Studies on the Intestinal Absorption and Excretion of Calcium and Phosphorus in the Pig

1. A Critical Study of the Bergeim Technique for Investigating the Intestinal Absorption and Excretion of Calcium and Phosphorus

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Owing to the importance of calcium and phosphorus in nutrition it is not surprising that problems relating to the intestinal absorption and excretion of these elements have received considerable attention during the last 30 years or so. Nevertheless, there are a number of points on which information is limited and conclusions drawn from results reported in the literature are somewhat conflicting.

For instance, it is generally accepted that the absorption of Ca and P takes place in the small intestine, but Fournier (1951*a*) and Fournier & Dupuis (1953*a, b*) are of the opinion that the absorption of Ca and P from the stomach is of considerable importance. Also, the weight of experimental evidence obtained over the past years indicates that excess Ca is not excreted through the wall of the large intestine although a number of workers, using essentially the same technique, for example Bergeim (1926), Marek, Wellmann & Urbanyi (1935) and Fournier (1950*a, b*, 1951*a, b*) have obtained results which they consider to be evidence of such an excretion.

The present study was undertaken in an attempt to clarify some of the problems relating to intestinal absorption and excretion of Ca and P. The object of the experiment reported here was to investigate the suitability of the Bergeim (1926) technique for studying the intestinal absorption and excretion of Ca and P in the pig.

EXPERIMENTAL

The pigs and their diet

The experimental pigs, castrated male Saddlebacks, were placed in metabolism cages when 9–10 weeks old. Previously they had been reared out of doors on the University farm. The floors of the metabolism cages were constructed to enable urine to drain away quickly.

The pigs were fed twice a day, at 9.30 a.m. and 3.30 p.m. The composition of the basal ration in parts by weight was as follows: ground dredge corn 20, palm-kernel cake 20, middlings 15, bran 15, dried-grass meal 10, flaked maize 7·5, maize meal 7·5, yeast meal 5. Each pig received 625 g of this basal ration per meal, to which were added 20·0 g calcium carbonate and 0·5 g sodium chloride. The basal ration and mineral supplement were mixed thoroughly to a suitable consistency with 2000 ml. water before feeding. As soon as each meal had been consumed adequate drinking water was placed in the feeding troughs.

Technique and procedure

After the pigs had been kept on this régime for a week, two pigs (nos. 1 and 2) were slaughtered 2 h, two pigs (nos. 3 and 4) 4 h and two pigs (nos. 5 and 6) 6 h after the beginning of the morning feed. The pigs were slaughtered with a captive-bolt humane killer and the whole of the gastro-intestinal tract was removed and divided into the following sections as rapidly and carefully as possible:

	Section	Approximate length (cm)		Section	Approximate length (cm)
Stomach	1	—	Caecum	1	15
	2	—		2	25
Small intestine	1	270	Colon	1	30
	2	100		2	30
	3	100	Rectum	—	50
	4	75			
	5	75			

The first section of the stomach corresponded approximately to the area lined by cardiac glands and the second section to the areas lined by fundic and pyloric glands.

The contents of each section were removed as completely as possible and weighed. Immediately after mixing in a Waring Blendor, samples of fresh gastro-intestinal contents and of the feed were taken for the determination of pH, dry matter and soluble Ca and P. The remainder of the material was dried and ground before analysis for Ca, P, insoluble ash and phytate P. Common (1940) and Reid, Franklin & Hallsworth (1947) have shown that the above process of drying such material does not affect the phytate-P content.

On consideration of the results of this experiment it was decided to investigate the daily variation in the composition of the faeces excreted by two more pigs (nos. 7 and 8) housed and fed exactly as the first six pigs. After a week under these conditions the individual faeces were collected immediately after excretion, the time of excretion being noted. When the quantity permitted, individual stools voided at a particular time were divided into anterior and posterior portions. Faeces excreted between 7.00 p.m. on one day and 8.00 a.m. on the following day were collected together. The amount was usually quite small, since the experiment was carried out in the winter, when this period was one of almost total darkness. The faeces were dried and ground before analysis for Ca and P.

Methods of analysis

Dry matter was determined by drying a sample of the fresh material to constant weight in an oven at 100–105°.

pH was determined electrometrically on the undiluted material using a 'spear' type glass electrode.

Soluble Ca and P were determined as follows: 20 g fresh material were shaken with 60 ml. water for 5 sec, the resulting mixture was centrifuged at 1600–1700g for 10 min and the supernatant liquid poured off through a filter. An attempt to employ ultrafiltration in the determination of the soluble Ca and P in the gastro-intestinal contents was unsuccessful (Moore, 1952). A portion of the supernatant liquid was evaporated to dryness and ashed at 450°.

Total Ca and P were estimated in a nitric–perchloric acid digest (Gerritz, 1935) of the dried material obtained as described in the preceding section, Ca by precipitation as oxalate and titration with ceric sulphate using *o*-phenanthroline ferrous complex as an indicator (Wheatley, 1944), P colorimetrically with a Spekker absorptiometer by the method described by Koenig & Johnson (1942) and Kitson & Mellon (1944).

Phytate P was determined on a 0·5 N-trichloracetic-acid extract (Cruickshank, Duckworth, Kosterlitz & Warnock, 1945–6) of the dried material by the method described by Hill (1951).

Insoluble ash was determined on the dried material by the usual method (Association of Official Agricultural Chemists, 1950).

RESULTS

Gastro-intestinal contents

pH. The pH values of the feed and gastro-intestinal contents of pigs nos. 1–6 are given in Table 1. The pH of the contents of the stomach was always lower than the pH of the feed owing to the action of the gastric juice. As digestion proceeded, material remaining in the stomach became increasingly acid. Since the stomach was so divided for analysis that the fundic glands were situated in the second section it was not surprising that the pH of the contents of this section in all the pigs was considerably lower than that in the first section.

