

barium is removed by precipitation with sulphuric acid, filtered, and the precipitate washed. The filtrate is now ready for the usual application of the creatine method. When very small quantities of creatine are present, the usual difficulties of reading are experienced, but in most cases the readings ran from 2 mm. to 12 mm. on the Duboscq colorimeter. The percentage of peptones as determined is corrected by the figure thus obtained. It is of course necessary to determine ammonia in the original sample and make a correction therefore in the calculated percentage of peptones.

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### THE PHOSPHORUS CONTENT OF FECES FAT.<sup>1</sup>

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IN a recent communication<sup>2</sup> attention was called by one of us to the high phosphorus content of the feces fat of a man in normal health, under conditions where, according to the usual statements in the literature, little or no phosphorus should be found. The phosphorus, in the form of phosphoric acid, obtained from feces fat, as extracted by perfectly dry ether, has always been assumed to have its origin in bodies of the lecithin type, and the detection and estimation of lecithin here have been made to depend on the recognition and determination of phosphoric acid among the products of decomposition of the fat.

However, that all the ether-soluble organic phosphorus obtained through the extraction of dried feces may be assumed to come from a lecithin body may well be doubted, in view of the many conflicting results obtained in the last few years in the examination of fresh animal and vegetable tissues as well as of feces. All the more recent investigations on the subject agree in suggesting that what has been called "lecithin" is evidently a mixture, and as a preliminary to the discussion of the nature of the phosphorus-containing bodies in the feces we have thought it well to make a new series of phosphorus, and also nitrogen determinations in the fat extracted under certain conditions.

In the paper cited above the method of extracting the fat was referred to. In the work below the same plans were followed,

<sup>1</sup> Presented at the June (1906) meeting of the American Chemical Society at Ithaca.

<sup>2</sup> Long: This Journal, 28, 704.

but in one case when a large quantity of the fat was desired, the dried mass was rubbed up with fine crushed quartz and extracted in the Soxhlet apparatus in the usual manner. The ether used in the extractions was carefully purified and finally distilled from sodium. The evaporation of the ether after extraction left a crude fat which was purified by dissolving in dry ether, filtering from a slight residue always left and evaporating again. This treatment would exclude inorganic substances, and anything above a trace of glycerophosphoric acid, if present. The direct ignition of some of the fat thus purified left always a minute amount of residue of phosphate or phosphoric acid. On igniting the fat in the usual manner with sodium or potassium nitrate and carbonate the whole of the phosphorus was left as alkali phosphate which was always determined by the Pemberton method.

The table following gives the results obtained from the feces of seven individuals in normal health living on an ordinary mixed diet. There are also given, in No. 8, the results for nitrogen and phosphorus in the feces of the same man from whom sample No. 3 was obtained at an earlier period.

No. of sample.	Solids.	Per cent. of fat in dry material.	Per cent. of $P_2O_5$ in fat.	Per cent. of N in fat.	Per cent. of P in fat.
1.....	15.03	8.60	1.02	....	....
2.....	24.25	14.01	1.77	....	....
3.....	28.00	19.45	2.32	....	....
4.....	21.99	18.63	1.05	....	....
5.....	20.00	9.50	0.20	....	....
6.....	24.90	14.12	0.80	....	....
7.....	28.00	15.15	1.20	....	....
8.....	....	16.60	3.66	0.47	1.59

Extreme variations are shown in the percentage amounts of phosphoric acid recovered from the fat. As found before, these amounts are not increased by prolonged extraction. It will also be noted that these results are very high in samples Nos. 3 and 8, which, as mentioned, were from the same individual. It should be stated, in addition, that the very high phosphorus content referred to in our earlier paper was found in feces fat from the same man. As yet we have not been able to discover an explanation for this peculiarity.

All the results in the above table are relatively high and if calculated to ordinary lecithin it is plain that the lecithin content of normal human feces must be considered as high instead of

low as frequently stated. Some references to this subject may be found in the excellent book by Schmidt and Strasburger<sup>1</sup> where certain authorities are quoted. It must be remembered that 1 per cent. of phosphorus corresponds to 26 per cent. of distearyl lecithin, or 1 per cent. of  $P_2O_5$  to over 11.3 per cent. of the same complex fat. The relations for the other possible lecithins are not greatly different. On the assumption that this phosphorus content is due to a pure lecithin solely, and that no other soluble nitrogen compounds are present, a check on the phosphorus content would be secured through a determination of the nitrogen. In ordinary distearyl lecithin the nitrogen and phosphorus stand to each other in the ratio 14:31, but the above table shows a phosphorus percentage much higher than this. The discrepancy may be due to the presence of other bodies, containing nitrogen or phosphorus, or both, in the ether extract, or it may be due to variations in the composition of lecithin itself. Wintgen and Keller<sup>2</sup> give some figures for lecithins prepared from soy beans, and purified by acetone precipitation, in which the nitrogen content is relatively high, in some cases 50 per cent. higher than could correspond to a simple formula, while the phosphorus content is low. It is evident that under the name "lecithin" a number of substances are included which may be only remotely related to bodies of the simple distearyl lecithin type, and for the present we can do no better than refer to them as *lecithams* or, less distinctively, as *phosphatides* as suggested by several writers. There is a very considerable literature on the occurrence and nature of these bodies, the extent of which may be gathered from recent papers by Schulze and Winterstein,<sup>3</sup> and Koch and Woods.<sup>4</sup> It may be well to recall that Thudichum,<sup>5</sup> in discussing the organic phosphorus compounds of the brain and other organs of the body, described a number of substances, which he classed as phosphatides, in which the phosphorus and nitrogen ratios were quite variable. Some of these bodies appeared to contain two atoms of phosphorus in the molecule. In other compounds he found a great excess of nitrogen, from which the ratio of N:P appeared

