglobulin antibody is evenly distributed and mixed with agar. The standard curve is built by adding (on each well) increasing amounts of a standardized serum. Afterwards, the plate is covered and kept diffusing at room temperature, for 16-18 hours, followed by nigrosin staining. Using semilogarithmic paper the diameter of the precipitation shadows or halos (abcises), expressed as 0.1 mm units and the α_2-macroglobulin concentration (mg %) are plotted (Figs. 1, 2).

Quantitative albumin determinations. Depending upon the amount of albumin present in the urine, two cases have to be done:

1) Cases in which the amount of albumin may be determined by conventional electrophoresis and therefore densitometry reading can be applied.

2) Cases containing a small amount of albumin only detectable by using special methods of determination, such as immunodiffusion. In these cases the quantification was performed according to the method of MANCINI *et al.* (10). By making a central and four lateral wells (according to the template), on a cellulose acetate membrane, the method begins. On the central well 2 μl of antialbumin antibody are placed. After diffusing for 24 hours, 2 additional μl of serum antibody are added, followed by the deposit of 2 μl of antigen (concentrate urine or

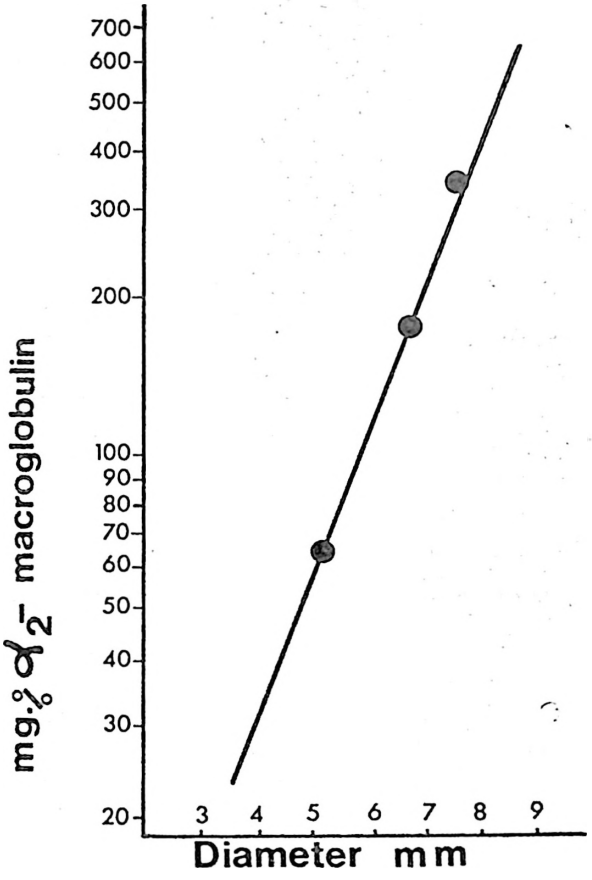

Fig. 1. *Standard α_2-macroglobulin concentration curve.*

The mg % α_2-macroglobulin concentration are plotted towards the mm diameter of the antigen-antibody well halo. On Hylland welled immunoplates, containing evenly distributed α_2-macroglobulin antibody (Fig. 2), different amounts of a standardized serum were deposited in the wells. After diffusion and staining were performed, as described in «Methods», the diameter of well halos were measured.

serum), on each external well. A second 24 hours diffusion period, then, begins. The standard curve is performed with four albumin dilutions respectively containing: 534, 267, 133 and 66 mg %. On semilogarithmic paper the precipitation travel distace (abcise) and the albumin concentration (mg%) are plotted (Figures 3, 4).

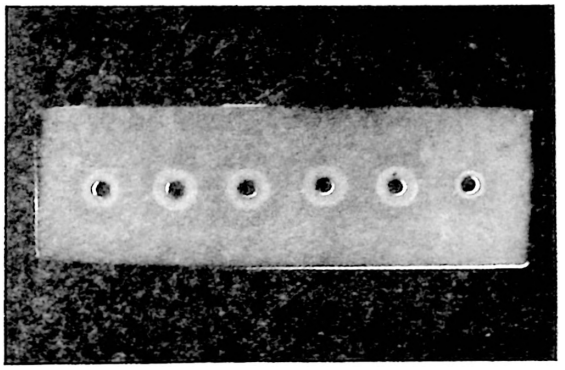

Fig. 2. *Typical α_2-macroglobulin immunoplate run.*

Sample application and runs were performed as described on «Methods».