Table 1. *pH of the feed and gastro-intestinal contents of pigs nos.* 1–6

	Time after feeding (h)					
	2		4		6	
	Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6
Feed	6·26	6·26	6·26	6·26	6·26	6·26
Stomach: section 1	5·80	5·68	5·07	4·96	5·04	4·47
section 2	4·70	4·74	3·81	3·21	2·79	1·97
Small intestine: section 1	6·23	6·23	6·14	6·16	6·00	6·12
section 2	6·89	6·64	6·48	7·44	7·06	6·14
section 3	7·32	7·30	7·16	7·75	7·35	7·78
section 4	7·34	7·52	7·00	7·53	7·45	7·43
section 5	7·46	7·80	6·57	7·26	7·71	7·42
Caecum: section 1	6·44	6·58	5·87	6·14	6·00	5·83
section 2	6·60	6·72	5·77	6·09	5·95	6·14
Colon: section 1	6·69	7·17	5·77	6·07	6·02	5·85
section 2	7·28	7·77	6·10	6·69	6·17	5·78
Rectum	7·80	7·91	6·76	7·14	6·65	5·90

For description of sections see p. 64.

Irrespective of the time after feeding or the pH of the contents of the second section of the stomach, the pH of the contents of the first section of the small intestine of all the pigs varied only from 6·00 to 6·23, owing presumably to the neutralizing action of the alkaline juices secreted into this segment. There was a gradual increase in pH of the contents along the remainder of the small intestine of pigs nos. 1, 2 and 5, but this increase continued only up to the third section of the small intestine of pigs nos. 3, 4 and 6. Although there was some individual variation there was no obvious difference between the reaction in corresponding sections of the small intestine as time after ingestion increased. Generally these results agreed with those given by Long & Fenger (1917) and Møllgaard (1946) for contents of stomach and small intestine in pigs.

Without exception there was a marked increase in acidity when the first section of the caecum was reached, the pH of the contents of this section being lowest in pigs nos. 3–6. The low pH observed in caecal contents may have been a function of the intestinal wall or it may have been due to bacterial activity. Vartiovaara & Roine (1942) have reported bacterial decomposition of cellulose and production of butyric and acetic acid in the caecal contents of pigs.

The contents of the colon and rectum tended to become more acid as time after ingestion of the last meal increased, particularly during the 3rd and 4th h. Again, this might have been due, at least in part, to microbial activity.

Calcium. The percentage of Ca in the dry matter and the ratio Ca: insoluble ash in the feed and gastro-intestinal contents are given in Table 2. The total weight of Ca in each segment and the percentage solubility of Ca in the feed and gastro-intestinal contents are given in Table 3.

From the percentage Ca in the dry matter and the ratio Ca: insoluble ash of the stomach contents of pigs nos. 1 and 2 it was obvious that Ca was removed more rapidly from both sections of the stomach during the first 2 h of digestion than the bulk of dry matter or insoluble ash. During the following 2 h Ca was also removed from both sections of the stomach more rapidly than insoluble ash, but was only removed more rapidly than dry matter from the second section. The preferential removal of Ca from the stomach seemed to have ceased during the 4–6 h period, since the percentage Ca in the dry matter of the contents of the first section of the stomach of pigs nos. 5 and 6 was greater than in the feed, and the ratios Ca: insoluble ash for the contents of this section of the stomach of pigs nos. 5 and 6 were greater than the corresponding ratios for pigs nos. 3 and 4.

The relatively high percentage of Ca in the dry matter and the ratios Ca: insoluble ash observed in the contents of the upper small intestine of pigs nos. 1–4 were due mainly to the preferential removal of Ca from the stomach during the earlier stages of digestion, but also possibly to the Ca contained in the secretions entering the lumen of this particular section of intestine. The corresponding values for pigs nos. 5 and 6 were considerably lower than in pigs nos. 1–4 owing to the cessation of the preferential removal of Ca from the stomach during the 4–6 h period after feeding.

Some discussion on the interpretation of the values for the percentage of Ca in the dry matter and the ratios Ca: insoluble ash in the contents of the remaining sections of the small intestine of the experimental pigs is perhaps needed at this stage, since factors other than the absorption of Ca affected their magnitude. For instance, the absorption of dry matter at a greater rate than Ca would be, in part, responsible for the general increase in the percentage of Ca in the dry matter of the contents of the

Table 2. *Percentage of calcium in the dry matter and ratios calcium: insoluble ash in the feed and gastro-intestinal contents of pigs nos.* 1–6

