A RAPID CLINICAL METHOD FOR THE ESTIMATION OF TOTAL FAT IN INFANTS' STOOLS *

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One of the great disadvantages in the study of the digestive disturbances of infancy is our inability to determine quickly the quantity of fat present in an infant's stool. Qualitative tests, while of value, do not suffice, for under normal conditions we may encounter neutral fat, free fatty acids and soaps in an infant's stool. Our requirements will not be fulfilled until we have some approximate method for determining the neutral fats, free fatty acids and the soaps as they exist in the stool when it leaves the body, or, in other words, as they exist in the intestine uninfluenced by external agencies.

In pursuance of this need we began last September to study the various methods now in use for the estimation of fat in stools with the hope that we might find a way of shortening some of the existing methods, or of combining the different principles involved into a new or modified method. The methods we have investigated more in detail are the Kumagawa and Suto, Friedrich Müller, Keller's modification of Röhmman's and the Folin-Wentworth method. Hoffman's copper acetate method, a qualitative method with approximate relative quantitative possibilities, did not give us satisfactory results.

From our investigation we believe the Folin-Wentworth method comes the nearest being accurate of any of the methods considered. We base our opinions not on long or continued use of any of these methods, but on the principles involved. The Folin-Wentworth method, however, does not give us what we want. It determines only the neutral fat, the fatty acids, including the fatty acid of the soaps, and the total fats. If we regard the free fatty acids in the stool of little importance, as some have done, we may then regard the figures obtained for fatty acids as representing the soaps. We believe, however, that we are not justified in disregarding the free fatty acids, for under some conditions they are not insignificant. The Folin-Wentworth method is not a clinical method. It requires from twenty-one to twenty-four hours to complete it, providing the extracting apparatus can be kept running the full twenty-hour period. It is suitable for careful metabolism work, but even here there

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* Read at the meeting of the American Pediatric Society, Washington, D. C., May, 1913.

are times, as we shall show later, when it fails to extract all the fat from a sample of stool.

One of us had been endeavoring for some time to develop a centrifugal method for the estimation of total fat. Being unable to get uniform results, we abandoned this method and directed our attention to an investigation of the rate of extraction with acid-ether according to the Folin-Wentworth method. Our results will be found in Table 1.

TABLE 1.—RESULTS OF EXTRACTION OF FAT FROM STOOLS BY THE ACID-ETHER METHOD

Sample 3. Pulverized Stool After Extracting.	Neutral Fat, Per Cent.	Fatty Acids, Per Cent.	Total Fat, Per Cent.	Total Fatty Substance, Per Cent.
Fractional Experiment*—				
One hour	1.73	17.69	19.42	37.20
Five hours more.....	0.02	22.36	22.38	42.87
Fourteen hours more.....	0.50	9.90	10.40	19.92
Total	2.25	49.95	52.20	99.99
Sample 3 extracted the full twenty hours	4.49	45.49	49.98	
Difference in total result...	+ 2.24	— 4.46	— 2.22	

* In this experiment a new weighed flask was substituted at the end of each run. The extraction was kept running the full twenty-hour period.

From this experiment it will be seen that the bulk of the neutral fat appears to be extracted at the end of the first hour, and the bulk of the fatty acids (produced by the action of the acid ether on the soaps) is extracted at the end of the sixth hour.¹ In other words, 76.88 per cent. of the neutral fat comes out in one hour, 82 per cent. of the fatty acids in six hours, and 80 per cent. of the total fat in six hours. It will be further observed that in the sample undergoing undisturbed extraction for twenty hours, over twice as much neutral fat is obtained and the amount of total fat is over 2 grams less than that obtained in the frac-

1. The formation of fatty acids from soaps is what is termed a substitution process. It is readily effected with weak acids. The following experiment shows how completely the acid ether extracts a sample of soap. We took of

Pure castile soap, gm.....	1.1035
Extracted 12.5 hours with N/10 acid ether. Weight of substance after extraction, gm.....	1.1142
Weight of substance unextracted by petroleum ether, gm.....	0.0412
Fatty acids recovered from the soap. By subtraction, gm.....	1.0623
By titration, gm.	1.0516

tional experiment. From this we conclude that the cold acid-ether process of extraction cannot be utilized for clinical purposes in the sense of giving us data on the three varieties of fat encountered in an infant's stool. We thought some importance might attach itself to the fact that the bulk of the neutral fat came out during the first hour's extraction, but the difference between the neutral fat figures for the fractional extraction experiment and the undisturbed twenty-hour extraction experiment is so great we feel that this process cannot be depended on for quick estimation of the neutral fat in a stool.

We next set ourselves the task of endeavoring to shorten the drying period. We encountered stools which dry more readily than others, and stools, which, in spite of many hours drying in the closed hot-air oven and subsequently many months (nine) desiccation over H_2SO_4 , fail to remain in powdered form after passing through a forty-mesh sieve. We constructed a drying oven² through which a constant current of hot air passed, the stool sample being placed on a shelf over and under which the air circulated. By this method we were able to reduce the drying time one-half. Drying the stool with alcohol also shortens the period, and in many instances, but not in all, this method proved to be the shorter one. By this means the water is taken out with the boiling alcohol and the alcohol finally carefully evaporated off.

