STUDIES ON CALCIUM AND PHOSPHORUS METABOLISM

PART I. THE EXCRETION OF CALCIUM AND PHOSPHORUS

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IN contrast to the large amount of biochemical work which has been undertaken on general problems affecting nutrition, the process of ossification, which is certainly one of the most important of these, has received but little attention. Most researches on this subject have been concerned with the growth and development of bone on histological lines. Investigations into the nature of the biochemical conditions which govern normal ossification have been few. The earlier observers confined themselves chiefly to studies in the composition of bone, largely restricting their field of inquiry to the distribution of the salts of the bone ash. While much of this work is of fundamental importance, our knowledge of the metabolic changes which finally lead to the deposition of the various salts constituting the mineral matter of formed bone has remained fragmentary.

A study of the process of ossification must necessarily involve consideration of the metabolism of calcium and phosphorus, since these constitute the chief elements of the bone-salts. Recently, attention has been directed to their importance in various pathological processes apart from those involving the skeleton. Bosworth, Bowditch, and Giblin (1), Holt, Courtney, and Fales (2), and others in America have demonstrated the necessity for special consideration of calcium in intestinal disorders in infants.

In spite of the increased stimulus given to studies of the metabolism of calcium and phosphorus by the recognition of their importance in many pathological processes, our knowledge concerning the absorption, transport, utilization, and excretion of these elements in the human subject has advanced somewhat slowly, and much of it is based on data derived from experiments on animals.

The present contribution contains the results of observations on the metabolism of calcium and phosphorus in infants fed on cow's milk. It is presented in two parts: (1) The excretion of calcium and phosphorus is first discussed. For the sake of clearness, the experiments are described in sections as follows: (*a*) The influence of diet on the amount of faeces excreted. (*b*) The effects of variation in the intake of calcium and phosphorus on their elimination. (*c*) The distribution of fatty derivatives in the faeces. (*d*) The relation of

phosphorus to fat excretion. (2) The relative retentions of calcium and phosphorus in normal and rachitic infants.

(a) *The relation of faecal solids to diet.* In normal infants on a diet of cow's milk, the mineral matter and fatty derivatives constitute together about one-half of the dried faeces, the composition of which is illustrated by the following analyses :

Analyses of Normal Dried Faeces in Infants (per cent.).

	Diet: Cow's milk.				
	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.
CaO	10.9	10.0	10.1	7.8	8.05
P ₂ O ₅	7.7	8.6	7.7	5.75	5.82
Neutral fat	1.07	1.81	1.62	3.5	3.34
Free fatty acids (as oleic acid)	11.13	10.99	10.43	15.2	16.21
Combined fatty acids	19.15	16.35	17.90	17.1	14.2
Total fat	31.35	29.15	29.95	35.8	33.75
CaO as phosphate	8.94	8.34	8.28	6.06	6.61
CaO as soaps	1.96	1.66	1.82	1.74	1.44

Calcium is eliminated almost entirely by the faeces in combination with phosphorus and as soaps, and one of the first considerations to arise in the study of excretion of the two elements is the relation of ingested mineral matter and fats to the faecal solids. According to Lusk (3), 'common observations would seem to justify the popular supposition that normal faeces are made up of the undigested residues of the food-stuffs. In truth, however, this is very far from the fact. The faeces are chiefly the unabsorbed residues of intestinal secretions.' The evidence upon which this conclusion was based was derived from the experimental studies of Voit and others on animals, and from observations during starving experiments on men.

The following experiments were conducted to test the accuracy of this generalization in the particular case of infants fed on cow's milk, by determining to what extent excretion by the bowel was dependent on the amount of milk solids ingested.

Methods: Each subject was placed on a 'metabolism' bed (4) and the urine and faeces collected separately every twenty-four hours, during periods of several days. The faeces were dried on a steam bath, then weighed, finely ground, and preserved for subsequent examination in air-tight stoppered bottles. The data derived from the analyses of these specimens of urine and faeces are discussed in the following sections.

In the initial period the intake consisted of fresh undiluted cow's milk. During the second period one-half of this measured quantity of milk was given, the bulk being kept constant by dilution with water, and the calorific value by addition of cane sugar. Observations over a third period in which the diet consisted of diluted whey (prepared by rennet coagulation) were also made. The reduction of the milk solids in the diet to such a small concentration (fat, 0.8 per cent.: CaO, 0.04 per cent.: P₂O₅, 0.05 per cent.) generally resulted in the

onset of diarrhoea, and consequently abnormal stools. In these cases, the average daily faecal weight was of little importance for comparative purposes and is not recorded in the tables.

TABLE I
Relation of Faecal Weight to Diet.

Case 1. O. M., aged $1\frac{3}{12}$ years. 8.3 kilos.

Period I. Diet: 1,000 c.c. cow's milk daily.

Day	1	2	3	4	5	6	7	8
Weight of dry faeces	21.1	6.9	20.1	18.9	4.7	18.7	7.3	13.6

Average daily weight = 13.9 gm.

Period II. Diet: 500 c.c. cow's milk and 500 c.c. water.

Day	1	2	3	4
Weight of dry faeces	8.9	6.4	2.4	6.8

Average daily weight = 6.1 gm.

Case 2. A. Q., aged $\frac{8}{12}$ year. 6.5 kilos.

Period I. Diet: 1,000 c.c. cow's milk daily.

Day	1	2	3	4
Weight of dry faeces	11.2	16.6	9.2	16.2

Average daily weight = 13.3 gm.

Period II. Diet: 500 c.c. cow's milk and 500 c.c. water.

Day	1	2	3	4
Weight of dry faeces	8.2	6.5	9.1	10.0

Average daily weight = 8.4 gm.

