Nitrogenous Compounds in Hemodialysate

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Patients with uremia usually improve after dialysis therapy, but often it is not possible to correlate clinical and chemical changes, other than those in urea and electrolytes. Analyses of blood have shown that several constituents including phenols, guanidines, unidentified anions, and undetermined alpha-amino ninhydrin reactive substances have been found elevated in uremia. Dialysis has succeeded in reducing some of these materials, but none has yet been positively established as clinically toxic. Alternatively, the dialysates may be examined for the presence of substances of possible clinical significance. This has been attempted for aromatic compounds, but the methods are cumbersome. Analysis of extracted organic substances has been hindered by large volumes of dialysates, low concentrations of contained materials, and the presence of glucose and salt.

A previous communication described a new, safe technic employing the Kidney artificial kidney, which provides adequate dialysate material for analytical studies of nitrogenous substances. In brief, 1,000 ml of Ringer's solution is temporarily substituted for the usual dialysate compartment, which contains from 100 to 370 liters. In 30 to 60 minutes, 6 to 9 liters of blood are accessible to the dialysis membrane and exchange of low molecular weight compounds is rapid. During six hemodialyses, for example, urea concentrations in these dialysates ranged from 52 to 85 per cent of initial blood concentrations. Although dialysis to equilibrium, which could be accomplished with a small amount of blood in vitro, is not achieved, this new technic permits the collection of a small amount of dialysate from the entire circulating blood volume in vivo.

The analytical studies performed on the dialysates are an attempt to define nitrogenous substances removed by hemodialysis.

Methods

For analysis of amino acid composition, 100 ml of dialysate were desalted electrolytically or by ion-retardation chromatography to osmolar concentrations below 25 mOsm./liter. After concentration under vacuum to 10 ml, the dialysates were lyophilized and then reconstituted in a sodium citrate-citric acid buffer (pH 2.875). Amino acid analysis was performed by the gradient elution method of Piez and Morris. Hydrolysis was accomplished by 6 N hydrochloric acid at 110°C for 24 hours.

Chromatography was performed on Sephadex gel columns and with a Vanguard automatic recording apparatus at 280 m

Experimental dialysis procedures in vitro utilized acetylated Visking membranes, size 18/32, and were modified from the technic of Craig. Amounts of nitrogenous materials were estimated by ninhydrin and Folin-Lowry phenol reactions.

Results

A representative chromatogram of ninhydrin reactive substances is presented in figure 1. Unknown peaks are present, ranging from 35 to 38 in various specimens. Acidic amino acids predominate and most of the peaks of unknown ninhydrin reactive materials also fall in this range. The amino acid composition of dialysate before and after acid hydrolysis is presented in figure 2. All amino acids increase after hydrolysis, with the exception of methionine and cystine, which are partially destroyed by this procedure. The increases combined with concomitant reductions in the amounts of unknown materials indicate destruction of peptide linkages and degradation of peptides or conjugated amino...
acids. Figure 2 represents the amino acid removal during a patient's first clinical dialysis. In figure 3 is presented the same material collected during the eighth clinical dialysis. The removal of amino acids is decreased; this decrease applied also to the unknown substances.

During hemodialysis, circulating protein materials are accessible to the dialysis membrane. Although partially dependent on shape for rate of transfer, the membrane pores of approximately 25 Angstrom units \(^{15}\) would be expected to permit passage of materials up to approximately 6,000 molecular weight.\(^{16}\) In the hope of separating small from larger molecular weight materials, dialysates were subjected to chromatography on Sephadex columns. These cross-linked dextran gels, which are prepared in different sizes, act by steric hindrance to exclude materials of large molecular weight and to trap selectively low molecular weight substances within their internal volume. On Sephadex G-25, the presently available gel with the lowest molecular weight exclusion of about 4,000, the nitrogenous materials in the dialysate eluted consistently from the internal volume (fig. 4).