	Time after feeding (h)											
	2				4				6			
	Content in dry matter (%)		Ratio, Ca:insoluble ash		Content in dry matter (%)		Ratio, Ca:insoluble ash		Content in dry matter (%)		Ratio, Ca:insoluble ash	
	Pig no. 1	Pig no. 2	Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6	Pig no. 5	Pig no. 6
Feed	1·67	1·67	3·61	3·61	1·67	1·67	3·61	3·61	1·67	1·67	3·61	3·61
Stomach: section 1	1·51	1·55	3·02	3·06	1·61	1·54	2·74	2·31	1·86	1·81	3·04	2·99
section 2	1·39	1·27	2·47	2·08	0·63	0·71	0·81	0·93	0·66	0·72	0·77	0·85
Small intestine: section 1	2·17	2·39	5·16	5·98	1·61	2·25	4·02	4·59	0·98	0·78	1·64	1·32
section 2	2·38	2·52	5·24	6·01	2·47	3·54	4·38	6·41	1·34	1·12	2·20	1·84
section 3	2·55	2·36	5·31	5·24	2·52	3·40	4·31	5·74	1·78	1·75	2·61	2·71
section 4	2·64	2·47	4·98	4·88	2·56	3·13	4·20	5·27	2·22	2·23	3·23	3·21
section 5	2·88	2·93	5·12	5·24	2·36	2·83	3·51	4·31	1·89	2·53	2·48	3·61
Caecum: section 1	1·34	2·15	1·15	2·62	2·11	2·78	2·67	3·64	2·22	3·02	2·19	3·94
section 2	1·96	1·77	2·31	2·02	2·38	2·73	2·70	3·03	2·62	3·36	2·64	4·58
Colon: section 1	2·55	2·55	2·53	2·35	2·41	2·45	2·53	2·41	2·70	3·29	2·71	4·55
section 2	3·24	3·25	3·27	3·24	2·03	2·99	1·86	2·65	2·59	2·56	2·57	2·99
Rectum	3·48	3·58	3·44	3·54	2·37	3·73	2·02	3·47	2·51	1·84	2·31	1·69

For description of sections see p. 64.

Table 3. *Weight of calcium and percentage solubility of calcium in the feed and gastro-intestinal contents of pigs nos.* 1–6

	Time after feeding (h)											
	2				4				6			
	Weight of Ca in section (g)		Solubility of Ca (%)		Weight of Ca in section (g)		Solubility of Ca (%)		Weight of Ca in section (g)		Solubility of Ca (%)	
	Pig no. 1	Pig no. 2	Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6	Pig no. 5	Pig no. 6
Feed	—	—	4·10	4·10	—	—	4·10	4·10	—	—	4·10	4·10
Stomach: section 1	2·50	2·63	10·4	11·3	1·61	1·97	43·9	39·2	1·67	1·47	32·0	34·9
section 2	1·07	1·16	35·3	41·6	0·38	0·34	77·3	57·1	0·45	0·27	47·6	38·0
Small intestine: section 1	0·36	0·23	29·5	30·8	0·14	0·28	49·7	40·2	0·21	0·05	39·2	35·6
section 2	0·68	0·35	12·4	19·8	0·27	0·39	29·9	31·3	0·15	0·12	35·2	24·6
section 3	1·14	0·73	4·45	5·31	0·59	0·92	21·9	19·8	0·22	0·27	27·4	23·3
section 4	0·97	1·25	3·07	7·08	0·72	1·10	19·7	15·1	0·38	0·63	27·3	26·2
section 5	0·97	1·25	1·48	2·59	0·76	0·63	8·00	10·9	0·50	0·14	16·8	18·2
Caecum: section 1	0·29	0·47	21·7	16·4	0·33	1·28	14·7	13·8	0·39	0·58	28·8	15·4
section 2	0·46	0·69	14·2	18·9	0·96	1·62	16·7	14·6	1·41	1·26	26·2	16·1
Colon: section 1	2·23	2·66	15·1	14·6	1·53	1·66	17·3	19·7	1·22	2·36	24·4	11·8
section 2	1·93	2·16	8·82	6·64	1·36	2·70	19·6	15·1	1·74	1·28	20·5	11·5
Rectum	0·95	2·31	6·56	5·17	0·53	1·17	16·6	9·36	0·93	0·95	13·8	21·6

For descriptions of sections see p. 64.

small intestine from sections 1–5 of pigs nos. 1 and 2. It has already been noted that Ca tended to be removed from the stomach more rapidly than the bulk of the dry matter and insoluble ash during the early stages of digestion, and that this tendency decreased as digestion proceeded. This would also result in a higher percentage of Ca in the dry matter and a higher ratio Ca:insoluble ash in the contents of the lower than of the upper small intestine. Again, it is possible that if Ca passed more rapidly through the pylorus it might continue to move along the small intestine at a greater rate than the bulk of dry matter and insoluble ash.

Therefore, whereas simple absorption of Ca in the small intestine would result in a decrease in the percentage of Ca in the dry matter and in the ratios Ca:insoluble ash in the contents of the small intestine from sections 1–5, the circumstances discussed above would tend to result in an increase in these values. The extent to which these factors affected the percentage of Ca in the dry matter and the ratios Ca:insoluble ash in the contents of a particular section of small intestine at a particular time was difficult to decide and no conclusions concerning the absorption of Ca can be made from these figures alone.

From this discussion it will also be apparent that the first residues of a meal to reach the large intestine would be richer in Ca than residues entering the large intestine at a later stage. Hence one might expect that just before the last meal before slaughter the percentage of Ca in the dry matter and the ratio Ca:insoluble ash of the contents of the large intestine would be highest in the rectum and lowest in the caecum. A considerable proportion of Ca derived from this meal would have left the stomach 2 h after the last meal in advance of dry matter and insoluble ash and proceeded to some point along the intestine. At this particular point, seen to be the caecum of pigs nos. 1 and 2, the colon of pigs nos. 3 and 4, and the rectum of pig no. 6 there would be an appreciable decrease in the percentage of Ca in the dry matter and the ratios Ca:insoluble ash of the contents. The Ca present in the large intestine below the caecum of pigs nos. 1 and 2 and below the colon of pigs nos. 3 and 4 was, most probably, exogenous in nature and derived mainly from the last meal but one.

The percentage solubility values for Ca in the feed and gastro-intestinal contents of the pigs are given in Table 3. The solution of Ca by the acid gastric juice was very evident from the percentage solubility of Ca in the second section of the stomach of pigs nos. 1 and 2.

