Pakistan J. Pharm. Sci.1(2): 103-111, 1988.

PEPTIDIC URAEMIC TOXINS

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ABSTRACT

A systematic investigation concerning uraemic toxicology has been made using modern analytical methods. The interest is especially focused on substances which are present in uraemic subjects in high concentrations, the so called "uraemic toxins". The samples from biological fluids have been screened with special regards to the presence of peptides and low molecular proteins these peptidic uraemic toxins are thought to be responsible for certain physiological disturbances when present in uraemic subjects.

Introduction

The search for uraemic toxins is presently selective. It is mainly confined to the assessment of the toxicity, which is attributed to the uraemic solutes. The nonspecific nature and the complexity of uraemic syndrome have made this study crucial.

The significance of the molecular weight of uraemic solutes has increased importance since the theoretical derivation of the "Square-meter-hour" hypothesis by (Babb, et al. 1971) and its subsequent modification to middle molecule hypothesis (Babb, et al. 1972) and trade of hypothesis (Bricker, N.S. 1972). The square-meter-hour hypothesis relates the efficiency of dialysis concerning number of hours of dialysis of middle molecules per week and the active membrane surface area (McGonigle, et al. 1983, Millora, et al 1972).

One group of compound often categorized as uraemic toxins are the so called middle molecules which have the molecular weight range of 350 to 3000 daltons, which are described under middle molecule hypothesis. Various reports (Man, et al. 1974, Funck-Brentano, et al 1976, Zimmerman, et al 1980, Cristol, et al 1938, Salisbury, et al 1957, Bock and Gerok 1961, George 1961, First, et al 1972, Garrote, et al 1984, Menyhart and Graf 1977) are in support of "trade of hypothesis", in which uraemic toxicity is attributed to polypeptides and proteins.

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Materials and Methods

Thirteen samples of uraemic patients and nine samples of non-uraemic patients have been analysed. Various techniques have been used to isolate and characterize the samples. The techniques used in this study are permeation chromatography, Amino Acid Analysis, Ion-exchange-Chromatography, and Peptidic Analysis.

Permeation Chromatography:

A new separation technique was developed applying the principles of high permeation chromatography. This method, designated high speed gel permeation (HSGP), permits to achieve better resolution than is possible with standard techniques of gel filteration. A high degree of sensitivity is also achieved, that has permitted the development and use of "micro columns of sephadex G-I5 and micro fluid sample volumes". The column is coupled to a dual channel ultraviolet absorptiometer (LKB, Uvicord III). The absorption was measured at 206 nm using Unicam AR-25 linear recorder. Using phosphate buffer of pH 7.6 molarity 0.1 M, the sample fractions of I ml each were collected using LKB Ultra-rac 7000 fraction collector. The column was calibrated with standards of known molecular weight.

Amino Acid Analysis:

The fractions obtained from high speed gel permeation (HSGP) (0.5 ml each) were hydrolysed under vacuum with 6N HC1 (0.5 ml) at 110°C for 24 hours. The solutions were then evaporated to dryness in a vacuum desicator over the sodium hydroxide pellets. the amino acid analysis of hydrolyzed samples was then carried out using automated amino acid analyser.

Jon-Exchange-Chromatography (1EC):

In order to achieve better separation, the technique of gradient elution on sephadex DEAE-25 micro-columns was adopted. Ion exchange chromatography of different fractions obtained from HSGP separation mostly of peak 6 was carried out. By using phosphate buffer, the gel permeated fraction of peak 6 was eluted from the column at a flow rate of 5 ml per hour with phosphate buffer (0.1 M, pH 8 to 13) using a linear gradient of 0 to I M sodium chloride.

Peptidic Analysis:

For peptidic analysis fraction 6d which was obtained from ion-exchange-chromatography was analysed for its amino acids. A similar procedure was used as has been performed for amino acid analysis prior to ion exchange chromatography.