Kidney handling of plasma proteins. The glomerular filter behaviour is analyzed by using three different molecular protein size clearances, corresponding to albumin (60,000 m.w.), gammaglobulins (150,000 m.w.) and α_2-macroglobulin (900,000-1,000,000 m.w.). Molecular clearances were calculated according to the general formula (9) used for other compounds.

The apparent protein sieving coefficient for each protein is defined by the specific protein clearance over the glomerular filtration rate (G.F.R.) ratio. The G.F.R. was identified with creatinine clearance.

Results

Each study of the proteinuria is based on six different groups of data: filtration glomerular rate, specific protein clearances, particular protein sieving coefficient (11), number and kind of protein components existing in the urine, 24 hours proteins excretion and various type of curves expressing the kidney protein handling.

Information obtained from the glomerular filtration rate or creatinine clearance does not need further comment.

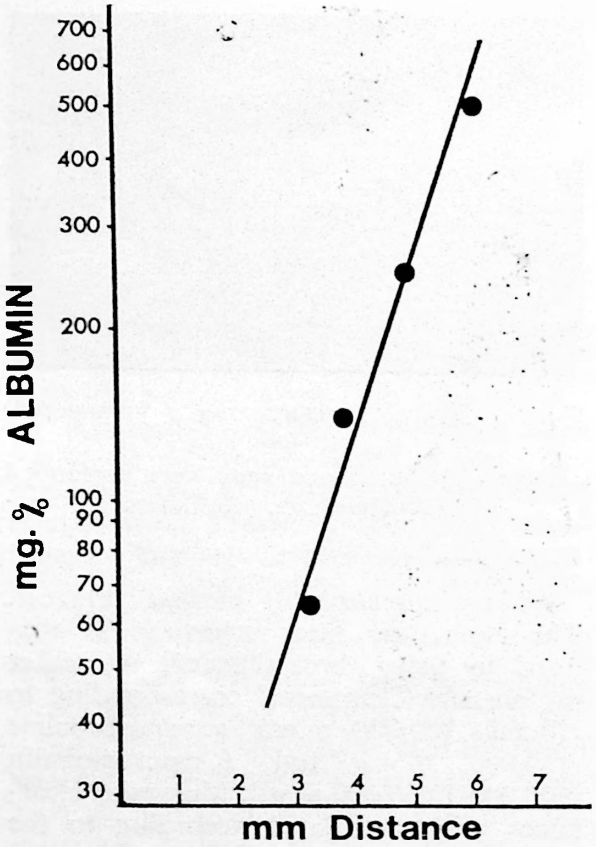

Fig. 3. *Standard albumin concentration curve.* Albumin concentration (mg %) are plotted towards the travel distance of the precipitation line. Procedure performance was done as described on «Methods».

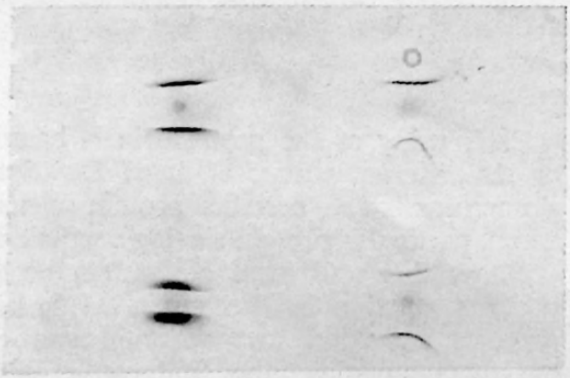

Fig. 4. *Typical albumin-immunodiffussion plate appearance.* Sample application and runs were performed as described on «Methods».

