A METHOD FOR THE DETERMINATION OF FAT IN DRIED FECES AND ITS DISTRIBUTION AS SOAP, FREE FATTY ACIDS AND NEUTRAL FAT

AN APPLICATION TO FECES OF THE ROESE-GOTTLIEB METHOD FOR DETER-
MINING FAT IN DRIED AND CONDENSED MILKS *

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The investigation of the fat metabolism of infants and young children has been for some time an important subject of study in this laboratory. In furtherance of it, a large number of stools have recently been collected from the wards of the hospital, from other institutions and from various private patients. Careful data have been obtained regarding the food of the patients from whom stools were collected and so far as possible representative samples of the daily food were procured for analysis. Total fat has been determined in food and feces, and in the latter the distribution of the fat as soap, free fatty acids and neutral fat. In most cases total ash and calcium content of food and feces were also determined. The results of these investigations have been incorporated with many analyses which we had obtained in previous metabolism studies. It is our intention to present in a series of papers these data and to discuss their bearing on various problems of fat metabolism in infants and young children, especially the relation of the amount and the kind of the fat in the food to the quantity and distribution of the fat in the feces and the influence of fat on calcium metabolism.

In this preliminary paper we present the method used in obtaining the fat values. Previous to the autumn of 1915 a modification of the Soxhlet method was used for fat determination in this laboratory. This method has numerous disadvantages. It involves a forty-eight hour extraction with an elaborate equipment, which requires much attention. Moreover, in order to obtain complete extraction of the fat it is necessary to use very small quantities of material with a consequently high percentage error. The need of a shorter and simpler method led us to apply to the study of feces the Roesse-Gottlieb method for the determination of fat in dried and condensed milks,

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as practiced in the Dairy Laboratory, Bureau of Chemistry, Department of Agriculture.¹

As adapted to the determination of total fat and fat distribution in feces, the method which we have employed, is as follows:

METHOD

One-gram samples of thoroughly dried and powdered material are weighed into small evaporating dishes. One half of these samples are used for the determination of total fat and the other half for the determination of the unsaponified fat; that is, the neutral fat and the free fatty acids. To each sample which is to be used for the determination of total fat there is added about 10 c.c. of dilute hydrochloric acid (one part of concentrated hydrochloric acid to three parts of water) and to those which are to be used for the determination of the unsaponified fat there is added the same volume of water; that is, about 10 c.c. The dishes are then placed on a water bath and left there until the contents are thoroughly heated and disintegrated. The homogeneous mixture from each dish is then transferred to a Röhrig tube (see illustration) and washed in, first with sufficient water to bring the contents of the tube exactly to a mark designating half the capacity of the portion below the faucet, then with 95 per cent. alcohol until the tube is filled to the level of the faucet. There is then added 25 c.c. of ethyl ether, free from nonvolatile matter, and the tube is shaken vigorously for a half minute; there is then added 25 c.c. of petroleum ether, boiling below 60 C., and the tube is again shaken for a half minute. The mixture is allowed to stand until the upper liquid is perfectly clear. The layer of ether, containing the extracted fat, is then drawn off by the faucet through a quick acting filter paper into a bottle of known weight. To make the extraction complete the process is repeated with 20 c.c. of both kinds of ether. For extreme accuracy a third extraction is made with 30 c.c. of the mixed ether, which has been recovered from previous determinations. To insure against loss of fat, the tip of the faucet, the funnel and filter paper are washed with about 10 c.c. of mixed ether.

The ether is distilled from the combined extracts by means of an electric stove. The distillate, composed of mixed ethyl and petroleum ether, is condensed and used for further determinations. The fat residue is dried over night on a water bath, then for several hours in an oven at 98 C., desiccated and weighed.

1. Patrick: Roese-Gottlieb method. Circular 66, U. S. Dept. of Agric., Bureau of Chem.

In order to determine the free fatty acids the extract from the nonacidified samples is used. After the fat has been dried and weighed, it is dissolved in benzol, heated and titrated while very hot with tenth-normal alcoholic sodium hydrate. Phenolphthalein is used as an indicator and the titration is carried to the maximum color. The alkali used is standardized by stearic acid, dissolved in hot benzol.

The difference in weight between the extract from the acidified material (the total fat) and that from the nonacidified material (the neutral fat and free fatty acids) is the weight of soap fat. The difference between the weight of the nonacidified extract and the weight of the free fatty acids, computed from titration figures, represents the neutral fat. The following is an example:

	Gm.
A. Weight of extract from acidified sample.....	0.38
B. Weight of extract from nonacidified sample.....	0.24
Difference between A and B = fat as soap.....	0.14
C. Titration of B.....6.0 c.c.	
Equivalent weight of free fatty acid.....	0.17
(1 c.c. tenth-normal NaOH = 0.0284 gm. stearic acid.)	
Difference between B and C = neutral fat.....	0.07

In the Roese-Gottlieb method ammonia is used to facilitate the separation of the fat from the protein. This step is necessarily omitted in the determination of fat in feces on account of the presence of the free fatty acids, which would be saponified by the ammonia.