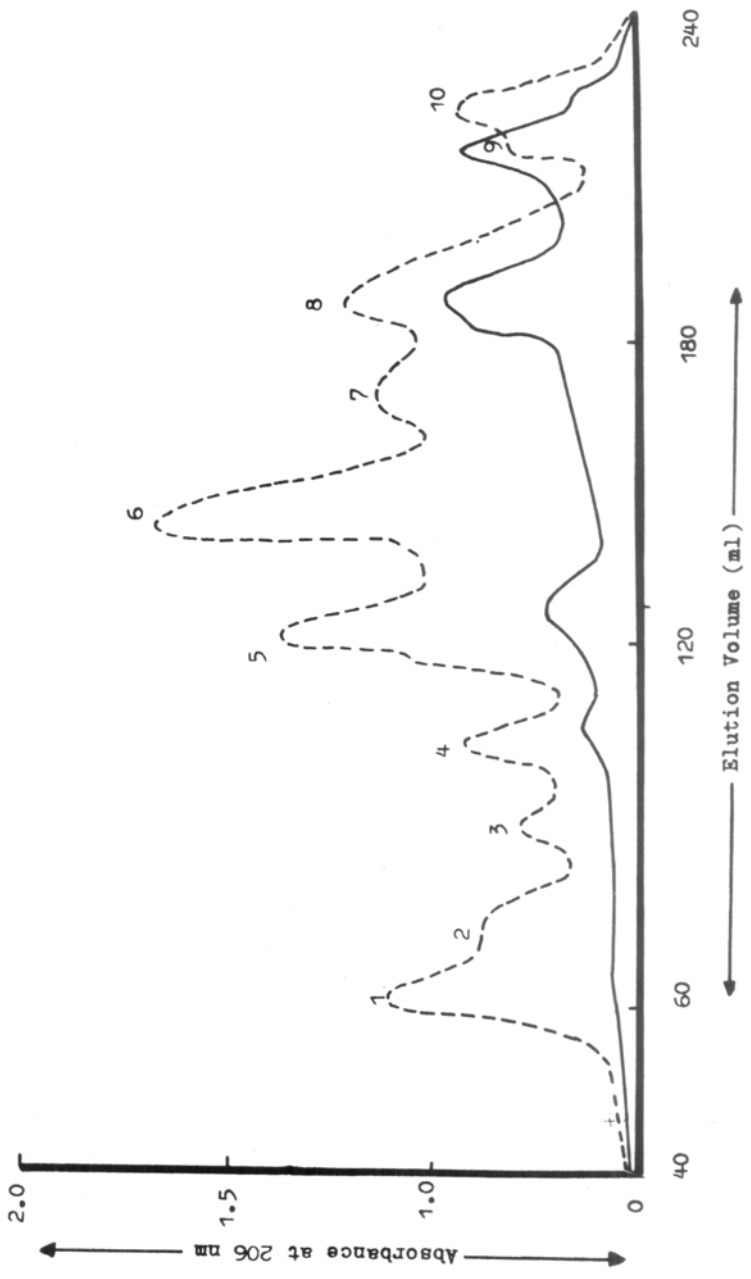

Fig. 1: Separation of Normal plasma (-) and Uraemic plasma (---) on Sephadex G-15.