<sup>1</sup> "Die Faeces des Menschen," p. 160.

<sup>2</sup> Abs. Biochemisches Centralblatt, 5, 272 (1906).

<sup>3</sup> Z. physiol. Chem. 40, 101 (1903).

<sup>4</sup> J. Biol. Chem. 1, 203 (1906).

<sup>5</sup> Thudichum: "Die chemische Konstitution des Gehirns des Menschen und der Thiere," Tübingen, 1901.

to be 4:1 instead of 1:1, as in the simple lecithins. Quite recently Winterstein and Hiestand<sup>1</sup> have described a new group of vegetable products which appear to consist of a combination of a lecithin and a carbohydrate. Former investigators had included this with the lecithins of low phosphorus content, but the authors now point out the true relation of these bodies.

However, we are not now concerned with the question of the variation in the composition of these lecithin bodies, but with that of their occurrence in the fat of feces. There can be two general sources for these phosphorus-containing fats. They may represent unchanged substances from the original animal or vegetable foods, or they may represent products of metabolism within the human body. Probably each source contributes a share, but if we consider a large fraction as coming from the food it will be necessary to assume that lecithins are more resistant to the digestive operations than are the other fats since the proportion found in the feces fat is relatively higher. The behavior of the lecithin fats in this respect has been pointed out by Stassano and Billon.<sup>2</sup> Estimating from the phosphorus content about 4 grams daily of lecithin in the feces would be indicated by the maximum of the results in the table above, while the other results are much lower. From a vegetable diet alone it is scarcely possible to account for this largest value, but from a mixed diet containing eggs the difficulty is less. But a large part of the crude fat of the feces is known to have other origin than that of the original food directly. Some comes from the destruction of the tissues lining the intestinal tract, some from the bile and some from the bacteria of the intestine. The importance of the bile in this respect has been generally overlooked, since the older analyses in general placed the organic phosphorus content very low. But the investigations of Hammarsten<sup>3</sup> on the biles of different animals have placed the matter in a new light. Hammarsten shows that a very considerable portion of the alcohol-soluble fraction of the bile solids may be classed in the lecithin or "phosphatide" group. The phosphorated fat found in the bile must have its origin in the

<sup>1</sup> *Z. physiol. Chem.* 47, 496 (1906).

<sup>2</sup> *Jahresbericht über die Fortschritte der Thier-Chemie*, 33, 76 (1903).

<sup>3</sup> See, especially, article in the *Ergebnisse der Physiologie*, 4, 1 (1905); "Zur Chemie der Galle."

disintegration of liver tissue, the lecithin content of which is relatively high.

It must be remembered, further, that bacterial cells make up a considerable fraction of the dried feces. Nothing very definite is known of the fat content of the intestinal bacteria, but of certain bacteria from other sources it has been shown that the fat content is high. This is especially true of the tubercle bacillus,<sup>1</sup> but of the minute composition of such fats not much is given in the literature. In view, however, of the general nature of bacterial cells it is fair to assume the presence of lecithin-like bodies in their fat.

Since numerous investigations of recent years have shown the great importance of the lecithins in relation to problems of nutrition, metabolism, and immunity, we have undertaken a continuation of this work with the hope of defining more closely the nature of the phosphorus compounds in the feces fat.

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## DISCOLORATION OF FRUITS AND VEGETABLES PUT UP IN TIN.

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A NUMBER of cases of discoloration of fruits and vegetables put up in tin have come to the attention of the writer. In most cases the discoloration was undoubtedly due to sulphides of the heavy metals, the discoloration in some cases being confined to the container, in others affecting the fruits and vegetables as well. The source of the hydrogen sulphide varied in different cases. Some micro-organisms are capable of evolving hydrogen sulphide through breaking down of proteid matter, but this would result only with goods which were not properly sterilized and is not so frequent as some other causes. Also such goods are unfit for consumption so that the discoloration is of minor importance. In other cases hydrogen sulphide resulted from the use of sulphites in connection with fruits, reaction having taken place between the sulphites and vegetable acids with the liberation of sulphurous acid, which, acting upon the tin of the container, had produced

<sup>1</sup> See among other authorities, De Schweinitz: *This Journal*, 25, 354 (1903).