Since it appeared from these studies that the nitrogenous substances were smaller than molecular weight 4,000, an experiment was performed to compare dialysis in vitro with that of urea and glucose. The concentration of dialysant compared to the original concentration was plotted against time. Concentration gradients were maintained steep by frequent dialysate changes so that only mi-

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migration across the membrane was critical. Prior studies\textsuperscript{17} have shown that membranes of the size used permit passage of materials up to 1500 molecular weight. The results are shown in figure 5. Dialysant concentrations of urea (M.W. 60) were lowest, followed by all ninhydrin reactive materials including amino acids, and next by glucose (M.W. 180). The highest concentrations of dialysant were recorded by the Folin-Lowry phenol reaction, which measures peptides and proteins more sensitively than it does free amino acids. The dialysis of these nitrogenous materials was not much different from that of glucose, however, thus implying only a small difference in size.

![Figure 2](image)

Amino acids in dialysate before and after acid hydrolysis.

![Figure 3](image)

Amino acids in dialysate from a later treatment with the artificial kidney.

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![Graph](image)

**Figure 4**

Elution of dialysate from a Sephadex G25 column measuring 22 by 2.5 cm. Albumin is excluded from the gel particles and eluted from the external volume of the bed whereas the dialysate materials are eluted from the internal volume.

**Discussion**

The data indicate the presence in hemodialysate of small peptides or conjugated amino acids in addition to the expected free amino acids. It has not been possible to define the upper limit of molecular weight of these materials. Performance on Sephadex gel columns and dialysis across acetylated membranes suggest molecular weights considerably below 4000. These studies do not, however, exclude the possibility that larger, and possibly biologically important peptides are present but not detectable by these technics.

Of additional interest in one patient was the decrease in removal of some amino acids and unknown nitrogenous materials from the first to the eighth hemodialysis. This patient, who is reported elsewhere, suffered from progressive malnutrition, and it is conceivable that removal of essential nutriments by dialysis contributed to this condition.

The clinical significance of these findings is still uncertain. It has now been well demonstrated that life may be maintained by repeated dialysis therapy in the absence of significant renal function, and it has seemed quite reasonable to assume that toxic materials are removed by passage across dialysis membranes in the process. The application of the technics described herein for additional studies, including a systematic survey for peptides with biological activity, seems promising.

**Summary**

Correlation of clinical and chemical changes in patients with uremia has not been possible for substances other than urea and electrolytes. Since clinical improvement is frequently seen following hemodialysis, examination of the dialysate for substances of possible importance seemed warranted. With use of the Kiil artificial kidney, small volumes of dialysate have been obtained and found suitable for analytical studies. Amino acid analyses performed after salt removal revealed a preponderance of acidic amino acids and 35 to 38 unknown ninhydrin reactive peaks. Following acid hydrolysis, the unknown peaks decreased in height and known amino acids increased, indicating a degradation of peptides or conjugated amino acids. Elution from the internal volume of Sephadex G-25 columns suggested molecular weights of less than 4000. Confirmatory evidence of low molecular weights was obtained in vitro by estimation of ninhydrin and Folin-Lowry phenol reactive materials remaining in the dialysate compared to glucose and urea. These results suggested that besides free amino acids, the nitrogenous compounds in the dialysate include conjugated amino acids or very small peptides. Of additional interest in one patient was the decrease in removal of some amino acids and other nitrogenous materials.

![Graph](image)

**Figure 5**

Dialysis of protein substances compared to glucose and urea.

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from the first to the eighth clinical dialysis, suggesting a relationship to progressive malnutrition observed in this case.

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References


William Heberden 1710-1801

William Heberden, the elder (1710-1801) B.A., St. John's College, Cambridge, 1728; senior fellow, 1749; M.D. 1739, F.R.C.P., 1746; Goulstonian (1749) and Croomian Lecturer (1760); Harveian Orator (1750) and censor, F.R.S. 1749; practiced in London from 1748; first described angina pectoris; attended Johnson, Cowper and Warburton. His works (edited in Germany by Soemmering) include, "Commentarii de Morborum Historiâ et Curatione," and contributions to Transactions of the College of Physicians and Royal Society.

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