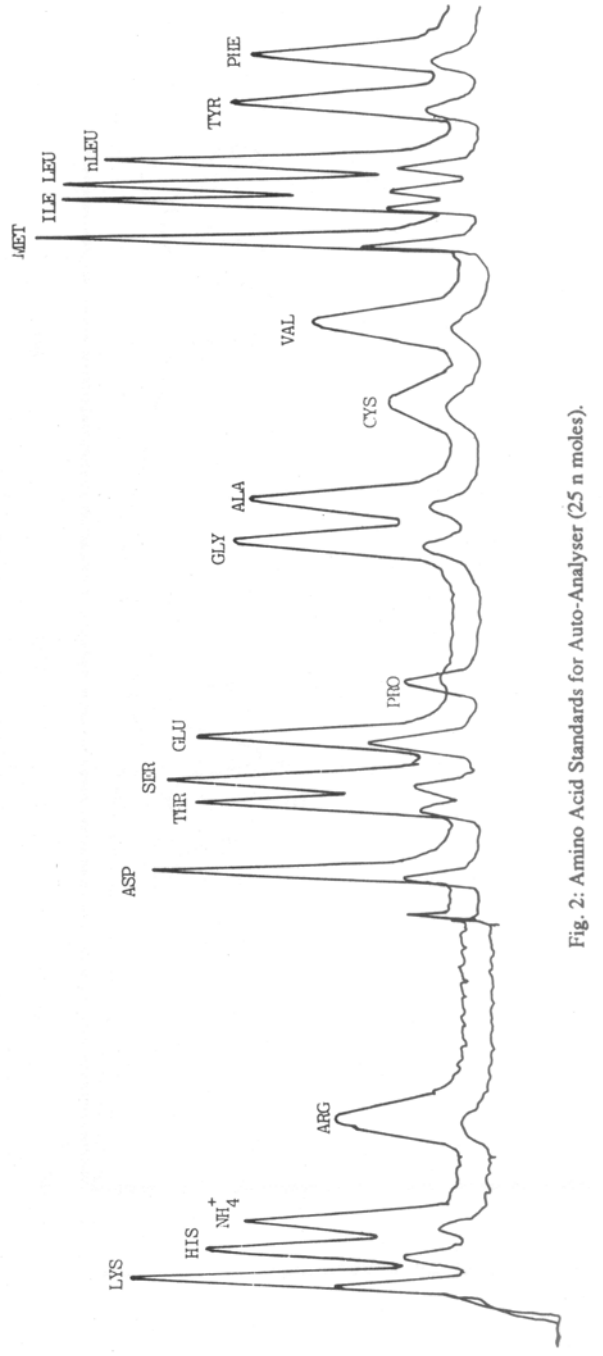

Fig. 2: Amino Acid Standards for Auto-Analyser (25 n moles).

Results and Discussion

Using permeation chromatography method various uraemic body fluids have been separated into 10 compounds UV-absorbing peak, based primarily upon differences in molecular weight and configuration (Figure-1).

Distinct and readily recognisable difference in these peaks have been detected when plasma and urine from patients with uraemic am compared with normal plasma and urine. Significant abnormalities have been identified in uraemia in a peak representative of dialyzable solutes of "middle molecular weight", which appear to have a relationship to the severity of the disease. This peak 6 (Figure-I) has corresponded to the molecular weight of about 1250 daltons. Since it occupies a place between the known standards of molecular weight 1200 and 1350 that is of angiotensin and vitamin B_{12} (Zimmerman, et al 1980) which conclude that the molecular weight of the solute in peak 6 approximates from 1150 to 1400.

The amino acid analysis of peak 6 before and after acid hydrolysis have shown the presence of plypeptides (Figure 2, 3).

Figure-1 illustrates schematically the UV peaks detected at 206 nm from normal and uraemic serum. As mentioned before peak 6 in the middle molecular range is prominent in serum from uraemic individuals. The estimated quantitative magnitude of peak 6 from uraemic controls is presented in the lower part of figure-1.

By ion-exchange-chromatography method, peak 6 has been further separated into four different subpeaks. Typical gradient elution chromatograms from uraemic and normal scrum are illustrated in figure-4. These peaks, which were labelled 6a, b, c, d, are found consistently in severe uraemia. Only three of the subpeaks were detected in normal serum although in much lower quantities than in uraemia. One of the subpeaks, especially 6 d was very prominent in uraemia.

Peptidic analysis of subpeaks 6d showed 8 different amino acids in various concentrations which are lysine, histidine, aspartic acid, glutamic acid, glycine, methionine, isoleucine and leucine (Table-1, Figure-3).

