XXXVII. THE INFLUENCE OF FREE FATTY ACIDS IN THE INTESTINAL CONTENTS ON THE EXCRETION OF CALCIUM AND PHOSPHORUS.

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(Received April 12th, 1921.)

WITHIN recent years a considerable amount of work has been done on the changes in the metabolism of calcium and phosphorus in infants, especially in relation to the pathological processes which directly involve the skeletal system. Part of this work has been concerned with the study of the effects of fat in the diet on the retention of these elements.

The results of Orgler, Meyer, Steinitz, Rothberg and Freund, quoted by Findlay [1918] in his survey of rickets seemed to indicate that there is a close relationship between the nature of the fat in the diet and the degree of calcium retention. These workers found a much greater retention with cod-liver oil and sesame oil than with butter fat. Their results, however, have not received full confirmation from later investigations. The work of Holt, Courtney and Fales [1920] has also served to direct attention to the relationship between calcium absorption and fat digestion. These workers found that in infants "the best absorption of calcium was obtained when the calcium intake bore a definite relation to the fat intake." Bosworth *et al.* [1918] have concluded from their observations that impaired fat digestion in infants, artificially fed, is due to the excessive quantity of calcium which cow's milk contains. These workers hold that the calcium soaps formed in the intestine occlude a certain quantity of neutral fat and free fatty acids which thus escape absorption. The exact nature of the relation between fat digestion and calcium retention is, however, still undetermined.

During the course of a series of observations on rachitic children, some information was particularly desired of the influence of the fatty acids set free in digestion on the degree of retention and mode of excretion of calcium and phosphorus. To obtain this, it was obviously important to compare the excretion of calcium and phosphorus in normal digestion with their excretion when a large excess of free fatty acids persisted unabsorbed in the intestine.

It was shown by Noël Paton and Balfour [1891] in their study of the fat absorption in a case of biliary fistula in a woman, that the absence of bile from the gut interferes considerably with the absorption of the products of fat

digestion. Their results agreed with those of Munk and other previous observers who worked on dogs with complete biliary fistulae.

Hutchison and Fleming [1920] in a recent paper have also shown, from observations on a case of congenital biliary atresia, that the exclusion of bile from the gut is associated with a large and persistent excess of free fatty acids in the intestinal contents.

The mode of excretion of calcium and phosphorus under the conditions in which bile was completely or even partially absent from the intestine was therefore thought to be worthy of consideration, as some light might be thrown upon the relation of fats to calcium under normal and pathological conditions.

The following are the details of studies made in two cases of congenital biliary atresia, with complete exclusion of bile from the gut, and in one of jaundice in an infant suffering from syphilitic hepatitis, with partial exclusion of bile. The analytical data of these three cases are compared with those obtained in the examination of a normal infant.

METHODS.

The subjects were placed on a "metabolism" bed, devised by Findlay, so that the urine and faeces could be collected separately and quantitatively. The faeces were dried on a steam-bath till constant in weight, then finely ground, or if pasty, thoroughly mixed in a mortar and preserved in air-tight bottles. Each daily quantity of urine was measured, acidified with nitric acid, and the bulk diluted to one litre. The diet in all cases consisted of fresh cow's milk containing approximately 3 % fat.

The calcium and phosphorus were determined in the ash of the dried faeces by the usual gravimetric methods. The neutral fat and free fatty acids were extracted directly from the dried faeces with ether. The combined fatty acids were obtained by splitting the soaps in the residue with hydrochloric acid and extracting the liberated fatty acids in a separating funnel.

RESULTS.

In Table I the daily amounts of urine and dried faeces are recorded, together with the quantities of CaO and P_2O_5 which these contained. In Table II, which shows the distribution of the fat in the dried faeces, the values given for free fatty acids were obtained by dissolving the ether extract (containing fat and free fatty acids) in neutral alcohol, titrating the free acids with $N/10$ NaOH , and calculating to oleic acid. The neutral fat was found by difference. In Table III the values recorded for CaO existing as soaps in the dried faeces were calculated from the weight of combined fatty acids found by experiment. An experimental determination of the mean molecular weight of the insoluble fatty acids of butter fat combining with calcium was found to give the value 274, and the coefficient $\frac{28}{274}$ or 0.102, was therefore used in calculating the percentage of CaO existing as soap from the weight of combined

fatty acids found. The difference between the total CaO and that existing as insoluble soap was taken as the CaO existing as phosphate of calcium.

CASE 1. NORMAL SUBJECT. J. H., AET. 4 MONTHS.

Intake: 720 cc. cow's milk daily.

Table I.