As material left the stomach and entered the small intestine a decrease in the solubility of Ca was to be expected owing to the increase in pH. Nevertheless, an average of 30·2% of the Ca in the contents of the first section of the small intestine of pigs nos. 1 and 2 was soluble, 44·9% in pigs nos. 3 and 4 and 37·4% in pigs nos. 5 and 6. The solubility of Ca in the contents of the small intestine of all the pigs generally tended to decrease from section 1 to 5. This decrease took place fairly rapidly in pigs nos. 1 and 2 but more slowly in pigs nos. 3–6. As a result, the solubility of Ca in the lower sections of the small intestine increased with time after feeding. It appeared from these results that the upper sections of the small intestine were most suited to Ca absorption.

Phosphorus. The percentage of P in the dry matter and the ratios P:insoluble ash in the feed and gastro-intestinal contents are given in Table 4. The total weight of P in each segment and the percentage solubility of P in the feed and gastro-intestinal contents are given in Table 5.

The rate at which P left the stomach decreased as digestion proceeded and only during the first 4 h after feeding was P removed from the stomach more rapidly than dry matter and insoluble ash.

As with Ca, the high percentage of P in the dry matter and the ratios P:insoluble ash in the contents of the upper sections of the small intestine of pigs nos. 1–4 were mainly due to removal of P from the stomach during the first 4 h of digestion and to a lesser extent to the secretion of P-containing juices into the upper small intestine. Although the ratios P:insoluble ash of the contents of the small intestine of pigs nos. 1–4 might be interpreted as indicating the absorption of P along the length of the small intestine it was evident that, as with Ca, factors other than absorption influenced the results.

The changes in the percentage of P in the dry matter and the ratios P:insoluble ash of the contents of the large intestine of the pigs paralleled the corresponding changes in Ca values closely and may be accounted for by a similar explanation (see p. 69).

From the percentage solubility values for P in the gastro-intestinal contents (Table 5) it appeared that the conditions in the upper three sections of the small intestine were those most favourable for P absorption.

Assuming that no absorption of phytate P took place in the small intestine, the increase in the ratios phytate P:insoluble ash (Table 6) in the contents from sections 1–4 of the small intestine of pigs nos. 3 and 4, for instance, must have been due either to the phytate P passing more rapidly than insoluble ash along the small intestine or to the fact that the ratios phytate P:insoluble ash in the material leaving the stomach decreased as time after feeding increased.

Similarly, the increase both in the percentage of phytate P in the dry matter and in the ratios phytate P:insoluble ash in the contents of the large intestine of pigs nos. 1 and 2 from the caecum to the rectum must have been due to an accumulation of the phytate P derived from the last meal but one. It was quite unreasonable to suppose that phytate P was excreted into the large intestine, and it was assumed that extensive absorption of dry matter did not take place in this region.

The ratios non-phytate P:insoluble ash of the contents of the first section of the stomach of pigs nos. 1 and 2 (Table 7) were greater than the ratio in the food, whereas the ratios in the contents of the second section were about the same as in the food. These findings could be explained if insoluble ash was removed from the first section of the stomach more rapidly than phytate P during the first 2 h of digestion and from the second section of the stomach at about the same speed. This explanation is unlikely, particularly in view of the high ratios non-phytate P:insoluble ash of the contents of the first section of the small intestine. A more probable explanation was that hydrolysis of phytate P took place in the stomach during the first 2 h after feeding, the rate of hydrolysis being sufficient to prevent a decrease in the ratios non-phytate P:insoluble ash in the stomach contents although non-phytate P was almost certainly

Table 4. *Percentage of phosphorus in the dry matter and ratios phosphorus:insoluble ash in the feed and gastro-intestinal contents of pigs nos.* 1–6

	Time after feeding (h)											
	2				4				6			
	Content in dry matter (%)		Ratio, P:insoluble ash		Content in dry matter (%)		Ratio, P:insoluble ash		Content in dry matter (%)		Ratio, P:insoluble ash	
	Pig no. 1	Pig no. 2	Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6	Pig no. 5	Pig no. 6
Feed	0·78	0·78	1·68	1·68	0·78	0·78	1·68	1·68	0·78	0·78	1·68	1·68
Stomach: section 1	0·75	0·73	1·51	1·45	0·69	0·54	1·17	0·82	0·76	0·59	1·25	0·98
section 2	0·62	0·59	1·10	0·96	0·36	0·30	0·46	0·40	0·29	0·24	0·34	0·29
Small intestine: section 1	1·17	1·48	2·79	3·70	1·51	1·12	3·77	2·29	0·76	0·75	1·28	1·28
section 2	1·22	1·43	2·69	3·41	1·54	1·38	2·73	2·47	0·69	0·76	1·14	1·25
section 3	1·24	1·29	2·59	2·87	1·26	1·53	2·15	2·58	0·98	0·76	1·44	1·18
section 4	1·23	1·30	2·34	2·57	1·29	1·41	2·11	2·37	0·99	1·17	1·43	1·69
section 5	1·23	1·27	2·18	2·28	1·12	1·37	1·67	2·09	1·04	1·44	1·36	2·06
Caecum: section 1	0·72	1·02	0·62	1·25	1·11	1·23	1·41	1·61	1·16	1·44	1·14	1·88
section 2	0·93	0·94	1·10	1·07	1·12	1·19	1·32	1·33	1·39	1·57	1·40	2·15
Colon: section 1	1·34	1·44	1·33	1·33	1·14	1·07	1·20	1·05	1·39	1·49	1·40	2·06
section 2	1·56	1·66	1·58	1·65	1·06	1·35	0·96	1·20	1·35	1·14	1·34	1·34
Rectum	1·76	1·89	1·74	1·87	1·24	1·70	1·06	1·58	1·31	0·99	1·21	0·90

For description of sections see p. 64.