The molar ratio of these amino acid (table 1) gave approximate molecular weight of 1268 (calculated from the molecular weight of Amino Acid, subtracting water molecules), which is an other evidence of peptidic middle molecule compound being of molecular weight of about 1250.

The analytical system (HSGP + IEC Sephadex-DEAE gradient elution) proved to give stable and reproducible values. The quantification is performed by integrating the area of individual peaks.

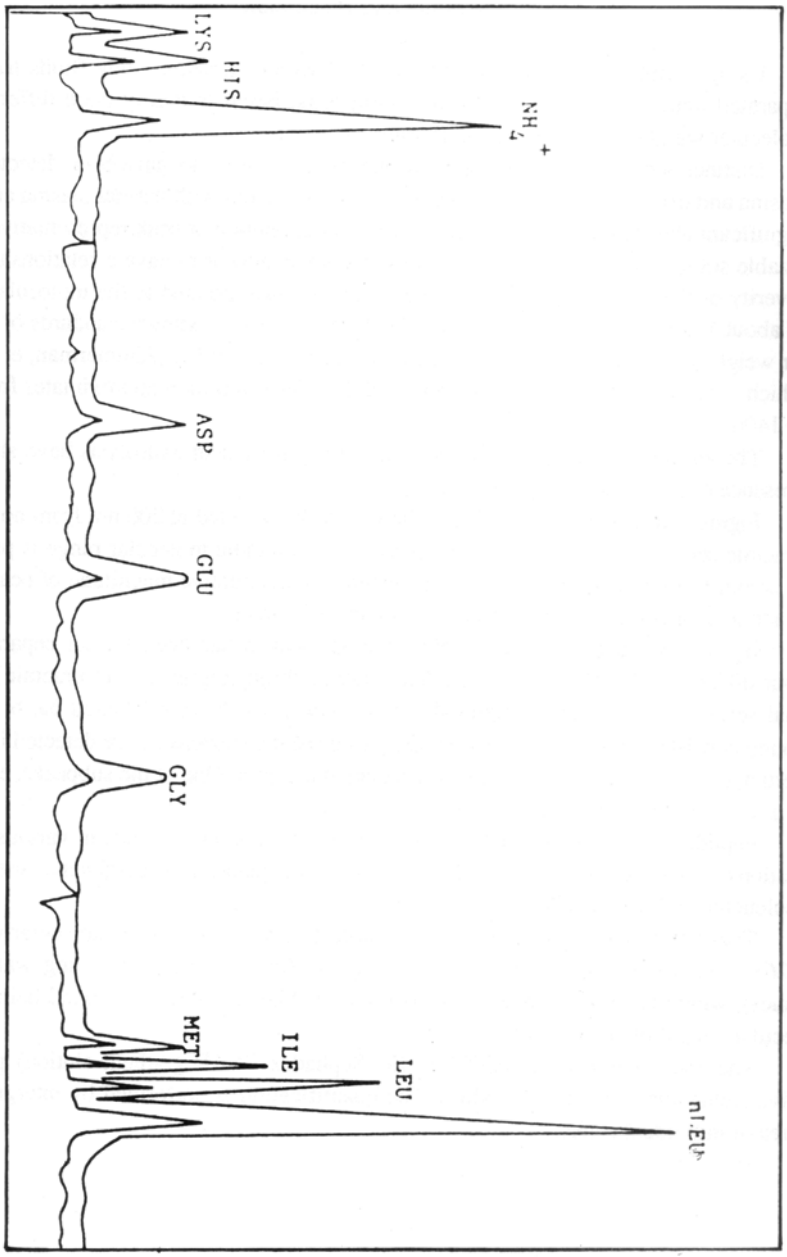

Fig. 3: Amino Acid Analysis of fraction 6d after Acid hydrolysis.

