This apparatus could also be used for making a determination of carbon dioxide from a carbonate.

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FURTHER OBSERVATIONS ON THE NATURE OF FECES FAT.[1]

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In two earlier papers[2] figures were presented showing the amount of phophorus contained in the normal feces fat of a number of individuals. From the conditions of the experiments this phosphorus content, and the nitrogen found at the same time in one set of examinations, suggested the presence of a body or bodies of the "lecithin" type, using this term in the broader sense as describing the fat like "phosphatides" or "lecithans." Inasmuch as a lecithin content in feces fat has been frequently denied[3], while other authorities have maintained the reverse to be true[4], and have spoken of a high percentage amount of these bodies, it appeared that further work in this direction was desirable. In the following pages some observations on these and other points will be given.

For our experiments the mixed feces from a number of men in normal health were collected, dried and extracted with absolute ether, after rubbing up with fine ignited quartz. In the drying of feces in the ordinary way on the water-bath it is not possible to avoid the loss of some nitrogen, in non-protein combination, but the loss may be reduced somewhat by keeping the temperature low. On the other hand, the slow drying, with long contact with the air, occasions some change through oxidation processes. The loss of nitrogen from the fat-like bodies is the most serious of the objections to the water-bath method of drying, but in the working up of large quantities of feces it is practically the only method available and was followed, therefore, in our work.

By extraction in the Soxhlet apparatus we secured two lots of "fat" of about 40 grams each, which will be referred to as samples A and B. These crude fats were purified by solution in absolute ether, filtering and

[1] Presented at the New York meeting of the Am. Chem. Soc. Dec. 31st, 1906.

[2] Long, this Journal, 28, 704. Long and Johnson, this Journal, 28, 1499.

[3] Hoppe-Seyler, Physiolosische Chemie, p. 337. Hoppe-Seyler, Chemische Analyse für Aerzte, 6th Ed. p. 480. Bokay, Z. physiol. Chem., 1, 157.

[4] For example, Deucher, Maly's Jahresber., 1898, p. 606. Also, F. Oefele, Maly's, Jahresber., 1904, p. 457.

slow evaporation of the ether. There was a slight loss in this resolution in each case, possibly because the first extraction carried down traces of inorganic substances by aid of traces of water remaining in the dried feces. With the fat so secured, which was, of course, a mixture of all bodies present which are soluble in absolute ether, the following tests were made. Many of these tests were carried out on the purified fat and also on a fraction precipitated from its ethereal solution by acetone. The results from the original fat will be given first.

Experiments with Original Fat.

Sample A was used for most of the tests, and B mainly for confirmation. The two lots of fat are not absolutely comparable, but practically so in most respects, since they were obtained from the feces of the same individuals, with an interval of a few weeks between the collections.

Melting Point.—A constant melting point of such a mixture cannot be expected, from a consideration of the various factors which enter to modify it. Some of the single fats present are soft, with a low melting point, which cholesterol and the related koprosterol, which are always present in relatively large amounts, have high melting points, the first melting at 145° and the second at about 96°. The lecithin-like bodies have all a high melting point and when they are present the general consistence and behavior of the fat is greatly modified.

In our experiments with fat A the actual softening of the fat which could be designated as incipient fusion did not begin below about 65°, which value is higher than that usually quoted in the literature. The sample B showed even a higher fusion point, and not below 70°. The high melting point of crude feces fat depends not alone on the presence of cholesterol and other bodies mentioned above which may have their origin in the bile, but also on the nature of the fatty bodies of diet. When these fats are very hard with a high melting point their absorption is relatively imperfect and a considerable fraction may pass down the intestine to be lost with the feces, as was long since pointed out by Mueller[1] who showed also that the feces fat may have a melting point of 85° higher in the mean, than the melting point of the food fat.

Phosphorus Content.—The recognition of the phosphorus in the crude fat is a very simple matter, but the determination of the compound or compounds in which it occurs present greater difficulties. The practical difficulty is greatly increased by the fact that different methods of extraction yield crude fats with very different phosphorus contents, and when these are considered in relation to the nitrogen contents found at the same time, no uniformity can be found, as a rule, as will appear in the remarks on nitrogen content, below.

The many extractions we have made show that the amount of phos-

[1] See reference in Schmidts und Strasburger, "Die Faeces des Menschen," p. 149.

phorus present in the crude fats from the feces of different individuals is extremely variable, but thus far no relation has been found connecting this amount with the diet or other factors. In our earlier papers referred to above, we noted in several crude fat samples amounts of phosphorus varying from 0.087 per cent. to 1.59 per cent. In the present case from fat A we found again a high phosphorus content of 1.17 per cent., which if calculated according to the old method to lecithin would correspond to about 30.5 per cent. of the distearyl product. On the other hand from fat B, which we expected to be in general similar to A, we found only 0.51 per cent. Then wide variations are in themselves sufficient to suggest the importance of more extended investigations to connect the phosphorus with other factors in individual cases.

