IV. A WET ASHING METHOD FOR THE ESTIMATION OF SODIUM IN FAECES

By DAVID STIVEN

*From the Department of Physiology, University College, Dundee,
University of St Andrews*

(Received 21 November 1937)

IN the course of investigations in this laboratory on the excretion of Na in tuberculous patients, there arose the problem of dealing with many specimens of faeces. The dry ashing method recommended by Butler & Tuthill [1931] has its disadvantages and to avoid these the following method was devised; it has been found to be relatively rapid and to give accurate results. The essential differences in procedure are that wet ashing is employed and that baryta and alcohol are added to the faecal suspension to remove soluble phosphate and organic matter. Since all the Na (and K) salts are soluble in dilute alcohol, the use of a fraction of the alcoholic filtrate from the faecal suspension is considered to be justified, on the assumption of a uniform distribution of the Na between the precipitated faeces and the supernatant fluid.

Procedure

The faeces are transferred from the containers to a 2 l. conical flask fitted with a rubber stopper through which pass an ordinary funnel with a cotton wool plug to act as a dust filter and a bent tube connected to an aspirator. The water escaping from the aspirator is led by tubing down to the bottom of the trap of the sink. During the whole process of heating the faeces a current of air is sucked through the flask to prevent the escape of any odour into the room. In transferring the faeces to the flask and washing out the containers, not more than 300 ml. water should be used. It is a disadvantage to use any more water than is just required to give a suspension that will not "catch" on the bottom of the flask during boiling. If too much water is used, the concentration of alcohol in the diluted faecal suspension will be too low to precipitate the organic matter and the result will be slow filtration and a turbid filtrate. The mixture of faeces and water is now brought to the boil, the flask being rotated at intervals to aid the maceration of the faeces. Only gentle boiling must be allowed on account of frothing. A few minutes' boiling usually suffices to give a uniform suspension and at this stage about 5 g. $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ dissolved in hot water are poured into the mass and the whole is well shaken. After a few minutes' boiling the reaction to phenolphthalein is tested and if it is not alkaline more baryta should be added. The flask is now cooled and the suspension transferred quantitatively to a 1 l. flask; the 2 l. flask is rinsed out and the volume is made up to the mark with rectified spirit. If the stool is small and maceration in about 150 ml. water has been possible, it can be made up to 500 ml. with alcohol. This alcoholic suspension is allowed to stand overnight and after adjusting the volume to the mark it is filtered through an ordinary filter paper. About 800 ml. of filtrate can as a rule be obtained, but centrifuging gives a larger volume.

So low is the Na content of faeces as a rule, that it will be found necessary to use as much of this filtrate as can be obtained. The whole stool may contain

only 15 mg. Na. Since 2–3 mg. Na are a convenient amount to use for precipitation as the triple salt from 20 ml. of the solution of uranyl zinc acetate, and this amount of Na must be contained in not more than 2 ml., a considerable volume of the alcoholic filtrate is necessary for each estimation. The method that has been found most practicable is to ash the residue from 700 to 800 ml. of the filtrate, make this up to about 15–20 ml. and use 2 ml. of this for precipitation of the Na. If the Na content is too high, 1 ml. can be used with 20 ml. of the solution of the double salt.

To carry out the ashing, transfer 700–800 ml. of the alcoholic filtrate back to the 2 l. flask and boil off the alcohol with the air current running. Remove all the alcohol and reduce the volume to about 100 ml. At this stage, with the solution still boiling, add drop by drop 10 ml. 10*N* H₂SO₄ to precipitate the excess barium as sulphate. If frothing should start it can be checked by adding a few drops of conc. HNO₃. When the solution begins to blacken, it is transferred quantitatively to a 300 ml. Kjeldahl flask and the wet ashing is completed in the usual manner by well-timed additions of conc. HNO₃. To the pale yellow solution are now added about 5 ml. water and the mixture is again gently boiled to expel nitrous fumes and to aid the reprecipitation of the barium sulphate that tends to redissolve in the concentrated acid. The contents are now transferred by pipette to a stoppered graduated 25 ml. cylinder. The Kjeldahl flask is rinsed out with 2 ml. lots of water which are transferred each time to the stoppered cylinder by the same pipette. This rinsing is continued until 15–20 ml. in all have been collected in the cylinder.

The volume is noted, the contents are shaken up thoroughly and the solution is filtered through a fine paper to remove the barium sulphate. The Na in 2 ml. lots of this filtrate is estimated by the method described by Butler & Tuthill [1931] for urines of ordinary Na content.

The Na in the sample of faeces used can be calculated thus:

$$\text{Total Na in stool} = \frac{\text{Wt. triple salt}}{67} \times \frac{\text{Vol. to which ashed material is made up}}{2} \times \frac{1000}{\text{Vol. of alcoholic filtrate used}}$$

An estimation carried out on the reagents used in this laboratory in the quantities described above showed that about 3 mg. Na were added from this source. This amount must be deducted from the result to get the true Na content of the faeces.

The recovery of added Na was investigated by taking a 2 days' stool, macerating it and measuring out two equal volumes of the suspension. To one was added an accurately weighed amount of pure fused NaCl. Both samples were treated as described above. Of 48.4 mg. Na added in one experiment, 47.6 were accounted for in the estimation; in a second experiment the recovery was 54.2 mg. from a sample to which had been added 53.5 mg.

The daily excretion of Na in the faeces of normal individuals has been found to range from 16 to 75 mg.

SUMMARY

The Na content of faeces has been estimated by removal of free phosphate as barium phosphate, precipitation of the organic matter by alcohol and wet ashing of the alcoholic filtrate.

REFERENCE

Butler & Tuthill (1931). *J. biol. Chem.* **93**, 171.