Case 3. R. A., aged $\frac{3}{12}$ year. 9.4 kilos.

Period I. Diet: 1,000 c.c. cow's milk daily.

Day	1	2	3	4
Weight of dry faeces	15.6	18.2	19.6	23.3

Average daily weight = 19.1 gm.

Period II. Diet: 500 c.c. cow's milk and 500 c.c. water.

Day	1	2	3	4
Weight of dry faeces	8.0	10.6	9.8	8.2

Average daily weight = 9.1 gm.

Case 4. A. G., aged $\frac{6}{12}$ year. 6.45 kilos.

Period I. Diet: 1,000 c.c. cow's milk daily.

Day	1	2	3	4	5	6	7
Weight of dry faeces	7.8	4.5	14.5	nil	13.2	21.8	9.5

Average daily weight = 10.19 gm.

Period II. Diet: 500 c.c. cow's milk and 500 c.c. water.

Day	1	2	3
Weight of dry faeces	6.0	3.8	8.6

Average daily weight = 6.1 gm.

Period III. Diet: 500 c.c. whey and 500 c.c. water.

Day	1	2	3	4
Weight of dry faeces	Nil	Nil	2.1	5.8 (enema)

Average daily weight = 1.9 gm.

TABLE I (*continued*).Case 5. G. K., aged $\frac{9}{12}$ year. 5.2 kilos.

Period I. Diet: 1,000 c.c. cow's milk daily.

Day	1	2	3
Weight of dry faeces	17.2	19.8	18.6

Average daily weight = 18.5 gm.

Period II. Diet: 1,000 c.c. whey daily.

Day	1	2	3
Weight of dry faeces	3.5	2.3	5.3

Average daily weight = 3.7 gm.

Case 6. J. C., aged $\frac{4}{12}$ year. 5.6 kilos.

Period I. Diet: 780 c.c. cow's milk daily.

Day	1	2	3	4	5	6
Weight of dry faeces	10.8	11.0	11.8	14.1	14.0	20.9

Average daily weight = 13.7 gm.

Period II. Diet: 780 c.c. cow's milk daily and 1.0 gm. CaO as calcium lactate.

Day	1	2	3	4
Weight of dry faeces	18.3	26.0	29.0	12.7

Average daily weight = 21.5 gm.

Results : It will be seen that the daily average weight of dried faeces shows a definite decrease with the diminished intake of milk solids. When the solids of the milk in the diet were halved, the faecal weight as shown in the second period was found to be correspondingly reduced. The effect of reducing the milk solids in the intake by using diluted whey is strikingly shown in Case 4, in which no diarrhoea resulted. On the last day of this period the contents of the large intestine were washed out by means of a plain water enema. The substitution of whey for the diet of unmodified cow's milk in Case 5 resulted also in an extreme reduction of the weight of faeces.

Bischoff and Voit (quoted by Lusk, *loc. cit.*) found that the production of faeces in dogs was not proportional to the amount of meat ingested. The faeces of herbivora, however, consist largely of undigested residues of the food, and consequently the weight of dry faeces depends on the intake of solids.

The conclusion to be drawn from the results recorded is, that in infants on a diet of cow's milk the production of faeces also depends on the amount of milk solids of the diet. In each case non-nitrogenous matter in the form of calcium phosphate, calcium soaps, and fatty derivatives formed about one-half of the faecal solids, and, consequently, the reduction of the average daily faecal weight observed must have been largely due to a correspondingly reduced evacuation of these from the bowel.

It was therefore of importance to ascertain how much of the non-nitrogenous matter of the faeces was derived directly from the elements of the milk solids. Further observations were accordingly made on the elimination of calcium,

phosphorus, and fats in relation to the amounts of these ingested, with the object of studying the conditions which govern their excretion. The large proportion of calcium salts in the non-nitrogenous matter of the faecal solids suggested that the amount of this element in the diet was one of the chief factors in influencing the faecal weight, e.g. the effect of adding 1 grm. of CaO daily (as calcium lactate) to the intake in Case 6 was a pronounced increase in the average weight of dried faeces excreted per day.

(b) *The effects of variation in the intakes of calcium and phosphorus on their elimination.* In the preceding series of experiments the mineral content of the diet during the first period (unmodified cow's milk) was double that of the second period (diluted milk) and approximately five times that of the third (diluted whey). Considerable variations were therefore made in the amounts of CaO and P_2O_5 ingested during these periods. The effects of variations in the intake thus made on the excretion of calcium and phosphorus, and on faecal weight, were also studied in two additional experiments. In one of these calcium was omitted almost entirely from the intake; in the other an attempt was made to ascertain the influence of fat and soluble phosphate on faeces formation, by their addition separately to a diet deficient in both.

Method: Faeces and urine were collected as already described. The total CaO and P_2O_5 in the diet and the excreta corresponding to each period were determined, the estimations being made by the usual gravimetric methods. The following results were obtained:

TABLE II.

Relation between the Amounts of Calcium and Phosphorus ingested and excreted (per Day).