Table 5. *Weight of phosphorus and percentage solubility of phosphorus in the feed and gastro-intestinal contents of pigs nos.* 1–6

	Time after feeding (h): 2				4				6			
	Weight of P in section (g)		Solubility of P (%)		Weight of P in section (g)		Solubility of P (%)		Weight of P in section (g)		Solubility of P (%)	
	Pig no. 1	Pig no. 2	Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6	Pig no. 5	Pig no. 6
Feed	—	—	15·8	15·8	—	—	15·8	15·8	—	—	15·8	15·8
Stomach: section 1	1·25	1·25	21·3	22·8	0·69	0·69	15·6	22·2	0·69	0·48	9·37	20·6
section 2	0·48	0·54	34·0	37·3	0·21	0·14	47·1	43·7	0·19	0·09	36·9	24·1
Small intestine: section 1	0·20	0·14	33·0	31·7	0·13	0·14	45·7	25·6	0·16	0·05	37·3	50·7
section 2	0·35	0·20	19·2	24·1	0·17	0·15	19·5	18·3	0·08	0·08	29·9	30·5
section 3	0·55	0·40	15·3	14·7	0·30	0·41	14·8	8·61	0·12	0·12	15·7	19·2
section 4	0·45	0·66	16·4	13·4	0·36	0·49	14·6	10·7	0·17	0·33	13·6	18·8
section 5	0·42	0·54	12·5	12·6	0·36	0·31	14·1	15·5	0·27	0·08	11·3	12·1
Caecum: section 1	0·16	0·23	16·4	10·0	0·17	0·56	13·9	17·3	0·20	0·28	11·2	14·2
section 2	0·22	0·36	18·6	11·9	0·45	0·71	14·3	17·9	0·75	0·59	11·1	11·8
Colon: section 1	1·18	1·50	12·5	8·66	0·73	0·72	14·6	17·8	0·62	1·07	11·9	14·6
section 2	0·93	1·10	7·23	6·02	0·71	1·22	14·9	11·9	0·91	0·57	12·2	13·9
Rectum	0·48	1·22	6·85	6·12	0·28	0·53	11·5	8·13	0·49	0·51	12·3	17·0

For description of sections see p. 64.

Table 6. *Percentage of phytate phosphorus in the dry matter and ratios phytate phosphorus:insoluble ash in the feed and gastro-intestinal contents of pigs nos.* 1–6

	Time after feeding (h)											
	2				4				6			
	Phytate P content of dry matter (%)		Ratio, phytate P: insoluble ash		Phytate P content of dry matter (%)		Ratio, phytate P: insoluble ash		Phytate P content of dry matter (%)		Ratio, phytate P: insoluble ash	
	Pig no. 1	Pig no. 2	Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6	Pig no. 5	Pig no. 6
Feed	0·46	0·46	0·99	0·99	0·46	0·46	0·99	0·99	0·46	0·46	0·99	0·99
Stomach: section 1	0·29	0·37	0·57	0·74	0·27	0·19	0·46	0·28	0·39	0·23	0·63	0·38
section 2	0·22	0·17	0·38	0·29	0·13	0·13	0·16	0·17	0·17	0·08	0·20	0·09
Small intestine: section 1	0·46	0·51	1·10	1·27	0·18	0·26	0·44	0·52	0·31	0·24	0·52	0·41
section 1	0·59	0·45	1·30	1·06	0·46	0·48	0·81	0·86	0·42	0·38	0·69	0·62
section 3	0·73	0·58	1·52	1·28	0·63	0·72	1·07	1·22	0·61	0·60	0·89	0·92
section 4	0·69	0·74	1·30	1·46	0·74	0·82	1·22	1·38	0·77	0·83	1·11	1·19
section 5	0·73	0·76	1·29	1·36	0·75	0·71	1·11	1·08	0·71	0·82	0·93	1·17
Caecum: section 1	0·29	0·64	0·25	0·79	0·62	0·49	0·79	0·64	0·65	0·61	0·65	0·79
section 2	0·45	0·29	0·53	0·33	0·58	0·47	0·66	0·53	0·79	0·85	0·79	1·16
Colon: section 1	0·58	0·44	0·57	0·40	0·44	0·38	0·46	0·37	0·90	0·60	0·90	0·84
section 2	0·79	0·63	0·80	0·62	0·35	0·48	0·32	0·43	0·78	0·56	0·77	0·66
Rectum	0·85	0·74	0·84	0·74	0·50	0·80	0·43	0·74	0·67	0·41	0·62	0·38

For description of sections see p. 64.

leaving the stomach at a more rapid rate than insoluble ash. During the second 2 h period after feeding the hydrolysis of phytate P appeared to continue in the first section of the stomach of pig no. 3, but was either reduced or had ceased in the first section of the stomach of pig no. 4. There was little evidence that hydrolysis of phytate P occurred in the stomach after the 4th hour of digestion.

Table 7. *Ratios non-phytate phosphorus: insoluble ash in the feed and gastro-intestinal contents of pigs nos.* 1–6

	Time after feeding (h)					
	2		4		6	
	Pig no. 1	Pig no. 2	Pig no. 3	Pig no. 4	Pig no. 5	Pig no. 6
Feed	0·69	0·69	0·69	0·69	0·69	0·69
Stomach: section 1	0·94	0·71	0·72	0·53	0·62	0·59
section 2	0·72	0·68	0·29	0·23	0·14	0·19
Small intestine: section 1	1·69	2·43	3·32	1·76	0·76	0·87
section 2	1·40	2·35	1·92	1·61	0·45	0·63
section 3	1·07	1·59	1·08	1·36	0·55	0·26
section 4	1·02	1·10	0·89	1·00	0·32	0·49
section 5	0·90	0·92	0·56	1·01	0·43	0·89
Caecum: section 1	0·37	0·46	0·62	0·97	0·50	1·09
section 2	0·56	0·74	0·62	0·80	0·61	0·99
Colon: section 1	0·76	0·93	0·74	0·68	0·49	1·22
section 2	0·78	1·03	0·65	0·77	0·57	0·68
Rectum	0·90	1·14	0·63	0·84	0·59	0·52

For description of sections see p. 64.