Specific protein clearances. Protein clearances, in apparent normal people, show the appearance of albumin. In some cases, it is not unusual to find gamma-globulin (mainly IgG) associated to other lower molecular weight proteins. Standard values for specific protein clearance are given on table I.

Table I. *Typical values of protein clearances in normal individuals.*

	ml/min
Total protein clearance	2.6×10^{-4}
Albumin clearance	8×10^{-4}
φ_A	4×10^{-5}
Immunoglobulins clearance	1×10^{-4}
φ_I	1×10^{-5}
α_2-macroglobulin clearance	0
φ_M	0

	Blood g %	Urine g %	
Total protein	7.02		0.0300
Albumin	3.79		0.0050
α_1-globulin	0.37	Other	
α_2-globulin	0.60	proteins	0.0248
α_2-macroglobulin	0.32		
β-globulin	0.95		
γ-globulin	1.13		0.0002

Particular protein sieving coefficient. The appearance, in the urine, of a particular protein, depends upon two factors: glomerular pore size and tubular reabsorption capability. The way and amount of protein being filtered through the glomerular membranes depends upon; the molecular size, configuration and molecular hindrance, among other factors. Each protein is sieved through the glomerule depending upon a particular coefficient or φ. Real sieving coefficient may be calculated according to CHINARD *et al.* (2). However the specific protein clearance/ G.F.R. gives information on the sieving coefficient for any protein appeared in the urine (Fig. 4).

Curves expressing protein kidney handling. From above described information

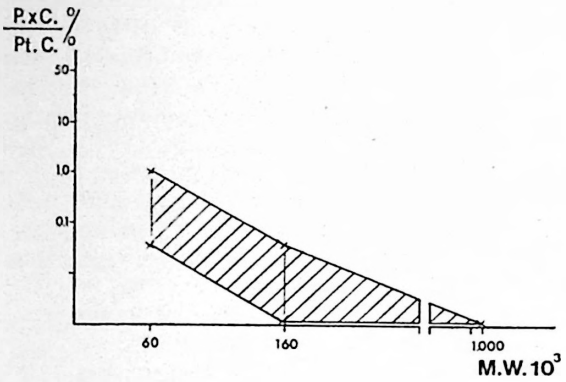

Fig. 5. *Standard curve relating the molecular size (abcise) and per cent, specific and total protein, clearances ratio.*
Curve is made up by using specific protein clearances of albumin, IgG and α_2-macroglobulin, from mean values corresponding to 30, apparent normal people.

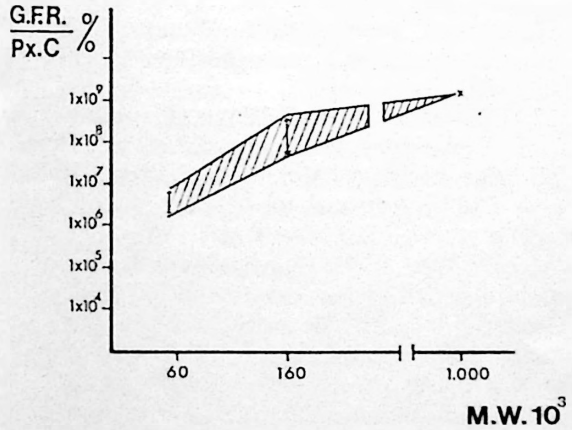

Fig. 6. *Molecular size and per cent G.F.R./ specific protein clearances ratio plotts.*

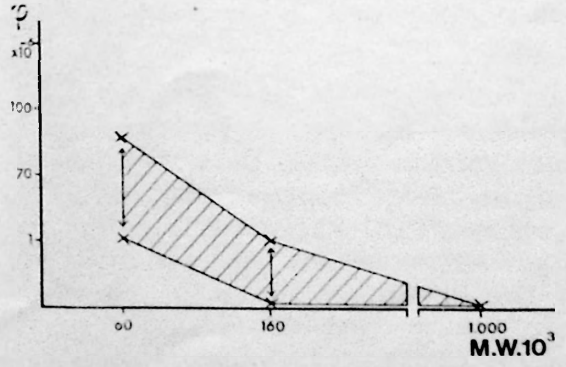

Fig. 7. *Molecular size (abcise)-sieving coefficient plotts.*

three different type of curves may be drawn: 1) Curve relating the molecular size and per cent specific/total protein clarances ratio (Fig. 5). 2) Curve in which the molecular size and per cent G.F.R./ protein clearances ratio are plotted (Figure 6). 3) Molecular size and sieving coefficient plotts (Fig. 7).