Case.	Period.	Intake.		Weight of Dried Faeces.	Excretion.					
		CaO grm.	P_2O_5 grm.		Urine.		Faeces.			
					CaO grm.	P_2O_5 grm.	CaO grm.	%	P_2O_5 grm.	%
I										
1. O. M. $1\frac{3}{4}$ years	8 days cow's milk	1.65	2.2	13.9	—	1.17	1.6	11.5	1.05	7.35
II										
	4 days diluted milk	0.82	1.1	6.1	—	0.93	0.67	11.1	0.33	5.43
I										
2. A. Q. $1\frac{1}{2}$ year	4 days cow's milk	1.65	2.2	13.3	0.003	0.93	1.22	9.2	0.94	7.06
II										
	4 days diluted milk	0.82	1.1	8.45	0.002	0.59	0.68	8.05	0.49	5.82
III										
	4 days whey	0.50	0.55	diarrhoea	0.002	0.48	0.16	1.61	0.04	0.43

TABLE II (*continued*)

Case.	Period.	Intake.		Weight of Dried Faeces.	Urine.		Excretion.			
		CaO gm.	P ₂ O ₅ gm.		CaO gm.	P ₂ O ₅ gm.	CaO gm.	%	P ₂ O ₅ gm.	%
3. R. A. $\frac{9}{12}$ year	I 4 days cow's milk	1.6	2.0	19.1	—	0.92	1.36	7.1	0.74	3.9
	II 4 days diluted milk	0.80	1.0	9.1	—	0.56	0.56	6.2	0.24	2.68
	III 4 days diluted whey	0.3	0.4	dia- rrhoea	—	0.25	0.23	1.9	0.11	0.9
	IV 3 days sugar and butter fat	0.02	0.02	dia- rrhoea	—	0.2	0.04	0.54	0.03	0.47
	V 3 days sugar and butter fat + 0.1 gm. CaO as calc. lactate	0.13	0.02	stools solid	—	0.05	0.14	0.69	0.06	0.30
	VI 3 days sugar and butter fat + 0.2 gm. CaO as calc. lactate	0.29	0.02	stools solid	—	Nil	0.29	1.9	0.05	0.35
4. A. G. $\frac{6}{12}$ year	I 7 days cow's milk	1.62	2.05	10.19	0.027	0.78	1.52	14.27	1.11	10.93
	II 3 days diluted milk	0.81	1.02	6.1	0.016	0.60	1.0	16.5	0.67	11.09
	III 3 days diluted whey	0.3	0.44	1.9	0.006	0.40	0.28	14.5	0.21	10.8
5. G. K. $\frac{1}{12}$ year	I 3 days cow's milk	1.66	2.22	18.5	0.024	0.40	1.48	8.05	1.45	7.89
	II 3 days diluted whey	0.21	0.43	3.7	0.02	0.25	0.18	5.5	0.24	6.55
	III Diluted whey + 1.8 gm. CaO as calc. lactate	2.01	0.43	4.9	0.032	0.10	1.03	20.9	0.27	5.88
	IV Diluted whey butter fat + 1.8 gm. CaO as calc. lactate	2.01	0.43	10.0	0.034	0.08	1.88	15.5	0.37	3.77
	V Diluted whey butter fat + 1.8 gm. CaO as calc. lactate + 1.25 gm. P ₂ O ₅ as NaH ₂ PO ₄	2.01	1.68	10.2	0.16	0.22	1.42	13.45	0.89	8.44
6. J. C. $\frac{4}{12}$ year	I Cow's milk 3 days	1.28	1.71	13.7	0.008	0.61	1.24	11.15	0.85	8.05
	II Cow's milk + 1.0 gm. CaO as calc. lactate	2.28	1.71	21.5	0.12	0.39	1.72	8.05	0.92	4.32

In each period the amounts of CaO and P_2O_5 ingested daily are compared with those found in the excreta, and with the corresponding average daily weight of dried faeces. It is hardly necessary to state that values for the retentions of the two elements cannot be derived from the data presented, since these are not balance experiments in a quantitative sense (see Part II, p. 65).

An examination of Table II shows that in all the periods, the elimination of CaO and P_2O_5 was simply dependent on the amounts of these in the diet, and further that with diminished intake there was a corresponding diminution of calcium and phosphorus in both urine and faeces. The variation in the average daily weight of dried faeces with the calcium content of the diet is also noteworthy. During the periods when the amount of calcium ingested was small, diarrhoea often ensued. The stools in these cases were abnormal owing to the escape of an excess of fatty derivatives, and the weight of faeces was not recorded.

Case 1. The percentage CaO of the dried faeces is almost the same in both periods, while the reduction in faecal weight during the second period is nearly proportional to the diminution in the amount of CaO ingested.

Case 2. The percentage of CaO during the second period is slightly less than that of the first. The difference is not proportional to the variation in the intake, however. The reduction in faecal weight again approximately corresponds to the diminution of CaO ingested. In Period III there was diarrhoea. The low values for the percentages of CaO and P_2O_5 in the faeces recorded are here, as in the other experiments, due to an excess of fatty derivatives in the faeces.

Case 3. The first three periods present the same features as in Case 2. In Period IV the diet was practically free from calcium and phosphorus. The stools were fluid. The extreme reduction of the two elements in the faeces is correlated with the low intake, the excretion of calcium especially being almost negligible over a period of three days. The ash of the dried faeces during this period was easily fusible and appeared to consist chiefly of alkaline salts. This result shows that there was practically no calcium excreted from endogenous sources, and extremely little phosphorus, if any.

The addition of a comparatively small quantity of calcium (0.1 gm. CaO) daily to the diet in Period V immediately resulted in the faeces becoming white and semi-solid. In this and the succeeding period (0.2 gm. CaO added) the corresponding increase in the excretion of calcium by the faeces is evident. With the addition of calcium to the diet of Period IV, the urine finally became phosphorus free. It should be noted that although the addition of the small quantities of calcium to the diet during Periods V and VI changed the consistency of the faeces from a fluid to a semi-solid condition, there was still an excessive loss of fat by the bowel. This change in the nature of the faeces is discussed later, when it will be shown to have been due to an increased formation of calcium soaps.

