XI. A RAPID PROCESS FOR THE ESTIMATION OF THE HIGHER FATTY ACIDS AND SOAPS IN FAECES.

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In connection with an investigation on the aetiology of rickets at present being carried out under the Medical Research Committee, the necessity was experienced of a rapid and accurate method for the determination of fat in the faeces. That such a method would prove of value in clinical work is obvious, as it would facilitate the regulation of the diet of patients under treatment. It was therefore with this end in view that the present work was commenced. Various processes for the estimation of the fat content were tried. The results obtained with the staining method were far from accurate and this was rejected.

The acid amyl alcohol separation used for milk did not give a satisfactory result owing to the difficulty of obtaining an average of the faeces when made liquid with water, as sedimentation of the insoluble matter rapidly took place which carried down with it some of the fat.

On further consideration it was thought that the first step in the determination of the fat content should be the use of a suitable solvent. A 2 % alcoholic solution of caustic potash was employed since by this treatment all the fat is converted into the corresponding soap from which the cloud of fatty acid is displaced and determined by the nephelometer [Kober and Graves, 1915].

The only work which I can find directly bearing on the present investigation is by Laws and Bloor [1916] who extract the faeces with acidified alcohol and ether and seem to obtain fairly consistent results, which they claim to be within 5 % of those obtained by older methods.

By employing an acid medium for extraction it is however possible that there may be, on dilution, a recombination of the calcium and magnesium with some of the fatty acids, thus giving a low result.

METHOD.

(1) *Total Fatty Acids, i.e. free and combined as neutral fats and soaps.*

An average sample of the faeces is taken, thoroughly mixed and 1 g. weighed on to a fat-free filter paper, the material being spread out so as to form a thin layer. It is then introduced into a conical flask, 50 cc. of 2 % alcoholic potash solution are added and the flask is connected with a reflux condenser. Boiling is continued for about 15 minutes, by which time all the fat becomes saponified. The solution is cooled and decanted into a 200 cc. flask, made up to the mark, thoroughly mixed and a quantity filtered into a dry beaker. Of the filtrate 2 or 5 cc., depending on the quantity of fat present, are pipetted off and placed in a 100 cc. measure, 5 cc. of hydrochloric acid are added and the whole made up to 50 cc. with distilled water and mixed.

The standard is prepared as nearly as possible at the same time by taking 2 or 4 cc. of a standard fatty acid solution (1 cc. = 0.0005 g. fatty acids) made by saponifying oleic acid¹ 50 %, palmitic acid 25 % and stearic acid 25 % with alcoholic potassium hydroxide, and treating with hydrochloric acid in the same way as the sample. Both volumes are then made the same and the measures are set aside for about five minutes to allow the clouds of suspended fat to develop and the clouds are then compared in the nephelometer. The quantity of fat in the faeces can then be calculated. There is no colour in the sample tube when the cloud is precipitated, which makes the comparison of the fields very easy.

RESULTS.

(1) *Test of the nephelometric process.* To ensure a good average for the test the faeces were dried, hence the high percentage of fatty acids. This was done by slow evaporation with alcohol on the steam-bath, care being taken not to overheat.

A. Sample from a child with rickets.

(1) Nephelometric { reading of standard ... 60 }
 method { ., ., sample (average) ... 68 } ,

$$\therefore \frac{60 \times 0.002}{68} = \text{quantity of fatty acids in amount of sample used}$$

$$= 17.60 \% \text{ fatty acids.}$$

(2) By Soxhlet's extraction = 17.68 % ,, ,,

¹ It was found that oleic acid was necessary to produce the fat cloud. Dilute soap solutions of palmitic and stearic acids when acted on by HCl flocculated and settled as a curd, but on addition of the slightest trace of oleic acid the cloud so formed remained in complete suspension indefinitely.

in this form. The average amount in the samples I have examined is about 0.2 % calculated as fatty acid.

(ii) Calcium and magnesium soaps (insoluble soaps).

There is no direct nephelometric method for the estimation of these insoluble soaps in the faeces. It is necessary to know the ether-soluble fatty acids, as well as the total fatty acids and the water-soluble soaps.

Example :

	Sample A	Sample B
Total fatty acids by nephelometer ¹ ...	18.4 %	20.3 %
Fatty acids by Soxhlet	17.9 %	19.9 %
= Total soaps (by difference)	0.5 %	0.4 %
Water-sol. soaps by nephelometer	0.2 %	0.2 %
= Ca and Mg soaps (by difference) ...	0.3 %	0.2 %

With regard to the time necessary to complete a series of these estimations it was found that from eight to ten samples could be undertaken in a day. Thus the process offers a very great advantage over the tedious method of extraction with ether.

Another point in favour of the method is that all the fatty material is extracted whether combined as neutral fats, fatty acids or soaps and estimated as total fatty acids. This overcomes the possibility of incomplete ether extraction which is a well-known difficulty.

CONCLUSIONS.

By the saponification process and the use of the nephelometer very rapid and accurate determinations of the fatty acid content in faeces can be made. To ensure good results the following points require attention.

(a) The clouds in the sample and standard should be precipitated at the same time.

(b) The same amount of HCl should be added to each.

(c) The same time (about five minutes) should be allowed for the clouds to form.

(d) A standard should be used which gives readings fairly close to those of the sample analysed.

The application of the method to substances other than faeces is being investigated.

¹ I have evidence in work on fat metabolism in rickets, as yet unpublished, to show that there is very little neutral fat present in the faeces. The fatty acids are for the most part free, with a very small quantity combined as soaps (see above).

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