Non-phytate P derived from the gastric and intestinal secretions and the preferential removal of non-phytate P from the stomach would account for the very high ratios non-phytate P: insoluble ash in the contents of the first section of the small intestine of pigs nos. 1–4. As only the concentration of this particular fraction of P could change as a result of P absorption, the pronounced decrease from sections 1–4 in the ratios non-phytate P: insoluble ash in the contents of the small intestine of pigs nos. 1–4 may be interpreted as being due to a net absorption of P. Absorption was not so evident from the values found in pigs nos. 5 and 6.

Faeces

Diurnal variations in the percentage of Ca and P in the dry matter of the faeces of pigs nos. 7 and 8 over a typical 4-day period are shown in Fig. 1.

Although the results were somewhat variable the percentage of Ca and P tended to reach a maximum in the overnight and early morning excretions decreasing to a minimum in the faeces excreted in the later afternoon. Such a variation can be expected if Ca and P passed along the gastro-intestinal tract of the pigs more rapidly than the dry matter, for the first residues of a particular meal to be excreted would be richer in Ca and P than the residues excreted later. However, as the pigs were fed twice a day the excretion of faeces with a low percentage of Ca and P should also have

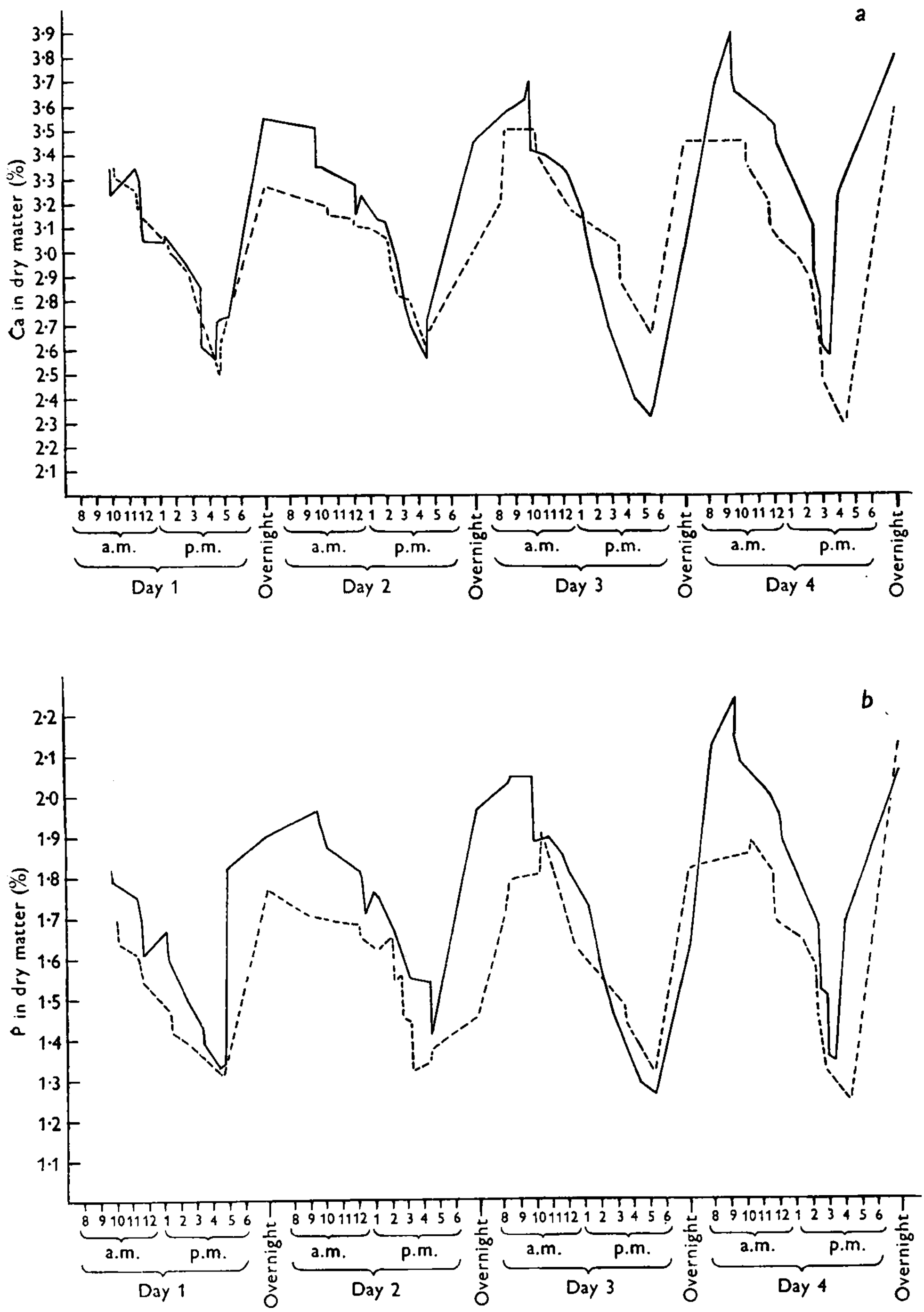

Fig. 1. Diurnal variations in (*a*) the calcium and (*b*) the phosphorus content of the faeces of pigs nos. 7 and 8. ——, pig no. 7; ----, pig no. 8.

occurred twice during the day, whereas it occurred only once. Reference to Tables 2 and 4 provides the data for a possible explanation. The contents of the large intestine possessing the lowest percentage of Ca and P were situated in the caecums of pigs nos. 1 and 2 (i.e. at 11.30 a.m.) in the colons of pigs nos. 3 and 4 (i.e. at 1.30 p.m.) and in the rectum of pig no. 6 (i.e. at 3.30 p.m.).