Day	Urine cc.	Faeces (dry) g.	CaO		P ₂ O ₅	
			Urine g.	Faeces g.	Urine g.	Faeces g.
1	350	10.8	0.016	1.18	0.44	0.83
2	440	10.7	0.016	1.07	0.57	0.92
3	350	10.2	0.016	1.03	0.49	0.78
4	390	11.7	0.02	0.95	0.57	0.73

Table II. *Distribution of fat in dried faeces (%)*.

Day	Neutral fat	Free fatty acids (as oleic acid)	Combined fatty acids	Total fat
1	1.07	11.13	19.15	31.35
2	1.81	10.99	16.35	29.15
3	1.62	10.43	17.90	29.95
4	6.08	12.12	12.20	30.40

Table III. *Distribution of CaO and P₂O₅ in dried faeces (%)*.

Day	CaO (total)	CaO (as soaps)	CaO (as phosphate)	P ₂ O ₅ (total)	% CaO as soaps of total CaO
1	10.9	1.96	8.94	7.7	17.9
2	10.0	1.66	8.34	8.6	16.6
3	10.1	1.82	8.28	7.7	18.0
4	8.1	1.24	6.86	6.2	15.3

CASE 2. CONGENITAL BILIARY ATRESIA. J. S. (MALE), AET. 3 MONTHS.

Intake: 720 cc. cow's milk daily.

Table I.

Day	Urine cc.	Faeces (dry) g.	CaO		P ₂ O ₅	
			Urine g.	Faeces g.	Urine g.	Faeces g.
1	310	28.0	0.036	1.14	0.91	0.4
2	430	24.4	0.038	0.936	0.87	0.34
3	420	23.4	0.046	0.88	0.95	0.28
4	420	30.5	0.048	0.956	0.99	0.26

Table II. *Distribution of fat in dried faeces (%)*.

Day	Neutral fat	Free fatty acids (as oleic acid)	Combined fatty acids	Total fat
1	17.47	33.13	25.75	76.35
2	14.44	34.26	25.45	74.15
3	12.63	31.72	30.15	74.53
4	8.2	40.6	29.35	78.15

Table III. *Distribution of CaO and P₂O₅ in dried faeces (%)*.

Day	CaO (total)	CaO (as soaps)	CaO (as phosphate)	P ₂ O ₅ (total)	% CaO as soaps of total CaO
1	4.07	2.63	1.44	1.42	64.6
2	3.83	2.60	1.23	1.35	67.8
3	3.76	3.08	0.68	1.19	81.9
4	3.76	2.99	0.77	0.85	79.5

CASE 3. JAUNDICE IN SYPHILITIC HEPATITIS. R. W. (MALE), AET. 17 WEEKS.

Intake: 300 cc. cow's milk daily.

Table I.

Day	Urine cc.	Faeces g.	CaO		P ₂ O ₅	
			Urine g.	Faeces g.	Urine g.	Faeces g.
1	90	9.0	trace	0.387	0.321	0.231
2	78	7.6	trace	0.281	0.228	0.174

Table II. *Distribution of fat in dried faeces (%)*.

Day	Neutral fat	Free fatty acids (as oleic acid)	Combined fatty acids	Total fat
1	15.6	29.9	25.05	70.55
2	12.63	29.32	29.75	71.70

Table III. *Distribution of CaO and P₂O₅ in dried faeces (%)*.

Day	CaO (total)	CaO (as soaps)	CaO (as phosphate)	P ₂ O ₅ (total)	% CaO as soaps of total CaO
1	4.3	2.56	1.74	2.59	59.5
2	3.7	3.04	0.66	2.3	82.1

DISCUSSION.

The most striking difference between the faeces of the normal subject and those in which bile was partly or completely absent from the gut lies in the fat content. Hutchison [1920] showed that approximately one-third of the dried faeces of a normal child consists of a fat and fatty acids. The results recorded in Table II of the series demonstrate that the normal fat content is increased by the absence of bile from the gut. In the case of congenital biliary atresia (Case 2), no bile was present in the faeces, but it was not completely absent in Case 3. In both, although a considerable amount of fat splitting had occurred, the absorption of fat had fallen markedly from the normal, and over 70 % of the dried faeces consisted of fatty derivatives in each case. These results confirm the findings of Hutchison and Fleming in their case of congenital biliary atresia.

The presence of this excess of fat, free fatty acids and insoluble soaps must naturally influence the bulk of the faeces excreted per diem. The naked eye appearance of these is characteristic. They are pale in colour and of a pasty

consistency. During the process of drying on a steam-bath they partly melt. Even when water-free they remain quite soft, and in marked contrast to dried normal faeces, which can be readily ground to a fine powder. Care has to be exercised in mixing such faeces so that uniform samples may be obtained for examination.