Discussion

Among the different ways of approaching the discussion, three main points may be emphasized. The first point may be related to the reliability of the methods and procedures used in this paper. Measurement and determination of different types of label protein components rate flow, through the kidney, appear to be the most reliable and perhaps sensitive procedure. The catabolic effects of the body on these compounds, in the case of protein components, and the nonphysiological effects of other label compounds such the Polyvinylpyrolidone-I^{131} are different considerations to have into account, when a procedure have to be chosen. Accuracy, physiological behaviour and resonable handling are the main advantages of present methodology.

Identification of place of origin, for the different protein components, may be the second point of discussion. The blood, glomerule, and renal tubule are the main sources of origin of the different proteins appeared in the urine. The isolation and identification of the glomerular proteins (appeared in the urine) using immunological procedures, it is on progress and will be published somewhere else.

Study of the renal tubular proteins by using acrylamide electrophoresis it is also being developed. Any compound filtered through the glomerular membranes, may be excreted as such, but mostly is taken up by the tubular cells by way of ingulfing. Though, much discussion and controversy it is going on, about, the single or

multiple sites of protein tubular reabsorption (3), the apparent sieving coefficient or φ, shows evidence related to the protein behaviour at tubular level. An increase of this coefficient indicates either a poor handling by the renal tubule or a loss of the sieving capability at the glomerular tufst level.

The third point to be discussed may be the significance of these type of studies. Implications on the glomerular hydrodinamic structure are beyond the scope of this paper. However, the protein clearances are one of the most accure procedures to get information on the behaviour of the different kidney portions, towards the various protein components.

References

1. CAIRNS, J.: «Symposium on Quantitative Biology». Vol. **32**, Cold Spring Harbor, L.I., p. 1, New Yok, 1967.
2. CHINARD, F. P., LAUSON, H. D., EDER, H. A., GREIF, R. L. and MILLER, A.: *J. Clin. Invest.*, **33**, 621, 1954.
3. CORTNEY, M. A., SAWIN, L. L. and WEISS, D. D.: *J. Clin. Invest.*, **49**, 1, 1970.
4. CROWLE, A. J.: «Immunodiffusion». Academic Press, p. 76, New York, 1961.
5. DETERMANN, H.: Gelchromatographie, p. 38; Springer Verlag, Berlin, 1967.
6. HAUGEN, H. N. and BLEGEN, E. M.: *Scand. J. Clin. Lab. Invest.*, **5**, 67, 1953.
7. HENRY, R. J.: «Clinical Chemistry. Principles and Technics». Harper and Row. Publ. New York, p. 152, 1968.
8. HENRY, R. J., SOBEL, L. and BERKMAN, S.: *Anal. Chem.*, **29**, 1491, 1957.
9. LANDIS, E. M. and PAPPENHEIMER, J. R.: In Handbook of Physiology, Vol. 2, Sect. 2, p. 961, Amer. Physiol. Soc. Washington, 1962.
10. MANCINI, G., VAERMAN, J. P., CARBONARA, A. O. and HEREMANS, J. F.: In «Proceedings of the 11th Colloquium, Protides of the Biological Fluids», Peeter's H. (ed.). Elsevier Publishing Co., Amsterdam. p. 370, 1963.
11. PAPPENHEIMER, J. R., RENKIN, E. M. and BORRERO, L.: *Am. J. Physiol.*, **167**, 13. 1951.
12. WORK, T. S. and WORK, E.: «Laboratory Techniques in Biochemistry and Molecular Biology», Vol. 1, p. 7, North Holland Publ., Amsterdam, 1969.