It was assumed that the digesta with a low Ca and P content marked the junction between the Ca and P derived from the last meal and that derived from the last meal but one, and that very little of the unabsorbed Ca and P derived from the 9.30 a.m. meal had been excreted in the faeces by 3.30 p.m. on the same day. Pigs nos. 7 and 8 received the second meal of the day at 3.30 p.m. so it appeared that the low Ca and P residues of the 9.30 a.m. meal would be supplemented with the rapidly moving Ca and P derived from the 3.30 p.m. meal well before these residues could be excreted in the faeces. When the period between feeding was much greater, i.e. between 3.30 p.m. and 9.30 a.m. on the following day, the low Ca and P residues of the 3.30 p.m. meal would be much further along the tract when the 9.30 a.m. meal was ingested on the following morning. The possibility of the Ca and P content of the last residues of the 3.30 p.m. meal increasing by the addition of unabsorbed Ca and P derived from the following 9.30 a.m. meal would therefore be less.

Thus it seems probable that the Ca and P contained in the faeces high in Ca and P excreted overnight and in the early morning were derived from both meals of the previous day, whereas the faeces low in Ca and P excreted in the later afternoon corresponded mainly to the last residues of the 3.30 p.m. meal of the previous day.

The results for pigs nos. 5 and 6 show that in the large intestine the material of low Ca and P content was situated in the rectum at 3.30 p.m. This material would have been excreted sometime shortly after 3.30 p.m., as indicated by the results obtained from the analysis of the faeces of pigs nos. 7 and 8.

DISCUSSION

Ideally, in this type of investigation it is necessary to include in the diet of the animals some reference substance physiologically and chemically inert that is neither absorbed nor excreted by the intestine and passes along the gastro-intestinal tract at exactly the same rate as the particular constituent of the diet of which the absorption and excretion are to be determined. To obtain a substance that fulfils this last requirement is perhaps the greatest problem. Iron oxide (Bergeim, 1926) and titanium dioxide (Fournier, 1950*a*, *b*, 1951*a*, *b*; Randoin, Susbielle & Fournier, 1951; Fournier & Dupuis, 1953*a*, *b*) have been used to study the intestinal absorption and excretion of Ca and P in rats, but it is doubtful if either of these substances is entirely suitable for this purpose. In the present experiment no reference substance was added to the food, although the food and gastro-intestinal contents were analysed for insoluble ash. It was realized that in no way could insoluble ash be regarded as an ideal standard, but it was considered that results expressed as insoluble-ash ratios might be of greater value, if interpreted with care, than results expressed as a percentage of dry matter.

During the early stages of digestion the removal of Ca and P from the stomach of the pigs was more rapid than the removal of dry matter and insoluble ash. This was attributed to a preferential removal of Ca and P from the stomach into the small intestine. Tyler (1946) found a similar effect in the gizzard of hens and offered the same explanation, but Fournier (1951*a*) considered that a ratio Ca:titanium dioxide in the stomach contents of rats lower than that in the food indicated that absorption of Ca had taken place from the stomach. Fournier & Dupuis (1953*b*) came to a similar conclusion about the absorption of P. It seems doubtful whether the interpretation these workers place upon their results is justified, although it must be pointed out that in preliminary experiments Fournier (1950*a*) and Fournier & Dupuis (1953*a*) claimed to have shown that Ca and P pass along the gastro-intestinal tract of the rat at the same rate as titanium dioxide.

Fournier (1950*b*) observed the entry of endogenous Ca into the lumen of the upper part of the small intestine of the rat and its subsequent reabsorption from the lower small intestine. The secretion of P into the upper small intestine of the pig has been reported by Marek *et al.* (1935), and there is considerable evidence that P is secreted into the upper small intestine of the rat (Bergeim, 1926; Fournier & Dupuis 1953*a*,*b*). The results of the present experiment indicated that a secretion of P into the upper small intestine of the pig was very probable, but evidence of a similar secretion of Ca was less definite.

When considering the particular section of the small intestine involved in the absorption of Ca and P the limitations of the Bergeim technique became particularly obvious. Direct evidence of Ca and P absorption from percentage figures and insoluble-ash ratios was not obtained because although Ca and P might well be absorbed from the first section of the small intestine the continuous passage of Ca- and P-rich material from the stomach into the small intestine, at least during the early stages of digestion, and the possible secretion of endogenous Ca and P completely obscure any indications of absorption. Further complicating factors are the possibility of the preferential passage of Ca and P along the length of the small intestine, the absorption of material other than Ca and P and also the absorption of a certain amount of silica.

From the solubility figures alone, it appeared that the upper section of the small intestine of the pigs was most suited to Ca and P absorption, as was found by Hagens (1943). It is interesting to note that the solubility of Ca in the contents of the lower small intestine of the pig increased with time after feeding. This finding may have some bearing upon the results of Harrison & Harrison (1951) which seem to indicate that absorption of radioactive Ca takes place from the lower small intestine of normal rats during the 4–24 h period after administration by stomach tube.

Whether or not there is an actual controlled excretion of Ca and P into the large intestine as a means of regulating the concentration of these elements in the body has been disputed for a number of years. On the whole, the results of the present work support the view that Ca and P are not excreted through the wall of the large intestine of the pig.