Case 4. No diarrhoea occurred during Period III (diluted whey) in this case, and the effects of reduction in the intake of CaO and P_2O_5 are clearly illustrated. The percentage of CaO and P_2O_5 in the dried faeces remained practically constant throughout the whole experiment, while the average daily weight of dried faeces was reduced in proportion to the amount of CaO ingested. The reduction of the calcium and phosphorus in both urine and faeces with the diminished intake is again to be observed.

Case 5. During the first period, the average weight of dried faeces was very high (18.5 gm.). The mineral matter in the intake and the fat were

excessively reduced in Period II by diluting the whey of the diet to twice its volume with water. The intake of fat was only 3.9 gm. per day. The extreme reduction of the faecal weight (3.7 gm.) will be observed.

The effects of an excess of a soluble calcium salt in a diet deficient in fat and mineral matter were followed by adding 1.8 gm. of CaO as calcium lactate to the diluted whey (Period III). Since the combining weight of fatty acids is approximately ten times that of calcium in the formation of calcium soaps, there was a large excess of CaO in the diet above that required to combine with all the fatty acids and phosphoric acid derivable from the diluted whey. Interest centred therefore in the mode of excretion of calcium during this period, and particularly in the question as to whether an excess of calcium induces a compensatory increased secretion of phosphoric acid into the intestine.

The dried faeces obtained were brick-hard, and effervesced strongly in dilute hydrochloric acid. Subsequent examination showed that about 70 per cent. of the total CaO of the dried faeces existed as carbonate of lime. The large increase of the percentage of CaO in the faeces is also striking. The amount of phosphoric acid excreted by the intestinal route was practically the same as that of the preceding period, though the urinary phosphorus was less. There was a slight increase in the urinary calcium. In spite of the large increase of calcium salt in the diet practically no change in the average daily weight of dried faeces resulted, and in order to ascertain whether this was due to the relative absence of fat or phosphoric acid in the diet, additions of these were made separately over the two subsequent periods.

During Period IV 15 gm. of milk fat were added daily to the diluted whey diet of Period III, the calcium content being unaltered. The average daily weight of dried faeces rose immediately from 4.9 gm. to 10.0 gm. On examination of the distribution of fat in the faeces (see later) it was found that nearly all the calcium of the faeces was in combination with fatty acids. No carbonate of lime was found.

An attempt was again made to increase the average daily weight of dried faeces by the addition of an excess of a soluble phosphate to the diet (Period V). 1.25 gm. of phosphoric acid as NaH_2PO_4 were added daily to the diet of Period IV.

The percentage of P_2O_5 in the faeces was increased though a portion of the added phosphorus had been excreted by the urine. The calcium content of the urine was considerably increased, probably, as Ott has stated, by the elimination of the calcium as CaHPO_4 . The faecal weight remained unaltered.

Case 6. This experiment consisted essentially in following the effects of an excess of calcium added to a diet containing adequate quantities of fat and phosphoric acid. In both periods the same amount of unmodified cow's milk was given, 1 gm. of CaO as calcium lactate being added daily to the intake during the second period. The effects produced by the addition of the calcium salt were the same as those observed in previous experiments. There was a considerable increase of faecal weight. More phosphoric acid was excreted by the faeces, and consequently less by the urine. An increased elimination of calcium by the urine as well as by the faeces occurred. There was a notable fall in the percentages of CaO and P_2O_5 in the dried faeces during the second period. This was subsequently found to be due to the presence of an increased amount of fatty derivatives.

Discussion. From these results there would appear to be little doubt that in infants fed on cow's milk the amount of calcium and phosphorus eliminated depends on the amounts ingested. With a diminution in the intake there was a corresponding fall in the amounts excreted, and when calcium and phosphorus

in the diet were reduced to minute quantities, the amounts of the two elements found in the excreta were correspondingly small.

When an excess of calcium salt was present in the diet there was an increased excretion of calcium by the faeces and a slight rise in the urinary calcium. The increase of calcium in the urine indicated that increased absorption had occurred. R. Berg (5), who investigated the retention of different calcium salts, found that the excretion of the excess took place almost entirely by the intestinal route.

The effect of an excess of calcium in the diet on the excretion of phosphorus is clearly shown. More phosphoric acid was found in the faeces and less in the urine. The calcium therefore served to restrict the phosphorus to the intestinal contents.

These conclusions confirm the work of previous observers on the elimination of calcium and phosphorus. An exhaustive review of the literature on the subject has been made by Forbes and Keith (6), from which the following references are taken. The decrease by one-half in urinary phosphorus, following the ingestion of 10 grm. of carbonate of lime per day, was found by Riesell, who experimented on himself. Similar observations were made by Lehmann, Strauss, and Herxheimer. Rüdell showed that the administration of calcium carbonate to children caused an increase in the urinary calcium, but not in proportion to the intake. Kochmann and Petsch working on dogs found that an increase of lime in the diet was followed by an increase of faeces phosphorus and a diminution of urinary phosphorus. Similar results were obtained by Bertram and Renvall. In an experimental study of calcification in dogs, with D. Noel Paton, the writer found that 88 per cent. of the phosphorus ingested was excreted by the urine. On the same diet, with the addition of a large excess of calcium lactate, only 15 per cent. of the ingested phosphorus appeared in the urine, a greatly increased amount being eliminated by the faeces.

By the addition of a large excess of calcium to a diet deficient in fat and phosphorus, the urine could be rendered phosphorus free, most of the ingested phosphorus being restricted to the intestine, and the excess of calcium eliminated by the faeces as carbonate.

Finally, the influence of calcium in the diet in controlling the amount of faecal solids excreted has been shown to be very great. This effect is apparently dependent chiefly on calcium soaps, and therefore on the extent of the combination of the ingested calcium with fatty acids in the intestine. It did not occur in the absence of an adequate amount of fat in the diet, or in the presence of an excess of phosphoric acid.