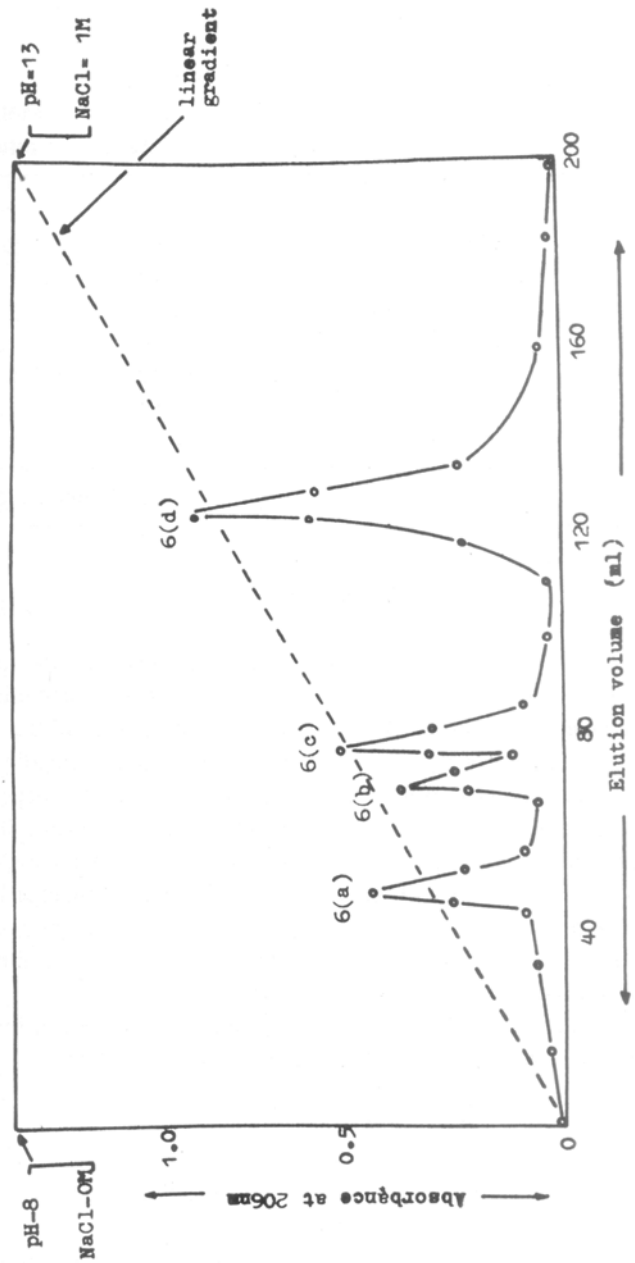

Fig. 4: Ion-Exchange Chromatography of fraction 6 on Sephadex DEAE-A-25.

Table 1: Concentrations of Amino Acid present in hydrolysed fraction 6(d).

Amino Acid	Amount (n moles*)	Molar ratio
LYS	3.68	1
HIS	6.93	2
ASP	3.72	1
GLU	7.05	2
GLY	3.09	1
MET	3.37	1
ILE	3.49	1
LEU	9.57	3
*per sample volume (20 μl)	12 (Total Amino Acids)	

Conclusions

Subsequent studies would be directed at attempting to correlate the absolute and relative magnitude of the sub-peaks of fractions 6 to uraemic symptology and clinical conditions. Quantitative analysis of these peaks could be used in evaluation of the influence of dialyser designs, membranes for the removal of naturally occuring "middle molecules" hypothesis for uraemic toxicity is still controversial, since it is largely dependent upon indirect measures for assessing its validity. In spite of hypothetical nature it has stimulated development of new dialysis membranes more permeable to middle molecules (Milutinovic et al 1974, Babb, et al 1975, Taraba, et al 1981, Buoncristiani, et al 1983, Oven, et al 1983, Itaratson, et al 1983, Shaldon 1966), and other modification of the dialysis operational procedures. This has proved that these molecules are of great importance for assessing the in-vivo characteristics of different dialysis system.

By comparing these results with the results from toxicity studies of different peptide fractions, the importance of middle molecules in uraemia may be defined.

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