Serum Proteins and Dialysis Treatment
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The kidney affects the metabolism of small-sized protein molecules, which, in normal conditions, pass through the glomerular filter, and are subse-
quently absorbed and catabolised by the tubules. This has been proved for
hormonal substances such as insulin (Rubenstein & Spitz, 1968), parathyroid
hormone (Martin et al, 1969), growth hormone (Salomon et al, 1964) and for
different polypeptides like ACTH (Richards & Sayers, 1951), vasopressin,
oxycocin (Kenny, 1960) and glucagon (Cox et al, 1957). In cases of tubular
nephropathies, the appearance in urines of small proteins, with molecular
weight between 10,000 and 40,000, shows the lack of reabsorption and of
renal catabolism. In uraemia with severe reduction of glomerular filtration
it is possible to have an increase in the blood of such substances and of dif-
f erent microproteins which cannot be eliminated by the usual dialysis tech-
niques. Their number, nature and the biological effects of the high con-
centrations we have recorded are not completely known.

We believe, therefore, that it is necessary to extend our work to include
several protein fractions in the blood of patients with chronic renal failure
due to different nephropathies, who have been treated by diet alone or by
periodic haemodialysis.

PATIENTS AND METHODS
We have studied 21 patients on regular haemodialysis treatment using Kiil
dialysers and Cuprophan membranes under standard conditions; the duration
of treatment has varied from a minimum of 5 months to a maximum of 40
months (average 21 months). The data we obtained have been compared with
those obtained in 126 patients with chronic renal disease who were not treated
by dialysis; of these, 42 were affected by glomerulonephritis (14 uraemic),
52 by a nephrotic syndrome (15 uraemic) and the other 35 patients were in
renal failure from chronic pyelonephritis (16 cases), polycystic kidney (9
cases) and nephrosclerosis (6 cases). The non-dialysed uraemic patients
were put on a diet containing 25-30 g of selected proteins of high biological value with a total intake of 2500 Calories per day. The dialysed patients were allowed a more variable diet, with higher protein content (60-70 g per day). The same investigations of plasma proteins were carried out in a group of 34 normal controls.

Eleven plasma proteins were studied: albumin (Alb), transferrin (Tr), alpha-macroglobulin (α2-M), haptoglobin (Hp), IgG, IgA, IgM, beta1C-globulin (β1C), alphalacid-glycoprotein α1AcGp), alphal-antitrypsin (α1Atr), and beta2-glycoprotein I (β2GpI). The measurements were made in sera from fasting patients, using Behringwerke immunoplates which employ the method of radial diffusion of Mancini et al (1964). Total plasma proteins were determined by the biuret method. All the values are expressed in mg/100 ml (numbers on the ordinates of the figures). In dialysed patients the blood was taken on the day of dialysis, just before its beginning.

RESULTS
The first phase of our study examined the effects of renal failure from two different viewpoints: (1) we compared patients in different stages of single nephropathies such as the nephrotic syndrome and chronic glomerulonephritis without such a syndrome; (2) we compared these data with results obtained from normal subjects. In the nephrotic syndrome (Figure 1) there is no significant effect associated with the presence of uraemia on the values for total proteins, albumin, transferrin and IgG which remain low; the values of IgA and IgM remain normal and the values for haptoglobin remain high. The increased values of alpha2-M fall with the development of uraemia to normal limits and there is also a fall in beta1C level. In contrast, alphal-acid glycoprotein increases with uraemia to levels above normal. The same phenomenon is observed in the group of patients affected by chronic glomerulonephritis without the nephrotic syndrome (Figure 2), while the other protein fractions are not affected with the advance of renal failure, whether they were low before the development of uraemia (such as albumin) or inside normal limits; only beta1C is reduced.

Regular haemodialysis (Figure 3) reduces or abolishes the differences in the concentrations of the protein fractions observed in the various groups of nephropathy. Thus the average values in patients on dialysis may be considered together in common groups including the relative standard deviations. These average values for those on dialysis are not far from those of non dialysed patients if the previous values were already normal: for example, albumin in patients with pyelonephritis, polycystic kidneys, nephrosclerosis; alpha2-M in patients with glomerulonephritis, pyelonephritis, polycystic kidneys, nephrosclerosis; haptoglobin, IgA, β1C in all the patients studied; IgG in patients with pyelonephritis and polycystic kidneys.