The weight of dried faeces daily obtained in the case of congenital biliary atresia (Case 2) is seen to be more than double that in the normal child of the same age. The amounts of cow's milk ingested were almost identical in the two cases. This increase in weight is evidently due to the presence of the excess of fatty derivatives. It will be observed that the weight of dried faeces per diem of the infant of 17 days suffering from jaundice does not fall much below that of the normal child of three months. Correlated with the presence of an excess of fatty derivatives (Cases 2 and 3), there is a diminution in the percentage of CaO and P_2O_5 as compared with the normal. This must be due largely to "dilution" by the fat, since it was found that there was no increased retention of calcium and phosphorus. The significance of the abnormally low percentage of phosphoric acid will be discussed later. In a series of analyses of the normal stools of infants on a diet of fresh cow's milk the calcium content of the dried faeces was found to vary only between very narrow limits and was generally about 10 % CaO . The CaO content of the faeces depends to a very large degree on the percentage of calcium in the diet. When the latter is increased there is a corresponding increase of calcium in the faeces. The results of Berg [1911], who added quantities of different calcium salts to his own diet and found that almost all the added calcium appeared in the faeces, demonstrate this clearly. Since the calcium content of cow's milk is very constant, the small percentage of CaO found in the dried faeces in Cases 2 and 3, must be due chiefly to the increased excretion of fat and fatty acids.

The manner in which the normal distribution of calcium in the dried faeces is altered by the presence of an excess of free fatty acids in the gut is evident from a comparison of the results in Table III of the series. The faeces of the normal subject contained less than 20 % CaO as soap, the remainder of the CaO existing as phosphate. In Case 2 the urine contained regularly more than twice the amount of P_2O_5 present in the faeces. The increased elimination of P_2O_5 in the urine is also evident, though to a less degree, in the case of jaundice (Case 3). In all three the intakes of calcium and phosphorus were in excess of the amounts found in the excreta, so that there was a positive retention of both elements.

The deviation of the greater part of the phosphoric acid from the normal excretory route is probably related to the fixation of calcium as soaps. This condition again is evidently dependent on the persistence of an excess of free fatty acids in the intestine, the non-absorption of these being due to the absence of bile. There has been no marked interference with fat digestion, in the two pathological cases. Even in the case of biliary atresia, in which bile was completely absent from the intestinal contents, fat splitting has been fairly

complete. Also, it is possible as Bosworth *et al.* [1918] have stated, that the presence of some neutral fat in the stools is partly due at least to mechanical occlusion of the fat in the mass of insoluble soaps. The increased amount of neutral fat in the stools of Cases 1 and 2 may have been dependent on this cause. The appearance of neutral fat in comparatively small excess of the normal in faeces consisting largely of insoluble soap, need not necessarily imply a defect in fat splitting.

It is of interest to note that Schabad [1910] in a series of observations on rachitic children, found a diminished excretion of phosphoric acid by the urine during the active stages of the disease, the increased elimination occurring by the faeces.

As the changes found in the mode of excretion of calcium and phosphorus appeared to be related to the persistence of an excess of free fatty acids in the intestine, the distribution of these elements in the excreta when the amount of fat ingested was varied seemed worthy of study. Further observations were therefore made on a case of congenital biliary atresia in a female infant (Case 4) with a view to ascertaining the effects of increasing concentration of fatty acids in the gut on the excretion of the two elements. The collection of the total urine and faeces quantitatively was found to be impossible in this case. The difficulty of obtaining specimens of faeces uncontaminated by urine could only be overcome by catheterisation before the bowel was evacuated. During an initial period of three days the milk given contained 3 % fat. Separated cow's milk, with the fat content reduced to 0.8 % fat was then substituted for ordinary milk over a succeeding period of three days. Finally an artificially prepared cream (5.3 % fat) was given during the last period. A sample of faeces at the close of each of these intervals was taken for analyses. The following results were obtained.

CASE 4. CONGENITAL BILIARY ATRESIA. J. SY (FEMALE), AET. 3½ MONTHS.

Intake: 720 cc. cow's milk daily.

Table I. *Distribution of fat in dried faeces (%)*.

	Period 2. 0.8 % fat	Period 1. 3.0 % fat	Period 3. 5.3 % fat
Neutral fat	10.68	5.50	15.26
Free fatty acids (as oleic acid)	25.34	39.29	48.43
Combined fatty acids ...	28.44	33.23	17.17
Total	64.46	78.02	80.86

Table II. *Distribution of CaO and P₂O₅ in dried faeces (%)*.

