TECHNICAL METHODS

A RAPID METHOD FOR DETERMINING FAECAL FAT

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The percentage of fat (split and unsplit) in faeces gives a useful indication of the efficiency of digestion and adsorption of fat by the intestinal tract. It is a definite help in the diagnosis of pancreatic disease, sprue, and other conditions causing steatorrhoea.

The methods recommended by standard textbooks for the determination of faecal fat are time-consuming, laborious, and extremely unpleasant because they foul the air in the laboratory, and usually three days are required to complete the test. The most odious procedure in the standard method is the drying of the faeces in an open dish to a consistency which permits the sample to be powdered.

The new method requires barely two to three hours from beginning to end. The faeces are dehydrated in a closed system and not even a fume cupboard is required to keep the laboratory atmosphere clean.

In common with the standard methods in this method, low molecular fatty acids, derived mainly from the decomposition of cellulose, are volatilized, ensuring more accurate results for clinical purposes.

The method is equally suitable for the determination of fat in food for the quantitative study of fat balances, when the proportion of fat intake to fat excretion is to be determined. Duplicates of the patient's food are passed through a mincer or another suitable disintegrator and after very thorough mixing the same method is applied.

Method

The wet faeces are treated with an exact amount of concentrated hydrochloric acid to liberate free fatty acids from soaps. They are then subjected to a distillation process using xylene to carry the water to a calibrated part of the apparatus, where it can be accurately measured. The fats and fatty acids go quantitatively into the xylene solution. After purification by means of petrol ether, the fatty residue is weighed and the amount of free fatty acid determined in the usual way by titration with alcoholic standard alkali.

Reagents.—The following are required: concentrated hydrochloric acid A.R.; xylene (any pure or commercial grade, boiling between 130–150°C., is suitable); petrol ether (boiling range 40–60°C.); N/10 sodium or potassium hydroxide solution.

Apparatus.—The apparatus used for the determination of water is shown in the illustration. It can easily be made from part of a burette. The reflux condenser may be connected to the apparatus by a standard ground joint, but a good cork is much preferable for connecting the flask since ground joints at this place show a tendency to stick badly.

The little funnel, which prevents droplets of water floating back with the returning xylene, rests loosely on three or four indentations put into the wider part of the apparatus.

The distance of the flask from the measuring part of the apparatus should not be less than 6 to 8 in. to avoid excessive heat radiating on this part. The tube connecting the apparatus to the flask is wide enough to give ample room to the glass rod, protruding from the flask during the distillation.

Procedure.—The specimen is thoroughly mixed until it is homogeneous, when 15–18 g. is transferred through a wide-neck funnel into a 500 ml. wide-neck flask avoiding smearing any of the specimen on to the sides of the flask. A glass rod protruding about 1 1/2 in. above the neck of the flask is tared with the flask. Exactly 3 ml. of concentrated hydrochloric acid is added and well mixed with the faeces by means of the glass rod, which is left in the flask during the distillation. About 200 ml. of xylene is added and the flask is connected to the apparatus. The distillation is carried out at a moderate speed (three to six drops per second) until no further water separates in the graduated part of the apparatus, usually requiring 25 to 45 minutes. With care, faeces can be heated with the xylene directly on a wire gauze. With food, however, local overheating easily causes scorching and a paraffin oil bath, heated to about 150–170°C., should be used.

The amount of water distilled is noted, and 2.7 ml. subtracted from this amount, allowing for the 3 ml. of concentrated hydrochloric acid added. The amount of dry faeces can now be calculated.
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The petrol-ether extracts are transferred by a Pasteur pipette to a centrifuge tube and the precipitate is spun down. If a stoppered, calibrated centrifuge tube is used, an aliquot part of the petrol-ether solution is used. Otherwise the precipitate is washed with two more portions of petrol ether (2-3 ml.), centrifuging each time. Filtration through filter paper is not advisable due to the difficulty of washing down fat which, by capillary action, collects at the highest part of the filter.

The petrol-ether solution is evaporated on a water bath in a tared flask of about 25 ml. capacity. Last traces of solvent and volatile fatty acids are removed by blowing a current of air (or any other gas available in compressed form at the laboratory) into the flask while it is still hot, spreading the fatty residue to a thin layer by rotating the flask, or keeping the flask under vacuum on a boiling water bath.

When constant weight is obtained, the free fatty acid may be determined by dissolving the residue in warm benzene and titrating the acids in the usual way with alcoholic caustic soda or potash to the end-point with phenolphthalein.

To comply with the standard textbooks 1 ml. of w/10 standard caustic solution is calculated to correspond to 0.0268 g. of fatty acid. The calculation is as follows:

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\% \text{ fat in dry faeces} = \frac{100 \cdot p \cdot X_1}{F \cdot X_a}
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when \( p \) = weight of fat residue from aliquot part of xylene; \( X_1 \) = weight of total xylene after removal of water; \( X_a \) = weight of xylene aliquot taken for fat determination; \( F \) = weight of dry faeces calculated from weight of wet faeces minus the amount of water distilled after allowing for the hydrochloric acid added (2.7 ml.).

Summary

A method is described for the determination of fat (split and unsplit) in faeces and food. It requires only two or three hours for a complete analysis. The results are quite accurate enough for all clinical purposes. The method was compared with that of Holt, Courtney, and Fales (1919), and the results agreed very well. The results of the new method were slightly lower (0.2–1% on the dry faeces), less inert material being extracted by the petrol ether than the ether-petrol-ether mixture used in the other method after the addition of a considerable amount of alcohol.

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Reference