The main evidence for the opinion that Ca can be excreted into the large intestine comes from the work of Bergeim (1926), Marek *et al.* (1935) and Fournier (1950*a*, 1951*a*,*b*). All these workers used the technique employed in the present experiment.

Bergeim (1926) observed that the ratios Ca:iron progressively increased in the contents of the large intestine of rachitic rats and concluded that Ca was excreted into the large intestine of rachitic rats. There appeared to be no excretion of Ca into the large intestine of rats on a similar diet but receiving cod-liver oil. If unabsorbed Ca passed along the intestinal tract and accumulated in the large intestine as in the present experiment with pigs, Bergeim's results might also be explained by a lower absorption of Ca from the small intestine of rachitic rats. This interpretation of Bergeim's findings would be consistent with the more recent results of Nicolaysen (1937*a*, *b*, 1951) and Greenberg (1945). Marek *et al.* (1935) also give results which show a progressive increase in the percentage of Ca and P in the dry matter of the contents of the large intestine of pigs. Here again it is possible that the increase was caused by unabsorbed Ca and P moving along the gastro-intestinal tract more rapidly than the bulk of dry matter and not by an excretion of Ca and P into the large intestine. Similarly, the conclusions reached by Fournier (1950*b*, 1951*a*, *b*) are open to criticism until there is more evidence that Ca and titanium dioxide pass along the gastro-intestinal tract of the rat at the same speed.

Thus, incontrovertible evidence of a controlled excretion of Ca and P into the large intestine of the rat and pig is yet to be obtained. However, the position may be different in other species. For instance, Cowell (1937) has shown fairly conclusively that Ca can be excreted through the wall of the large intestine of the rabbit.

The preferential movement of Ca and P along the gastro-intestinal tract of the pig is shown particularly well in the present experiment by the diurnal variations in the percentage of Ca and P in the dry matter of the faeces. Kane, Jacobson & Moore (1952) have noticed a similar variation in the faecal excretion of lignin and chromium oxide fed as markers to dairy cows.

It will be observed from Fig. 1 that the percentages of Ca and P in the dry matter of the faeces excreted at a particular time during the day by each pig follow one another fairly closely; in fact there is an approximately linear relationship between these two values, which must mean that the overall speeds of transit of Ca and P along the gastro-intestinal tract of the pig under the conditions of the experiment are about equal. This finding is rather surprising in view of the different types of Ca and P compounds that must be present in the contents of the tract.

Møllgaard (1946) has suggested that the enzymic cleavage of phytic acid due to the activity of the phytase present in cereal foodstuffs might take place in the stomach of the pig in the early stages of digestion. The results of the present investigation support this supposition. Hydrolysis of phytate appeared to occur readily in both sections of the stomach of the pig during the first 2 h of digestion and possibly to a limited extent in the cardiac region of the stomach during the second 2 h. These findings are not incompatible with the observed pH values of the stomach contents. Fleury & Courtois (1947) have shown that the optimum pH for phytase activity is between 5·0 and 6·0, and Hill & Tyler (1954) give a value of 5·0–5·1. The mean pH of the contents of the cardiac region of the stomachs of the pigs 2 h after feeding was 5·74 and in the pyloric region 4·72; 4 h after feeding it was 5·02 in the cardiac region.

In conclusion it must be stated that Bergeim's method of studying the absorption and excretion of Ca and P will be seriously limited until (*a*) an entirely reliable reference substance is discovered, and (*b*) a satisfactory method of distinguishing between endogenous and exogenous Ca and P is devised.

In the following paper (Moore & Tyler, 1955) it will be seen that some of the difficulties experienced in the present experiment may be overcome by using the method here described in conjunction with the use of radioactive isotopes.

SUMMARY

1. Pairs of pigs (9–10 weeks old) were slaughtered 2, 4 and 6 h after feeding, and the pH, total Ca and P, solubility of Ca and P, phytate P and insoluble ash were determined in the contents of the various sections of the gastro-intestinal tract. Results for Ca and P were expressed as insoluble-ash ratios as well as percentages of the dry matter.

2. The pH of the stomach contents became increasingly acid as time after feeding increased. There was little difference in the pH of the contents of corresponding sections of the small intestine of pigs killed at different times after feeding. A general decrease in the pH of the contents of the large intestine occurred as time after feeding increased.

3. In the earlier stages of digestion Ca and P were removed from the stomach at a greater rate than dry matter and insoluble ash. This preferential removal of Ca and P ceased during the third 2 h period after feeding.

4. Owing to the inadequacy of dry matter and insoluble ash as standards of comparison little information on the absorption of Ca and P was obtained from the percentage Ca and P in the dry matter and the ratios Ca and P:insoluble ash of the gastro-intestinal contents.

5. The solubility of Ca and P in the contents of the small intestine was greatest in the upper sections, and it was considered that absorption of Ca and P would most likely take place from these sections.

6. No evidence was obtained to indicate that Ca and P were excreted through the wall of the large intestine of the pig.

7. It was apparent that under the conditions of the experiment hydrolysis of phytate occurred in the stomach of the pig during the earlier stages of digestion before the pH of the stomach contents became sufficiently low to inhibit phytase activity.

8. There was a diurnal variation in the faecal excretion of Ca and P. The highest percentage of Ca and P was observed in the overnight and early morning excretions, whereas the lowest percentage of Ca and P was observed in the faeces excreted in the late afternoon.

9. The significance of the results obtained in the present investigation is discussed in relation to the results of other workers in this field.

10. It is concluded that the Bergeim technique for investigating the intestinal absorption and excretion of Ca and P as used in the present experiment possesses certain serious limitations.

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