After the trial of other methods of extraction, hot alcohol, etc., and titration methods without, as yet, satisfactory results, we again turned our attention to our centrifugal method which determines the total fat only. At times we had succeeded in removing comparatively large amounts of fat from infants' stools by this method, while at other times with samples of the very same stool, and apparently exactly the same manipulations, we got only blank or negative results. The principle of the centrifugal method is the conversion of all fat and soaps into fatty acids, which are later brought into the graduated stem of a centrifugal tube and the amount in cubic centimeters read off directly.

In our effort to obtain uniform results three difficulties presented themselves:

1. A whitish substance almost invariably appeared floating on the surface of the centrifugalized fluid, and later it appeared below or

2. The oven is easily constructed. One length of Russia iron pipe $3\frac{1}{2}$ to 4 inches in diameter is connected at each end with a circular elbow. At one end the elbow is turned down over a Bunsen burner, at the other the elbow is turned up and a thermometer suspended in it by means of a wire. An opening about 5 inches long is cut into the side of the pipe length and the flap turned into the pipe to form the shelf. A lid or door is hinged above the opening and is kept closed by means of a simple turn catch attached to the lower edge of the lid. The catch fits into a hole punched in the outer edge of the shelf flap. A brisk current of hot air passes through the pipe and the temperature is easily regulated with the ordinary Bunsen.

intimately mixed with the fat column in the stem of the tube after final centrifugalization. In some samples of stool in which no fat was present this substance rose to the top and sometimes resembled quite closely a poor fat layer.

2. Carbonization of the fat layer frequently took place because of an excess of acid. After a number of trials on different stools we were able to determine the maximum and minimum amount of acid necessary to burn the organic matter other than the fat and set the fat free, and to work out an optimum concentration which seems to be adaptable to most stools.

3. At times the fat column was difficult to read.

After milk fat passes through the intestinal canal certain physical changes take place. Before ingestion a centrifugal test shows a clear, fluid, yellow fat layer, which becomes more firm as it cools. It remains, however, in this clear condition for several minutes and is easily read. After the milk passes through the digestive tube a centrifugal test shows a denser and darker fat layer, which solidifies at a higher temperature and contracts in such a way as frequently to cause it to break in the center. A reading made when first taken from the machine is frequently a little greater than it is a minute or two later. If the tube is immersed in hot water the fat column melts and again occupies its former position in the tube. There is evidently a change in the specific gravity of the fat after it has passed through the intestinal tube. This difficulty seemed to be almost as insurmountable as the first one, which we will return to later. We thought to alter the specific gravity (lower the melting point) of the fat present in the stool by adding a known quantity of a fatty acid of low melting point. By deducting the difference in the result obtained we hoped to find the total fat content. We chose *oleic acid*. We were able to recover from stools and substances free from fat the amount of oleic acid added, and in stools with a fat content we were able to recover the oleic acid plus additional fat from the stool.³ The fat column was by this means made clear. Such a method we believe is not acceptable from a chemical or clinical standpoint, for we are introducing a factor. The introduction into a stool of a substance sought might easily lead to error. We found, as we have stated, that much of our trouble with the fat column came from carbonization induced by adding too concentrated acid.

At this point in our investigation we came across Kita's⁴ work on a similar method for the estimation of fat in meat. He had encountered the same difficulty with the appearance of a white substance on the top of the centrifugalized fluid, and found by adding a small amount of amyl alcohol complete separation of the fat layer was effected. We found

3. See foot note to Table 2.

4. Kita, T.: Arch. f. Hyg., 1904, li, 165.

this to be the case when we added amyl alcohol to our stool mixture. As is quite generally known, the addition of a small amount of 80 per cent. ethyl alcohol straightens out the fat column in the determination of fat in cheese by the Babcock method so that it can be easily read. Our experience with ethyl alcohol, however, has not been satisfactory. Because of its low boiling point (78 C.) when it is added to the hot acid stool mixture, some of the contents are often blown out through the stem of the tube. Kita gives no explanation for the use of amyl alcohol. The alcohol in either case effects a better separation of the fat, and we believe the advantage gained from the use of amyl alcohol lies in its very high boiling point (131 C.). By means of the addition of amyl alcohol, our fat layer now became as clear as we could wish it to be. By immersing the tube in hot water after centrifugalization the amount recovered is easily read off. As a precautionary measure we found it necessary that the amyl alcohol should be thoroughly mixed with the acid water stool mixture, otherwise it would come up into the stem of the tube, and, because of its acquired yellow color, appear as a fat column. We have worked out the following method, which has given us very uniform and apparently quite accurate results:

CENTRIFUGAL METHOD FOR ESTIMATION OF TOTAL FAT IN INFANT'S STOOLS

The Sample.—If pulverized stool is examined, one-quarter (0.25) gram should be carefully weighed. If fresh or moist stool is examined, one-half (0.5) gram. An inexpensive prescription balance is sufficiently accurate for clinical purposes. For more careful work an analytical balance is of course necessary. The stool, if dry, is best weighed in a poised watch glass and transferred to glazed paper. Moist stool is quite easily removed by means of a spatula from glazed paper on which it is weighed, or from the watch glass, by means of water. The sample is carefully rubbed up in a thin-lipped mortar. A maximum of 20 c.c. of warm (40 to 50 C.) distilled water is used to transfer the mixture to a Babcock milk bottle graduated in *fiftieths*. A little practice enables one to transfer the entire sample with the first 10 c.c. of water, leaving the remainder to rinse off the mortar and pestle.

1. To the sample, now thoroughly mixed, 17.5 c.c. H_2SO_4 (1.84) is added. Great care should now be taken to mix thoroughly by shaking the bottle back and forth vigorously (it should be remembered that all the organic material except the fat must be completely burned, or the test will be a failure).

2. One c.c. amyl alcohol is now added and thoroughly mixed.

3. The tube is now carefully counterpoised and centrifugalized for three minutes at high speed. Enough hot water is then added to bring

the fat into the graduated portion of the stem of the bottle. The bottle is again counterpoised, centrifugalized for one minute and the number of divisions on the stem occupied by the fat read off. Care must be taken to keep the stem hot and all readings must be made while it is hot. This is quickly accomplished by immersing the bottle in a pitcher or beaker of hot water.

Calculation.—If 0.25 gram of stool has been used, multiply the number of divisions on the stem of the tube occupied by the fat column by the factor 7.2; if 0.5 gram sample of stool is used, multiply by the factor 3.6; the result is the percentage of fat in the sample examined.

TABLE 2.—COMPARISON OF CENTRIFUGAL METHOD WITH FOLIN-WENTWORTH METHOD

Stool No.	Food Given.	Character of Sample	Percentage of Fat Obtained by the						
			Centrifugal Method. Analysis No.				Folin Wentworth Method. Analysis No.		
			1	2	3	4	1	2	3
1	Eiweissmilch..	Pulverized.	36.00	36.00	36.00		36.32		
3	Eiweissmilch..	Pulverized.	43.08	43.23			49.98	52.20	
8	Eiweissmilch..	Pulverized.	36.00	32.40	32.40	32.40	34.52	35.24	
9	Breast milk...	Moist*	16.20	16.20	16.20	16.20			
11	Breast milk...	Pulverized.	50.40	50.40	50.40		34.70	35.30	34.60
							Centrifugal test on sedi- ment left in extraction capsule.....		14.40
									49.00

* Oleic acid method. By substituting 0.1 c.c. oleic acid for the amyl alcohol and rubbing it well into the stool we obtained with a sample of this stool 34.20 per cent. fat. Subtracting the oleic acid value, 18 per cent., we obtained exactly the same result, 16.20 total fat.

To determine the amount of fat in the twenty-four-hour sample of stool, the stools may either be weighed when passed, or they may be kept in a moist chamber and weighed at the end of the period. The former is the better method, for, by this means any loss or gain in weight may then be detected and accounted for. The stool from which the sample is taken must be thoroughly mixed. From the known weight of the stool the total amount of fat is readily estimated. With a little practice a complete test can be made in ten minutes.

Sensitiveness of the Centrifugal Test.—Table 2 records a few comparative tests with the Folin-Wentworth method.

It will be observed that in most instances the centrifugal method gives practically the same reading on the same stool. It is not subject to as many variations as the extraction method. It will be further observed that for some unexplained reason at one time it gave a lower percentage than the extraction method. In another rather striking instance the centrifugal method gave a much higher reading than the extraction method. In the last instance we were able to demonstrate that the acid ether failed to extract 14 per cent. of the fat after twenty-six hours' extraction in the Soxhlet apparatus, and that the addition of this amount to that previously obtained by the extraction method was almost the same as the figures obtained by our centrifugal method. Furthermore, in the case of the third analysis (No. 11), Folin-Wentworth, only 0.5 gram sample of stool was used and run for twenty-six hours, which is the equivalent of fifty-two hours on 1 gram, and yet we were able to extract no more fat than in the other two cases.

We believe the true fat content of this sample of stool would not have been known had we depended on the Folin-Wentworth method. This is the breast-milk stool previously referred to, which resisted pulverization, even after nine months' drying over H_2SO_4 . After rubbing this sample through a fine sieve, it persisted in forming little balls and coffee-ground-like masses. These doubtless resisted extraction by the cold acid ether. The hot acid water mixture of course readily overcomes this difficulty. In the one case extraction is at work; in the other, liberation.

We believe a knowledge of the total fat content of stools may prove to be of value in the diagnosis and treatment of the digestive disturbances of infancy, and we hope this simple method, based on the Babcock principle, will help to further our knowledge in this field of medical research.