(c) *The distribution of fatty derivatives in dried faeces, and the relation between calcium soaps and faecal solids.* The distribution of the fatty derivatives is of interest in view of the governing influence which calcium soaps possess on excretion by the bowel. One of the best illustrations of the effects of an excess of calcium soap formation in the intestine is seen in jaundice. In a case of congenital obliteration of the bile ducts (7) studied by the writer, in which

fat-splitting was not appreciably diminished, though there was a persistent excess of free fatty acids in the intestine from non-absorption of digested fat, the average daily weight of dried faeces was consistently more than double that of a normal infant of the same age when the diets of unmodified cow's milk were identical. It was found that about 80 per cent. of the total calcium of the faecal solids was combined with fatty acids in the case of biliary atresia; the normal faeces contained only 20 per cent. of the total calcium in this form.

Examination of the distribution of the fatty derivatives in the dried faeces collected in the preceding experiments were made chiefly with the object of ascertaining the relation between the average daily weight of faecal solids and the amount of calcium soaps these contained.

Method: The neutral fat and free fatty acids were extracted directly with ether. After drying and weighing, the mixture was dissolved in neutral alcohol and the free acids titrated with $\frac{N}{10}$ NaOH, the latter being calculated to oleic acid. The neutral fat was found (approx.) by difference. The combined fatty acids were obtained from the residue of the first extraction by splitting the insoluble soaps with dilute hydrochloric acid and extracting with ether in a separating funnel. The weight of calcium soaps in the dried faeces was then calculated from the combined fatty acids, the mean molecular weight of these being taken as 274.0.

It has been shown by Holt *et al.* (2), who determined the distribution of fatty derivatives in dried and moist samples of a composite stool, that in the process of drying practically no change in composition occurs in the faeces of infants fed on cow's milk. The following results were obtained:

TABLE III

The Distribution of Fatty Derivatives and the Relation between Calcium Soaps and Faecal Solids.

Case 1. No analysis made of the faecal solids.

Case 2. A. Q.

	Period I.	Period II.	Period III.
	Milk.	Diluted Milk.	Whey.
Neutral fat	6.57	3.34	32.93
Free fatty acids (as oleic acid)	17.58	16.21	30.91
Combined fatty acids	17.10	14.2	3.12
Total fats	41.25	33.75	66.96
Average daily weight of faecal solids	13.3 grm.	8.4 grm.	Diarrhoea
Weight of calcium soaps excreted daily	2.41 grm.	1.26 grm.	—

TABLE III (*continued*).*Case 3. R. A.*

	Period I.	Period II.	Period III.	Period IV.	Period V.
	Milk.	Diluted Milk.	Whey.	Whey + 0.1 gram. CaO as calc. lactate.	Whey + 0.2 gram. CaO as calc. lactate.
Neutral fat	2.13	3.10	14.9	51.63	40.04
Free fatty acids	17.62	12.4	25.9	27.07	22.56
Combined fatty acids	31.2	21.7	5.9	9.4	13.0
Total fats	50.95	37.2	46.7	88.10	75.6
Average daily weight of faecal solids	19.1 gram.	9.1 gram.	Diarrhoea	Semi-solid stools	Semi-solid stools
Weight of calcium soaps excreted daily	6.30 gram.	2.11 gram.	—	—	—

Case 4. A. G.

	Period I. Cow's Milk.	Period II. Diluted Milk.	Period III. Whey.
Neutral fat	1.9	2.9	3.4
Free fatty acids	3.3	4.2	1.7
Combined fatty acids	31.2	35.2	20.1
Total fats	36.4	42.3	25.2
Average daily weight of faecal solids	10.19 gram.	6.1 gram.	1.9 gram.
Weight of calcium soaps ex- creted daily	3.26 gram.	2.2 gram.	0.3 gram.

Case 5. G. K.

	Period I.	Period II.	Period III.	Period IV.	Period V.
	Milk.	Whey.	Whey + 1.8 gram. CaO as calc. lactate.	Whey + 1.8 gram. CaO + butter fat.	Whey + 1.8 gram. CaO + butter fat + 1.2 gram. P_2O_5 as NaH_2PO_4 .
Neutral fat	3.0	1.2	0.81	2.0	1.7
Free fatty acids	21.1	5.1	0.49	3.6	7.6
Combined fatty acids	16.1	24.4	9.2	30.1	33.9
Total fats	40.2	30.7	10.5	35.7	43.2
Average daily weight of faecal solids	18.5 gram.	3.7 gram.	4.9 gram.	10.0 gram.	10.2 gram.
Weight of calcium soaps excreted daily	3.15 gram.	0.94 gram.	0.42 gram.	3.15 gram.	3.67 gram.

Case 6. J. C.

	Period I. (Milk.)	Period II. (Milk—1 gram. CaO as calc. lactate.)
Neutral fat	1.7	3.2
Free fatty acids	10.2	15.4
Combined fatty acids	29.6	26.6
Total fats	41.5	45.2
Average daily weight of faecal solids	13.7	21.5
Weight of calcium soaps excreted daily	4.3 gram.	6.1 gram.

Case 1. The distribution of the fatty derivatives was not determined in this case owing to accidental loss of the specimens.

Case 2. The excretion of total fat during the first period was slightly above the normal. The distribution of fatty derivatives during the second period shows that only minor changes in composition resulted when the intake of milk solids was reduced by one-half, the chief one being a reduction of the total fatty derivatives. The fall in faecal weight and diminution of calcium soaps are seen to be roughly proportional to each other. In Period III diarrhoea ensued, and the faecal weight was consequently without quantitative significance. The large excess of neutral fat and free fatty acids found in the faeces is characteristic of diarrhoea and is evident in all the other cases of the series. Correlated with the excessively reduced intake of calcium during this period, the percentage of combined fatty acids and therefore of calcium soaps in the faecal solids is very low.

