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Note

A relatively non-chemical approach to the isolation of peptide materials from plasma

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When peptide materials are to be isolated from body fluids, and used for *in vivo* studies in animals it is essential that the method chosen does not introduce chemical impurities, and is effective in removing amino acids and possibly drugs. This means that many of the methods[1,2] that are now available for separating peptides from amino acids are likely to be unsuitable, because of the chemical reagents used in these procedures, which could well render the peptides unsuitable for further studies. The essentially non-chemical approach described by Yanari *et al.*[3], in which peptides are separated from amino acids by eluting them from DEAE-cellulose with distilled water and carbon dioxide solution, is the type of method that might be considered.

In our own studies which are concerned with isolating peptides from pathological fluids, we have also separated peptides from amino acids, and drugs on a Sephadex C-25 column with distilled water. This technique when applied to a plasma ultrafiltrate, does enable plasma peptide materials to be rapidly isolated, which are relatively free of amino acids and drugs.

EXPERIMENTAL

Materials

Materials used were: Sephadex C-25, bradykinin triacetate, L-carnosine, glutathione, hexaglycine, lysine-vasopressin, oxytocin, glycyl l-alanine, paracetamol, amitriptyline, amphetamine, Valium, Nardil, Stelazine, barbituric acid.

Apparatus

Diaflo ultrafiltration Cell (Model 52) with a UM10 membrane was purchased from Amicon (Lexington, Mass., U.S.A.). Chromatographic column K9/60 and membrane were purchased from Pharmacia (Uppsala, Sweden).

Reagents

Indanetrione hydrate (ninhydrin) was 0.25% (w/v) in acetone (dissolved first in 1 ml acetic acid). All reagents were of AnalaR grade.

Method

50 ml of blood were collected by venepuncture, and the heparinised blood centrifuged. To the plasma was added ethanol (1:4) with stirring, and the solution was

pipetted into an ultrafiltration cell fitted with a UM10 membrane (retentivity, MW 10,000), and ultrafiltrated under nitrogen (25 p.s.i.).

The ultrafiltrate was evaporated down to a small volume (1 ml) at 37°, under reduced pressure, and the solution pipetted onto a Sephadex C-25 column (0.9 × 60 cm). This column was prepared by adding Sephadex C-25 (Na^+) to distilled water, and the suspension pipetted into the column, until a bed of 55 cm had settled. The ultrafiltrate was eluted from the column with distilled water (60 ml), and the eluates collected in fractions of 1 ml per 5 min. The column was finally eluted with 0.5 *M* sodium chloride (12 ml) solution. To each fraction was added the ninhydrin reagent (1.5 ml), and the solutions heated at 100° for 5 min, and then assayed on a Spectrophometer SP1800 at 570 nm. A paper chromatogram was prepared of the fractions directly from the column, before and after hydrolysis with 5 *N* hydrochloric acid for 24 h. The chromatogram was developed in the solvent system *n*-butanol–acetic acid–water (4:1:1) for 16 h, and then sprayed with ninhydrin solution.

Standard peptides (5 mg) amino acids (100 μg) and drugs (5 mg) were eluted separately from Sephadex C-25 with distilled water, in a similar manner to the ultrafiltrate. The fractions containing the peptides were detected by the method of Lowry *et al.*[4], and by the acid hydrolysis of the eluates followed by paper chromatography. The drugs were detected by thin-layer chromatography in which the silica gel plates were developed in the same solvent system as used for the amino acids, and then sprayed with potassium permanganate solution, and oversprayed with 0.5 *N* sulphuric acid; the plates were then heated at 80° for 5 min. Standard peptides and drugs were also added to blood specimens for detection by the above procedure.

RESULTS AND DISCUSSION

The fractions in which standard peptides, amino acids and drugs were eluted from Sephadex C-25 with distilled water, are summarized in Table I. These results show that there is a tendency for peptides to be eluted before either the amino acids or drugs with the exception of barbituric acid. It would therefore be expected that the same order of elution would apply to these constituents in a plasma ultrafiltrate. This was confirmed by the detection of a large number of fresh amino acid spots on the chromatogram in fractions 5–15 after acid hydrolysis, and by the location of the standard peptides in the fractions. The bulk of the plasma amino acids appeared on the chromatogram to be confined to fractions19–24, this was also confirmed by the amino acid elution profile curve (Fig. 1). The drugs were located in the fractions 23–26. The peptides in the ultrafiltrate appeared to be mainly confined to fractions 5–15, and a trace of peptide material was retained on the column.

Investigations are continuing into identifying the peptides in the material isolated from the bulked fractions 5–16, although it is highly unlikely that the material would contain amounts of physiologically active peptides, detectable by paper chromatography, because of their very low plasma levels. However since some peptides are at a higher concentration in the urine than in plasma it seemed worthwhile to apply the method to urine (20 ml), without ultrafiltration. The results obtained were similar to those of the plasma with a considerable quantity of peptide material being eluted before the amino acids. Thus the method enables peptide materials to be rapidly isolated from body fluids.

TABLE I

FRACTIONS IN WHICH PEPTIDES, AMINO ACIDS AND DRUGS WERE ELUTED FROM A SEPHADEX C-25 COLUMN

Compound	*Fraction no.*
Peptides	
Bradykinin	5–6
Vasopressin	8–9
Oxytocin	10–11
Glutathione	11–12
Angiotensin	13–14
Hexaglycine	14–15
Glycylalanine	17–18
Carnosine	21–22
Amino acids	
Alanine	8–9
Cystine	14–15
Cysteic acid	16–17
Aspartic acid	17–18
Methionine	17–19
Proline	19–21
Threonine	21–23
Tyrosine	21–23
Tryptophan	22–23
Drugs	
Barbituric acid	13–15
Acetylsalicylic acid	14–16
Paracetamol	24–25
Valium	24–26
Amitriptyline	24–26
Amphetamine	25–26
Nardil	26–27
Largactil	25–26
Stelazine	25–26

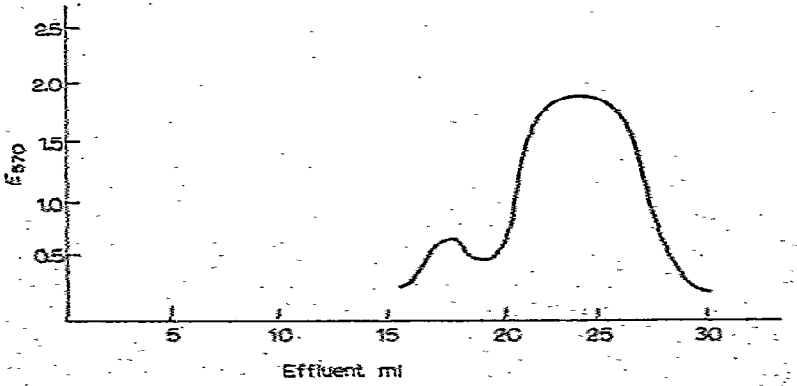

Fig. 1. Chromatography of plasma ultrafiltrate from a Sephadex C-25 column with distilled water. Ninhydrin colour developed only in fractions under the curve.

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