THE DESALTING OF URINES PRIOR TO AMINO ACID CHROMATOGRAPHY

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Introduction

THE need for a simple and inexpensive method for identifying urinary amino acids has been fulfilled to date by the use of paper chromatography. Before the urine can be chromatographed for amino acids, inorganic ions and large neutral molecules such as proteins must be removed. If left in solution they will seriously impair the spot separation. Several methods of effecting deionisation have been described and fall into three main categories:

Organic solvent extraction

Vergheze and Ramakrishnan (1957) described a method in which urine is evaporated to dryness and the amino acids extracted from the residue with a mixture of phenol and butanol. This becomes a lengthy procedure since dessication over-night is involved.

Electrolytic deionisation and dialysis

These two methods, described respectively by Consden et al (1947) and Wood (1956) are very efficient, but require equipment not readily available. They are expensive, taking into account the fact that since the Army is a relatively healthy community, identification of urinary amino acids is a comparatively uncommon request.

Ion exchange resins

Smith (1958) indicated how a cation exchange resin could be used to adsorb inorganic ions and amino acids from urine followed by selective elution, concentration and chromatography of the amino acids.

Thus a method for deionising urines based on this third category has been found suitable and is subsequently described.

Materials and Methods

This method depends on the fact that amino acids in solution are capable of acting as weak bases or weak acids.

They contain both basic ($-\text{NH}_2-$) and acidic ($-\text{COOH}-$) radicles.

Thus in acid solution they act as weak bases and are adsorbed onto the resin in a like manner to the inorganic cations.

Slightly acid urine is passed through the cation exchange resin "ZEOKARB 225". Proteins and anions are washed out of the column and the amino acids eluted with ammonia, leaving the inorganic cations absorbed on the resin. "ZEOKARB 225" is a

stable sulphonated polystyrene resin, operational over a wide pH range and is used for this purpose at a mesh size of 52–100. It is supplied by British Drug Houses in the sodium form so conversion to the hydrogen or acid form is necessary.

This is done by soaking a quantity of resin in twice its volume of warm 10 per cent v/v hydrochloric acid. The resin settles out and the hydrochloric acid plus any remaining floating particles is decanted to waste. This is repeated and followed by three similar washings in double distilled water. The resin may be stored at this stage in an airtight container, preferably polythene, as an aqueous slurry.

A column of 25 ml. of resin is set up in a burette after first plugging the bottom with a small wad of glass wool. Any bubbles trapped in the column may be disturbed by back flushing with water from a high reservoir connected to the nozzle. The water standing on the column is allowed to run away and the surface of the resin plugged with another glass wool plug. Cotton wool is unsatisfactory. At no time during or after use is the resin allowed to dry.

The deionisation of the urine is carried out as follows:—

5 ml. of slightly acid filtered urine (pH about 6.0) are pipetted onto the resin and allowed to percolate into the column. This is followed by 30–35 ml. of double distilled water which is allowed to run to waste. 30 ml. of 3N ammonia are poured into the column. As the ammonia travels downwards the resin will be seen to change colour from a light to a dark amber. When the ammonia approaches the nozzle the drops are allowed to fall onto red litmus paper in a 10 ml. measuring cylinder. On the litmus paper changing colour 1 ml. of the eluate is measured and discarded. The remaining eluate is collected. The ammonia remaining in the column is displaced with distilled water and collected. The total eluate should measure about 25 ml. This is evaporated down to 1 ml. on a boiling water bath or over a low bunsen flame, then cooled. This drives off the ammonia and concentrates the original sample content five times. The eluate can then be spotted onto the chromatography paper.

Results

Recovery experiments were carried out using solutions of amino acids in electrolyte solutions, in normal and known abnormal samples of urine. In between samples the resin column was recharged with 5 per cent v/v hydrochloric acid and washed with double distilled water. The subsequent chromatography was carried out “two way”, using Smith's n butanol/acetic/water and phenol/ammonia solvents with ninhydrin or isatin in pyridine and acetone as locating reagents.

It was found that all amino acids likely to occur in urine, normal and abnormal, were recovered from the column with the one exception of taurine. This was either completely absent or gave a very faint spot, depending on its original concentration.

The concentration of ammonia for elution was found to be critical. 2N ammonia gave excellent, discrete spots but failed to elute arginine. 4N ammonia gave the best recovery of arginine but the diffuse spots suggested that inorganic cations were also being eluted. 3N ammonia gave a reasonable recovery of arginine with good spots and is used routinely.

This method has been used routinely for amino acid chromatography of urines at the Royal Army Medical College for the past four years and has proved satisfactory.

Summary

Deionising methods for urine amino acid chromatography are briefly reviewed with the purpose of elucidating a satisfactory method using simple equipment suitable for routine use.

A method suggested by Smith (1958) using an ion exchange resin has been investigated, modified and found satisfactory.

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President of the Royal College of Physicians of London

Professor Max Leonard Rosenheim has been elected to succeed Sir Charles Dodds. Commencing a military career at an early age in Shrewsbury School O.T.C., Professor Rosenheim was not deterred from a brilliant career at Cambridge and at University College Hospital. The War of 1939-45 saw him involved as Officer in Charge Medical Divisions of General Hospitals at home and in the Middle East. Before the close of hostilities he applied his great knowledge and experience as a consultant in India in the rank of Brigadier. A much valued and readily given opinion at Millbank and a much acclaimed guest lecturer at R.A.M. College, the Journal congratulates him on attaining high office.

Chair of Medicine—Post Graduate Medical School, Hammersmith

Dr. Christopher Charles Booth is to succeed Professor Sir John McMichael.

As a rating in the Royal Navy in the 1939-45 War he was commissioned and later obtained a watch keeping certificate before turning to medicine at St. Andrews.

As lecturer in medicine at the Post Graduate Medical School he has given great support to Army Medical researches in sprue.