Case 3. In Period I the percentage of total fat is again abnormally high, and especially the combined fatty acids. The distribution of the fatty derivatives in the second period is, in this case, appreciably different from that of the first, and a strict correspondence between the reduction in faecal weight and calcium soaps was therefore not to be expected. The correlated fall in each of these with the reduced intake is fairly well shown, however. Diarrhoea ensued in the third period, and the distribution of the fatty derivatives presented all the features described in the preceding case. In Period IV the stools were rendered pasty in consistency on the addition of 0.1 grm. CaO as calcium lactate. In this and the succeeding period there was still an escape of a large excess of neutral fat and free fatty acids as in diarrhoeal stools in spite of the changed consistency of the faeces. The increase of combined fatty acids and therefore of calcium soaps in both periods is again correlated with the increasing additions of calcium to the diet. It would seem as if the increased formation of calcium soaps in the two last periods had been responsible for the physical change in the nature of the faeces. In this way the therapeutic effects of calcium salts in diarrhoea may be explained.

Case 4. The effects of the variations made in the intake are well illustrated in this case. The distributions of the fatty derivatives in the faeces of the first two periods show but slight differences; the notable result is the reduction of the faecal weight with the calcium soaps. The diminution in faecal weight and calcium soaps during the third period, following the substitution of whey for diluted milk in the diet, is very pronounced. The fall in percentage of combined fatty acids and total fat in this period is again correlated with the low content of fat and of calcium in the diet.

Case 5. All the features associated with the variations made in the calcium of the intake in the previous experiments are reproduced in Case 5. In Period II diluted whey was substituted for unmodified milk, so that both the fat and calcium of the intake were very considerably reduced. The fall in faecal weight and the corresponding diminution of the quantity of calcium soaps in the faeces are in consequence very pronounced.

In Period III the addition of a large excess of CaO to the diet is seen to have had no appreciable effect on the faecal weight. There is a striking fall in the percentage of combined fatty acids, evidently due to the low fat content of the whey. The greater part of the calcium was shown to have been excreted as a carbonate in the faeces (p. 52).

The fat content of the diet was then increased by the addition of milk fat to the whey, the calcium content remaining unaltered. The distribution of the fat in Period IV shows that a prompt increase in calcium soap formation had

occurred, the percentage of combined fatty acids in the faeces being more than three times that of the preceding period. The rise in the faecal weight, the percentage of total fatty derivatives, and the amount of calcium soaps excreted are evident.

The addition of acid sodium phosphate to the diet (Period V) made no appreciable difference in the average daily faecal weight, and the extent to which calcium soap formation proceeded remained practically unaltered.

Case 6. The last experiment of the series was designed to ascertain the effects of calcium added to the diet, when all the other constituents were unaltered. In the second period, 1 grm. CaO as calcium lactate was added daily to the unmodified milk diet of Period I. Adequate amounts of fat and phosphorus were therefore present, though calcium was in excess. No appreciable change in the distribution of the fatty derivatives resulted. The total amount of calcium soaps excreted, however, was increased, and there was a very pronounced rise in the average daily weight of dried faeces. These results serve to corroborate the findings of the previous experiments in which reductions in the amount of calcium ingested are made.

Discussion. Holt, Courtney, and Fales (2), who examined the fat distribution in the stools of a large number of infants, found that on a diet of cow's milk the average percentage of fat of the faecal solids was 36.2. Hutchison (8), working on fat absorption in children, showed that the total fatty derivatives generally formed about one-third of the dried faeces. In the first period of each experiment the total fat of the faeces was generally above the normal, and in Case 3 very considerably higher. The relation between an excess of fat in the stools and the mode of elimination of calcium and phosphorus is discussed later.

When diarrhoea ensued, the distribution of the fatty derivatives was completely changed. There was an excessive loss of neutral fat and free fatty acids. In all cases this change in the nature of the faeces was coincident with an extreme reduction in the calcium of the intake, to which must be attributed the smaller amount of calcium soaps in the faecal solids. Holt *et al.* (2), in their studies on fat excretion, constantly found a diminished percentage of calcium soaps in diarrhoeal stools. They also showed that the addition of calcium carbonate to the diet in diarrhoea led to an increased formation of calcium soaps, an observation which receives corroboration from the result of the three last experiments of the series. In one of these (Case 3) the addition of a small quantity of calcium to the diet was followed by an appreciable increase in the percentage of calcium soaps in the faeces. The alteration in the physical state of the stools is interesting in view of this chemical change. These became white and semi-solid, and diarrhoea immediately ceased, an occurrence which suggests that calcium soaps may play an important part mechanically in controlling excretion by the bowel. According to Bosworth, Bowditch, and Giblin (1) the presence of an excess of calcium soaps in the intestine may be the cause of severe constipation in young infants fed on unmodified cow's milk. These observers have demonstrated the insolubility of calcium salts of the higher fatty acids in the intestinal juices, and hold that neutral fat and free fatty acids are incorporated in the mass of insoluble soaps formed in the intestine. There is therefore an undue loss of fatty derivatives in the stools from diminished

absorption, in addition to the deleterious effects produced by constipation. Their conclusions as to the mechanical action of calcium soaps receive considerable support from the results recorded. In the second period of the experiments, when the intake of milk solids was halved, there was a corresponding fall in the faecal weight and the amount of calcium soaps excreted, and the composition of the faeces with respect to fatty derivatives approximated to the normal. Again, the ingestion of an excess of calcium in the absence of an adequate amount of fat in the diet had no effect on the faecal weight (Case 5), since the formation of calcium soaps could not take place, while the addition of calcium to a diet rich in fat was immediately followed by an increased excretion of insoluble soaps and consequently of faecal solids.