The advantages of this method for fat determination are evident. The technic is simple, the amount of ether required for each determination is not large, that is, it can be distilled off quickly, and the results can be obtained in from eighteen to twenty-four hours from the weighing of the samples. Moreover, the method gives the distribution as soap fat, free fatty acids and neutral fat.

Considerable confusion exists in regard to the interpretation of reported figures for fat in feces because of the different methods which have been used. Two, which have been much quoted, the Liebermann-Székely² and the Kumagawa-Suto³ methods, give a value for total fat only, stated in terms of fatty acids. This is obtained by saponifying the fat, then splitting it with acid before extracting with alcohol and ether. The extract obtained is purified by reextracting with petroleum ether. The Kumagawa-Suto method gives higher values than most methods, because the extract is never heated in drying higher than 50 C. Thus the lower fatty acids are in part held in the extract. As the lower fatty acids arise mainly from the carbohydrate of the food, results which include them are not to be desired in a study of fat metabolism, especially since their separate determination is very difficult and doubtful.

The Gephart-Czonka⁴ method involves the same principles as those mentioned, but the fatty acid value is obtained by titrating the

2. Liebermann and Székely: Pflüger's Arch. f. d. ges. Physiol., **72**:360, 1898.

3. Kumagawa and Suto: Biochem. Ztschr., **8**:212, 1908.

4. Gephart and Czonka: Jour. Biol. Chem., December, 1914.

petroleum ether extract instead of by weighing. This method requires considerable manipulation and the use of large quantities of ether. As in the methods just described, only total fat, as fatty acids, is obtained. These three methods avoid the slight objection which might be raised in regard to our own procedure, in which the total fat is weighed and reported as a mixture of fatty acids and neutral fat. This is not, however, of any practical importance, since the difference between the weight of the neutral fat and its corresponding fatty acid is so slight that the total fat value is not affected to the extent of 1 per cent. of the usual amounts weighed.

Folin and Wentworth⁵ also obtain their figures in this form, that is, the total fat as a mixture of fatty acids and neutral fat, and they obtain a value for the neutral fat by titrating the fatty acids and subtracting the result from the total fat. They claim that there is no significance in a figure for soap fat as separate from fatty acids, since both in the intestines and during the drying of the feces on the water bath "numerous different factors of no particular bearing on fat metabolism will materially affect the equilibrium between the soap fat and the fatty acids." Our experience, however, in the study of infants' stools of various types has led us to the conclusion that in spite of these objections the soap fat value, as determined, may be of significance in a study of fat metabolism. For this reason we feel strongly the advantage of a method by which it is possible to obtain this value.

In modifying the Roesse-Gottlieb method so as to obtain the fat separation, use has been made of the Folin-Wentworth procedure for titrating the fatty acids. As a matter of convenience sodium hydrate dissolved in 95 per cent. alcohol and standardized by stearic acid is used instead of sodium alcoholate. The solution we use quickly becomes cloudy from contact with the glass container, so that frequent restandardization is necessary.

Various authors believe that the ether extract of acidified material includes substances other than fat, notably coloring matter. Ordinarily the extract which we obtain is clean, homogeneous, mainly crystalline and dissolves clear in any of the fat solvents. Occasionally there is an appearance of sediment in the extract and in some cases, especially in the stools of breast fed infants, the extract shows marked color. In such cases the dried extract is dissolved in mixed ether and again filtered before drying and weighing. If care is taken to prevent drawing off any of the watery portion from the tube with the ether, the need of filtering will be lessened. This is very desirable since slight mechanical loss in the second filtration is scarcely avoidable.

5. Folin and Wentworth: *Jour. Biol. Chem.*, **6**:421, 1910.

The completeness of extraction has been tested with samples of stearic acid, saponified stearic acid and mixtures of these. In every case the recovery was 99 per cent. or more. In order to make sure that the results do not include the acids formed from carbohydrate, weighed samples of stearic acid were dissolved in mixed ether, lactic and acetic acid added and the whole evaporated on a waterbath, dried and weighed. Invariably only the stearic acid remained.

It is not claimed that the method here described gives results with an average error less than 1 per cent. The fat forms so large a proportion of the dried feces that the homogeneity of the sample is of great importance. When the fat amounts to 50 per cent. of the dried weight of the feces, there may be variations of 5 per cent. in duplicate analyses. This extreme error is largely due to the fact that stools with so large a proportion of fat are gummy and impossible to powder.

In some respects our method resembles that described by Saxon.⁶ His method gives the distribution as fatty acids, soap fat and neutral fat and he extracts by shaking. His determination is made on moist fresh feces and he does not use mixed ethers. The use of the Röhrig tubes in the Roese-Gottlieb method greatly facilitates the manipulation.

6. Saxon: *Jour. Biol. Chem.*, **17**:99, 1914.