Nitrogen Content.—The determination of the nitrogen by the Kjeldahl method presents no difficulties but the value of the determination is somewhat uncertain, in view of the loss of nitrogen which always occurs in the separation of the fat. In different series of experiments we have attempted the extraction of the fat in various ways, and each method seemed to be attended with a marked loss of nitrogen. If the feces are evaporated rapidly on the water-bath and extracted a product is obtained which is sometimes low in nitrogen; this is especially the case if the water-bath evaporation is followed by further drying in the air oven. A decomposition of the original nitrogen complex evidently takes place here.

On the other hand, the extraction of the original moist feces with boiling alcohol gives equally unsatisfactory results, as will be pointed out below. Boiling with alcohol produces a great change in the ratio of phosphorus to nitrogen. To secure a proper nitrogen value we believe the complete, but slow drying is necessary, followed by ether extraction, and this scheme we returned to after trying several others. From the fat sample A secured in this manner we obtained 4.16 mg. of nitrogen for each g. of fat, or nearly 0.42 per cent. From sample B, which was low in phosphorus, we obtained 2.82 mg. of nitrogen for each g. of fat, or 0.28 per cent. As compared with the phosphorus in the same sample this nitrogen is relatively high, and no simple relations appear to obtain in the two cases.

Iodine Number.—The iodine absorption of the crude part is relatively low. In fat A we found the value to be 22.2 per cent., while in fat B it was 24.2 per cent. These are about the values which are found in butter fat and are smaller than those for lard or tallow.

Saponification Number, or milligrams of KOH required for 1 gm. of the crude fat. This test was carried out in the usual manner with an alcoholic solution of potassium hydroxide and gave for fat A a value of 216.7 and for B 153.6. For the ordinary fats the second value is low.

but for such a mixture as might be here expected it is not abnormal. The crude feces fat always contains cholesterol, which does not saponify, besides other bodies of high molecular weight. The amount of cholesterol in the crude fat may run as high as fifteen per cent. or more, and would produce a marked decrease in the saponification value. For lecithins, on the other hand, the value is relatively high. From a good product made in this laboratory from eggs we found the number 335. For lard and tallow the value is about 200, and for butter fat about 225, in the mean.

A part of this alkali absorption is due to the presence of free fatty or similar acids. In an independent experiment with fat A it was found that 1 g. of the fat dissolved in alcohol required for neutralization 53 mg. of potassium hydroxide, as measured by phenolphthalein.

Experiments with the Acetone Precipitate.

Many methods have been proposed for the recognition of the lecithin-like bodies in fatty mixtures, and as applied to the feces they have frequently given such poor results as to lead to the impression that the lecithin content must be *nil* or very low. As lecithin combines with certain metallic salts readily, attempts have been made to precipitate it quantitatively from mixtures by such substances as cadmium chloride. Some years ago the details of a process for the separation from the yolk of eggs was described by Bergell[1] in which he extracted with alcohol, precipitated with cadmium chloride and recovered the lecithin from the precipitate by decomposition with ammonium carbonate. The results obtained from this process, even where applied to eggs, are far less satisfactory than would appear from Bergell's paper, and several attempts to duplicate his results gave always much lower figures. When applied to our feces fat extraction the results were always uncertain and irregular. The separation was abandoned as not suited to the purpose.

Much depends also on the original extraction method employed, and to illustrate the difficulties the following experiments may be cited. About 300 g. of feces was boiled under a reflux condenser with a considerable excess of absolute alcohol, enough to make the mixture contain about 85 per cent. alcohol. At the end of some hours the solution was filtered hot and the filtrate slowly evaporated to dryness. The fatty residue was taken up with chloroform, and the new solution filtered from a small insoluble portion. On adding an excess of acetone to the clear chloroform solution a precipitate was formed which was collected and tested for phosphorus and nitrogen, with these results:

$$P = 0.16 \text{ per cent.}$$

$$N = 3.96 \text{ per cent.}$$

The acetone-chloroform solution was evaporated and the fatty residue treated in the same manner, giving:

[1] Ber., 33, 2584.

P = 0.04 per cent.
N = 0.77 per cent.

These numbers have little meaning and certainly do not point to a lecithin content in the original feces. The prolonged boilings evidently effect a decomposition of phosphorus and nitrogen compounds. Somewhat similar results were obtained from a second extraction carried out with partially dried feces in the same manner from which it appears that the method is not satisfactory for the purpose, although it has been frequently employed in the search for lecithins as occurring in feces[1]. Some of the negative results reported in the literature are possibly due to this fact.

Our best results were secured by the acetone precipitation of the purified ether extract of fat A described above. A portion of this fatty mass was dissolved in ether and precipitated in a beaker by addition of a very considerable excess of acetone. The precipitate which formed consisted of two parts: the larger portion separated as a sticky, gummy mass which clung to the sides of the beaker after vigorous stirring, while a small amount of a yellowish white granular substance settled out also. This latter could be easily brought into suspension, and by washing with acetone by decantation was almost completely separated. It made up about 1.6 per cent. by weight of the original fat and showed a melting point of 150°. On analysis it gave:

P = 4.14 per cent.
N = 1.27 per cent.