	Period 2. 0.8 % fat	Period 1. 3.0 % fat	Period 3. 5.3 % fat
CaO (total)	8.8	5.37	2.12
CaO (as soaps)	2.9	3.38	1.75
CaO (as phosphate)	5.9	1.99	0.37
P ₂ O ₅ (total)	4.89	1.97	0.65
% CaO as soaps of total CaO ...	32.9	62.9	82.5

The abnormally high excretion of fat is again evident in this case of biliary atresia in which bile was totally absent from the gut. With increase in the fat content of the ingested milk there is an increase of fatty derivatives (Table I) in the faeces and a reduction of the mineral elements. The diminution of the phosphorus content is greater, however, than that of the calcium, which indicates that more phosphoric acid is being removed from combination with calcium as the concentration of fatty acids in the intestinal contents increases.

The increase of calcium soaps and the diminution of phosphoric acid (Table II) in the faeces is evidently correlated with the increased concentration of fat in the diet. Since the degree of absorption of the fatty acids in the absence of bile from the gut has presumably remained at the same low value throughout the three periods, it must be concluded that the changes in the mode of excretion of calcium and phosphorus observed are dependent on the concentration of free fatty acids in the intestinal contents.

In view of these results an explanation of the changes which the calcium and phosphorus of the foodstuff have undergone might be attempted. Calcium is present in cow's milk partly in organic combination, partly as soluble salts, and partly as phosphate. In the presence of the gastric juice the greater part of both the calcium and phosphorus passes into solution. No precipitation of these elements in insoluble form is possible at this stage owing to the presence of free acid. With the passage of the acid gastric contents into the duodenum neutralisation will begin as a result of contact with the alkaline pancreatic secretion and bile. As the reaction of the intestinal contents proceeds towards alkalinity prior to the onset of tryptic digestion the precipitation of insoluble phosphate of calcium before the commencement of fat hydrolysis will take place. In the normal subject a large proportion of the calcium and phosphorus is therefore limited to the gut in insoluble form and as such appears in the faeces. The existence of 80–90 % of the total CaO as phosphate in normal dried faeces lends support to the view. The small proportion of calcium which appears as soaps in normal faeces may be derived possibly from the calcium in combination with casein which undergoes disintegration after the onset of tryptic digestion.

The amount of phosphorus in cow's milk is in excess of the equivalent amount required to combine with all the calcium to form tricalcium phosphate. Although normally a considerable proportion of the calcium may be fixed in insoluble form as calcium phosphate, it does not necessarily follow that the latter will remain unacted upon by the products of digestion. Tri-calcium phosphate is soluble in the lower fatty acids and it can be shown by experiment *in vitro* that precipitated calcium phosphate is redissolved in an excess of the volatile water-soluble fatty acids of butter fat. The latter consist almost entirely of butyric and caproic acids. If the free fatty acids of fat hydrolysis persisted in excess in the intestine, as in the two pathological cases under discussion, the calcium phosphate, if initially precipitated, would tend to pass into solution. In the presence of the higher fatty acids and their soluble soaps

a secondary reaction may then result in the formation of insoluble calcium soaps and the liberation of phosphoric acid.

The calcium salts of the lower fatty acids are soluble and in this state calcium may be absorbed, as suggested by Bosworth *et al.* The phosphoric acid, which has been liberated from combination with calcium phosphate as a result of the precipitation of the calcium as soaps, may then be absorbed as it exists in soluble form. The increased elimination of phosphoric acid by the urine, as found in the two pathological cases, would naturally follow this increased absorption from the intestinal tract.

SUMMARY.

1. Biliary atresia and obstructive jaundice afford a means of studying the effect of non-absorption of fatty acids on the distribution of calcium and phosphorus in the faeces and urine and on the absorption of these elements.

2. In complete exclusion of bile from the gut over 70 % of the dried faeces may consist of fatty derivatives.

3. The non-absorption and persistence of an excess of fatty acids in the intestine is associated with a modification of the normal excretion of calcium and phosphorus. The calcium is found in the faeces as soap in large excess of the normal. The phosphorus, which normally is excreted by the bowel as tricalcium phosphate, is liberated from combination with calcium and is absorbed and eliminated by the urine.

4. The degree to which the normal excretion of calcium and phosphorus can thus be varied is shown to depend on the concentration of the free fatty acids in the intestinal contents.

This work was carried out in connection with the Child Life Investigation of the Medical Research Council. I desire to express my indebtedness to Prof. D. Noël Paton for much helpful advice and criticism.

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