These results also show that the percentage of fatty derivatives in the faeces depends on the quantity of fat ingested when this is beyond certain limits, but that their excretion is largely influenced by the degree to which calcium soap formation in the intestine proceeds.

With regard to the influence of calcium soap formation on faecal weight, it will be observed that this is not due to the weight of the soaps themselves, but apparently to the property which they possess of incorporating or occluding the other constituents of the intestinal contents in amount proportional to their mass. In this respect calcium soaps may be regarded as the basis of faeces formation. On a diet of cow's milk the composition of the dried faeces is approximately constant for the same individual, and within limits independent of the amount ingested. This fact may be explained on the assumption that the calcium soaps formed in the gut incorporate a proportional amount of the intestinal contents, the latter being derived chiefly from the milk solids, in which the mineral elements and the fat bear a constant relation to each other.

(d) *The relation of phosphorus to fat excretion.* Cow's milk contains an excess of phosphorus over the equivalent amount required to form tri-calcium phosphate with all the available calcium, a condition possibly correlated with the requirements of the growing tissues other than bone, in the building up of phospho-lipoids, nucleic acid, and other organic compounds of phosphorus.

In contrast to calcium, the excretion of phosphorus in the urine is considerable, though generally less than the phosphorus excreted by the bowel in combination with calcium, in the case of healthy infants on a diet of unmodified cow's milk. The greater part of the total calcium eliminated in the faeces is in combination with phosphoric acid, the remainder being in the form of insoluble soaps (p. 46). It was therefore of interest to follow the excretion of phosphoric acid in relation to that of fat when the derivatives of the latter in the faecal solids were found to vary.

Method: The mode of elimination of phosphorus in normal subjects was compared with that in a series of cases in which the excretion of fatty derivatives was excessive, the diet in all cases being unmodified cow's milk. The data were obtained as before by estimations of CaO and P_2O_5 in the separated urine and dried faeces. The results are arranged in Tables IV and V.

TABLE IV

Comparison of Excretion of Phosphorus by Urine and Faeces.

Diet: Unmodified Cow's Milk.				
	<i>Case 1.</i>	<i>Case 2.</i>	<i>Case 3.</i>	<i>Case 4.</i>
Period of observation	5 days.	5 days.	4 days.	5 days.
P ₂ O ₅ in urine	39.3 %	37.0 %	40.4 %	32 %
P ₂ O ₅ in faeces	60.7 %	63.0 %	59.6 %	68 %
Ratio of CaO to P ₂ O ₅ in Dried Faeces.				
	<i>Case 1.</i>	<i>Case 2.</i>	<i>Case 3.</i>	<i>Case 4.</i>
Period of observation	5 days.	4 days.	6 days.	5 days.
CaO to P ₂ O ₅	1.27	1.4	1.3	1.35
CaO %	9.86	9.8	8.62	7.8
P ₂ O ₅ %	7.72	6.9	6.44	5.75

Results: The ratio of CaO to P₂O₅ in the normal dried faeces, which contained approximately 30 per cent. of total fat, was relatively constant and gave an average of 1.3. This result is in agreement with the observation of Von Wendt, quoted by Keith and Forbes (6), that the greater part of the calcium in the faecal solids exists as tri-calcium phosphate. The ratios found in normal dried faeces are slightly higher than the ratio of CaO to P₂O₅ in the latter salt (1.18), since a small proportion, generally about one-fifth of the total calcium, is in combination with fatty acids as insoluble soaps (6).

Of the total phosphoric acid eliminated 30 per cent. to 40 per cent. appeared in the urine, the greater part of the phosphorus having been excreted in combination with calcium in the faeces. The absorption of phosphorus is therefore considerably in excess of the amount retained.

The distribution of phosphoric acid in the urine and faeces, when the fat content of the latter was abnormally high, is shown in Table V.

TABLE V

Distribution of Fat and Phosphoric Acid.

Diet: Unmodified Cow's Milk.								
No.	Duration of Observation.	Neutral Fat.	Free Fatty Acids.	Com- bined Fatty Acids.	Total Fatty Acids.	Total Fat.	CaO P ₂ O ₅ faeces.	% P ₂ O ₅ urine. % P ₂ O ₅ faeces.
1. Rickets	4 days	4.17	22.5	13.0	35.5	39.67	1.58	46.6 53.4
2. Rickets	4 days	6.57	17.58	17.10	34.68	41.25	1.31	49.8 50.2
3. Fat dys- pepsia	4 days	2.13	17.62	31.20	48.82	50.95	1.8	55.3 44.7
4. Marasmus	1 day	9.89	18.61	40.9	59.51	69.4	2.1	—
5. Congenital obliteration of the bile ducts	4 days	13.18	34.92	27.67	62.59	75.77	3.06	74.4 25.6

These results show that an increase in the percentage of fatty derivatives is associated with an increased amount of phosphoric acid in the urine and a diminution in the faeces. Consequently the ratio of CaO to P_2O_5 in the faecal solids is high (cf. Table IV). With the exception of the second case, these ratios form an ascending series corresponding to the increasing percentages of fatty derivatives in the dried faeces. A correlated deflexion of phosphoric acid from the faeces to the urine is also indicated. In Case 4 the urine could not be collected quantitatively, but the high ratio of CaO to P_2O_5 in the faeces show that the urinary phosphorus must have been considerably greater than the normal.

The chief conclusion to be drawn from these results is that the extent of the deviation of phosphorus from the intestinal route of excretion to the urine is dependent on the amount of free fatty acids which persists in the gut contents.