This substance redissolves easily in ether, chloroform and alcohol and on ignition leaves but a trace of ash. The small amount of substance available rendered other tests impossible, but it evidently belongs to the phosphatide group and represents a product higher in phophorus and lower in nitrogen than the typical lecithins. The numbers correspond almost exactly to the ratio:

$$P : N :: 3 : 2$$

It should be added that the same product was found in several later extractions, in small amount.

The larger part of the acetone precipitate made up about 35 per cent. of the weight of the purified ether extract as crude fat, and was used for a number of tests. It exhibited, after being washed with acetone and dried over sulphuric acid, the general appearance and behavior of the lecithin bodies and their action toward water.

Melting Point.— As in the case of the original fat this could not be accurately determined, but appeared to be slightly above the boiling point of water. In the capillary tube test the substance softened at the

[1] See Hoppe-Seyler, Chemische Analyse für Aerzte, 6th ed. p. 480.

boiling temperature, without actually melting. On heating to a higher temperature the mass gradually darkens and chars.

Phosphorus Content.—This was found in the usual manner by the Pemberton method of titration, after fusion with potassium nitrate and sodium carbonate. The titration gave 2.39 per cent. in sample A, which is too low for a real lecithin, undoubtedly, however, the acetone carries down various other products, which are free from phosphorus.

Nitrogen Content.—Some of the washed and dried acetone precipitate was subjected to the Kjeldahl distillation. The ammonia obtained, when reduced corresponded to 0 57 per cent. of nitrogen, which appears very low. It was found, however, that the ether-acetone filtrate from the precipitate was free from more than minute traces of phosphorus and contained considerable nitrogen. The recovered fat gave 0.31 per cent. of the latter element. This may have come originally from other substances than that containing the phosphorus, or it may have been split off, on the other hand, from the phosphorus containing complex during the treatment to which the fat was subjected. If then, assuming this point of view, the whole of the nitrogen could have been secured in the acetone precipitate along with the phosphorus, the nitrogen content would amount to about 1.13 per cent. This accounts fairly well for the whole nitrogen content, and in the acetone precipitate gives a ratio:

$$P : N :: 31 : 14.7, \text{ or } 1 : 1 \text{ atom.}$$

While these percentage numbers are low to indicate a true lecithin as making up the whole of the acetone precipitate, they certainly suggest that such a body may be present in relatively large amounts.

Saponification Number.—This was not found in the acetone precipitate, but was determined in the fat from the ether-acetone solution which was evaporated. The value found was 114.5 while that of the original fat was 216.7. It is evident, therefore, that a body of high alkali-combining power was removed in the acetone precipitation. To account for the low value from the ether-acetone residue this precipitated fraction would have to possess a value of 350 or over. Such numbers are found in the lecithins. The theoretical value for distearyl lecithin is 347. For some egg lecithin prepared in the laboratory we found 335 and for a commercial product containing some moisture and non-saponifiable matter a value of 231, equivalent to over 300 in purified condition. The phosphate group in this combination seems to be wholly indicated by phenolphthalein and in the calculation, therefore, five molecules of alkali were taken for one of the lecithin.

Iodine Number.—This could not be found directly for want of material, but from the value determined with the residue from the ether-acetone

solution it is evident that this factor is low. In both samples A and B, the fat recovered from the filtrate after precipitation with acetone showed a much higher iodine absorption than was found in the original fat and this can be the case only when the precipitated fraction possesses a low absorbing value.

Taking all these experiments as a whole they undoubtedly point to the pressence of a lecithin-like body in the crude extracted fat. As to the possible origin of this there may be several opinions. The conclusion of Bokay, cited above, that the feces fat contains no lecithin, is based apparently on a few direct feces tests, but largely on the results of some artificial digestion experiments in which he found that lecithin, while slowly attacked by pepsin and acid, is very quickly decomposed by pancreatic extracts, and therefore cannot pass down the intestinal tract as such. This argument probably holds true as far as the larger part of the food lecithin is concerned, but there is every reason to believe that a fraction, and perhaps a large fraction of that found in the feces does not come from the food but from the bile, as a product of the constant breaking down of cells of the liver. This may be discharged at a time and under such conditions that it is not readily attacked by the pancreatic ferment thrown into the intestine. The Bokay view is frequently quoted and is doubtless responsible for some of the misconceptions which obtain on the subject.

In our former paper[1] we called attention to the work of Hammarsten on the bile of different animals, where a high lecithin content is usually found. This fact has one important bearing on our discussion, and it is likely that we should, therefore regard the "phosphatide" or "lecithin" bodies of the feces as excretory products, rather than as waste or unabsorbed material from the food. In pathological conditions the food fat with its small lecithin content may find its way in part into the feces, but in health it is possible that the other source suggested is the more important.

Since the experiments described above were made an article by Erlandsen[2] has come to hand in which methods of extracting the lecithin bodies from certain tissues are very fully described. The author points out, as we have found, that the Bergell method of extraction and precipitation of lecithin gives unsatisfactory results. He finds, also, that acetone carries down nearly the whole of the phosphorus bodies from the ether extract, but holds the separation is not exact enough to be considered accurately quantitative.

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[1] Loc. cit.

[2] Z. physiol. Chem., 51, 71.