In nephropathies where levels without dialysis were lowered, normal
Nephrotic Syndrome with Renal Failure (Right Column) versus Without Renal Failure (Left Column)

The numbers at the bottom of each column indicate the statistical p differences of the nephrotic patients versus the normal cases (n.d. = Non Statistical Difference)

Figure 1. In this figure and in the next ones the columns show the mean values (line in the middle) and the standard deviations. The horizontal base lines define a band which include the mean values and standard deviations of the group of the normal controls.
GLomerulonephritis

WITH RENAL FAILURE (RIGHT COLUMN)  
VERSUS  
WITHOUT RENAL FAILURE (LEFT COLUMN)


Figure 2. The protein values of the patients with glomerulonephritis with and without renal failure are expressed as averages by the unbroken horizontal lines, and are limited by the dashed ones, which indicate the standard deviations.
Figure 3. All kinds of nephropathy are considered together: after a sufficient period of dialysis treatment, the differences in protein values found among the different types of patients cannot be observed any more.
values are observed during dialysis treatment: that is for total proteins, albumin, IgG in patients with the nephrotic syndrome, and for transferrin in all the nephropathies studied. Only alpha1-acid-glycoprotein is an exception which remains elevated in every kind of nephropathy with or without dialysis. Alpha2-M which was raised only in non dialysed nephrotic patients was normal during dialysis treatment.

Significantly raised levels not only of alpha1-acid-glycoprotein, but also of alpha1-antitrypsin and beta2-glycoprotein I are observed in groups of dialysed uraemic patients compared with normal controls (Figure 4).

**DISCUSSION**

Among the effects of uraemia on plasma proteins that we have studied, the reduction of alpha2-M in patients with a nephrotic syndrome may depend mostly upon depression of hepatic synthesis which was initially above normal in the absence of any great elimination through the glomeruli, because of the large molecular size. The fall in urinary protein losses which occur with progressive reduction in volume of filtrate, are probably less important because other macroglobulins, such as IgM, do not fall in parallel to alpha2-M with progression of the renal failure.

Notwithstanding, haemodialysis brings about a sparing of those proteins of average size which were lost in the urine through the increase of glomerular permeability that is characteristic of most nephropathies. This is because haemodialysis reduces progressively the urine flow by haemodynamic renal alteration (Migone et al, 1969) and thus renders normal the plasma concentrations of these protein fractions. On the other hand, the loss of aminoacids through the dialysis membrane probably does not reduce protein synthesis if it is balanced with a sufficient diet.
The most interesting data concern the increase of alphal-acid-glycoprotein in the different types of nephropathy; this is not corrected by dialysis. This protein is already known to participate in inflammatory reactions; but in uraemia an increase of synthesis through phlogistic reactive mechanisms does not seem probable, because haptoglobin, even though considered an 'acute phase reactant protein' and having a greater molecular weight (100,000), does not seem to be augmented at the same time. Other proteins, which have in common with alphal-acid-glycoprotein (molecular weight 44,000) a small molecular size, are also increased in the plasma: for example alphal-antitrypsin (molecular weight 45,000) and betal2-glycoprotein I (molecular weight 40,000): this last is independent of any reactive process (Cohnen, 1970).

Therefore it is probable that uraemia leads to retention in the blood of those microproteins which are normally filtered by the glomeruli but re-absorbed and catabolised by the tubules, and which are not eliminated through normal dialysis membranes. Our data are in agreement with similar reports that in uraemic dialysed patients there is retention in the blood of light chains of immunoglobulins (Epstein et al, 1968) and of various hormonal polypeptides such as parathyroid hormone (Berson & Yalow, 1966), growth hormone (Wright et al, 1968) and insulin (Hampers et al, 1968). For the betal2-microglobulin the increase in uraemic blood may reach values 150 times greater than normal (Peterson et al, 1969; Bernier et al, 1968).

Standard dialysis treatment purifies the body of toxic non-protein catabolites of small size, but does not affect pathological retention of microproteins. This leads us to investigate new procedures that can replace normal glomerular permeability more exactly.

REFERENCES

Cohnen, G. J. (1970) Journal of Laboratory and Clinical Medicine, 75, 212
Cox, R. W., Henley, E. D., Narahara, H. T., Vanarsdel, P. P. Jr. and Williams, R. H. (1957) Endocrinology, 60, 277
Experimental Biology and Medicine, 77, 161
Rubenstein, A. H. and Spitz, I. (1968) Diabetes, 17, 161
Journal of Clinical Investigation, 43, 103
Wright, A D., Lowy, C., Russel Fraser, T., Spitz, I. M., Rubenstein, A. H. and Bersohn, I. (1968) Lancet, 1, 798