It has been shown that an excess of fatty derivatives in the stools is associated with an unduly large formation of calcium soaps (Table III). The explanation of the partial deviation of phosphoric acid from the faeces to the urine would therefore appear to be that the normal restriction of phosphoric acid to the gut in the form of insoluble phosphate of lime is interfered with to an extent which is relative to the concentration of fatty acids in the intestinal contents. It has been shown that the opposite effect can be produced experimentally (pp. 52 and 53) by adding an excess of calcium salt to a diet containing a relatively small amount of fat. According to Bosworth *et al.*, the phosphoric acid existing as CaHPO_4 in milk is restricted to the intestine and appears as such in the faeces. The phosphorus combined in the casein is excreted by the urine. The latter can be rendered phosphorus free by feeding on calcium caseinate and calcium acetate.

A point of practical interest is the nature of the faeces, which are associated with this alteration in the mode of phosphorus excretion. The stools, since they contain an excess of calcium soaps, are bulky, and the average daily weight of dried faeces is high. The increased excretion of fatty derivatives leads to a diminished concentration of mineral matter, which in normal infants on a milk diet is about 22 per cent. of the dried faecal weight. The percentages of CaO and P_2O_5 found in the dried faeces are consequently very low (v. Table II). The stools are generally white and of a pasty consistency, and may actually melt in the process of drying on a water-bath. On ignition the dried faeces burn with a smoky, luminous flame, leaving a flaky scattered ash consisting chiefly of calcium carbonate in contradistinction to the compact ash of normal faeces, in which the lime is almost wholly in combination with phosphoric acid.

The series of cases under discussion is also of interest from the clinical point of view, since the presence of an excess of fatty derivatives in the faeces of infants fed on unmodified cow's milk is so frequently encountered. In Case 6 (congenital obliteration of the bile ducts) there was a very large excess of fatty acids in the faeces, a condition due to non-absorption of digested fat and invariably associated with the absence of bile from the gut (6). Jaundice was never present at

any time in the other cases of the series. The excess of fatty derivatives in the faeces and the deflexion of phosphorus from the faeces to the urine suggest, however, that in these hepatic insufficiency, varying in degree, may have been a feature common to all. According to Brodin (9) there may be severe functional insufficiency of the liver, characterized by a retention of bile in the blood without the appearance of jaundice. Since, as has been demonstrated, the excretion of calcium, phosphorus, and fats are so closely interdependent, attention might be profitably directed to the study of the liver functions in connexion with the absorption and retention of the mineral elements, and especially in diseases of infants such as rickets and fat intolerance, in which the aetiology is still obscure.

Summary. 1. The excretion of calcium, phosphorus, and fatty derivatives are interdependent, calcium being eliminated chiefly as phosphate and to a lesser extent as insoluble soaps, the relative amounts of these varying with the conditions of the intestinal contents.

2. 20 to 30 per cent. of the total CaO excreted normally exists in the form of calcium soaps.

3. A large proportion of the ingested calcium and phosphorus is restricted to the gut, and appears in the faeces as tri-calcium phosphate. Consequently the absorption of a considerable part of the intakes of these is normally prevented.

4. The total amount of calcium and phosphorus eliminated is nearly proportional to the intake. No evidence was obtained that mineral matter from endogenous sources was excreted in the faeces to any appreciable extent.

5. The urinary calcium is a very small fraction of the total calcium excreted. It is slightly increased by an increased intake of calcium salts, by the administration of acids salts, and, generally, by acid formation in the intestine.

6. In normal infants 40 per cent. of the total phosphorus excreted appeared in the urine, 60 per cent. in the faeces. With acid formation in the intestine, less phosphorus is excreted by the faeces, more by the urine. It was found that the degree of deviation of phosphorus to the urine was roughly proportional to the amount of fatty acids in the faeces, and consequently to the extent to which fatty acids had displaced phosphoric acid from its normal combination with calcium in the intestine.

7. It is argued that the phosphorus which is excreted by the faeces has been in greater part restricted to the intestine owing to the presence of calcium salts, and cannot be regarded as a re-excretion into the intestine. The excess of phosphorus over the equivalent amount of calcium forming tri-calcium phosphate is absorbed, and a part of this is retained, the remainder being excreted in the urine.

8. An excess of calcium salts restricts an increased quantity of phosphorus to the intestine. In a particular case, in which the intakes of fat and phosphorus were low, an excess of calcium in the diet rendered the urine phosphorus free.

9. With a persistent excess of fatty acids in the intestine a much greater proportion of calcium is excreted as soaps.

10. The influence of calcium soaps in controlling the bulk of faeces is discussed. The average daily faecal weight is shown to depend chiefly on the degree to which calcium soap formation proceeds. The calcium soaps act mechanically by incorporating in their mass fatty derivatives, the mineral residues of the diet, and the unabsorbed intestinal secretions, and consequently are regarded as the basis of faeces formation. The faecal solids of infants are shown to be derived in large part from the diet.

11. When both fat and phosphorus are very deficient in the diet, an excess of calcium in the intake may be excreted as carbonate.

12. No evidence of absorption of calcium in excess of requirements with subsequent re-excretion into the bowel was obtained. On the other hand, all the observations made seemed to indicate that the greater part of the calcium in the intake is restricted to the gut; and, except the small amount excreted in the urine, the amount absorbed is nearly equivalent to the amount retained.

13. The excretion of calcium and phosphorus in relation to fats is discussed in certain pathological cases, in which an excessive soap formation was a feature common to all. It is suggested that in these the common causative factor was a persistence of free fatty acids in the intestine, possibly secondary to some degree of hepatic insufficiency.

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