

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

3. The Effect of Beryllium Carbonate on the Absorption of Phosphorus

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Evidence has been produced by a number of workers (Branion, Guyatt & Kay, 1931; Jacobson, 1933; Guyatt, Kay & Branion, 1933; Kay & Skill, 1934; Duncan & Miller, 1936) that the addition of beryllium carbonate to the diet of animals decreases the absorption of phosphorus from the intestine. Since it is thought that a considerable proportion of the P absorbed from the intestine is absorbed as orthophosphate, it has been suggested that when beryllium carbonate is added to the diet absorption of P is limited owing to the formation of insoluble beryllium phosphate. However, a considerable amount of the P of cereal foodstuffs is present as phytate which must be hydrolysed before absorption of P can take place. Lowe & Steenbock (1936) compared the total and inorganic P content of faeces of rats on a diet containing phytate with the total and inorganic P content of the faeces of rats on an identical diet to which 3% beryllium carbonate had been added and concluded that the hydrolysis of phytate in the gastro-intestinal tract of the rat was greatly diminished by beryllium carbonate. Thus, it seems that two factors may contribute to the reduced P absorption: a precipitation of orthophosphate and a reduction in phytate hydrolysis.

In a series of experiments already reported (Moore & Tyler, 1955 *a, b*) the intestinal absorption and excretion of Ca and P in the pig were investigated. The technique used was essentially that of Bergeim (1926), in which animals were slaughtered at different times after feeding and the contents of the various sections of the gastro-intestinal tract analysed. Incidentally to the main study it was decided to investigate the mode of action of beryllium carbonate in decreasing the absorption of P from the intestine of the pig, since the above technique seemed particularly well suited to the problem.

EXPERIMENTAL

The two pigs (nos. 15 and 16) used in this experiment were of the same breed and age (i.e. 9–10 weeks) as those used in previous experiments (Moore & Tyler, 1955 *a, b*). The animals were housed, and fed twice a day with a basal ration supplemented with calcium carbonate and sodium chloride, as described previously (Moore & Tyler, 1955 *a*). Beryllium carbonate was introduced into the feed gradually with the original intention of attaining a final level of 3% in the diet, but increasing the beryllium carbonate beyond a level of 2.4% resulted in reduced food consumption. Lowe & Steenbock (1936) had noticed a reduction in food intake when beryllium carbonate

was included in the diet of rats at a level of 3%. In an experiment with dogs Duncan & Miller (1936) found that food was refused after 15 days on a ration supplemented daily with 0.75 g beryllium carbonate.

The ration containing 2.4% beryllium carbonate was therefore fed to pigs nos. 15 and 16 for a period of 10 days, and 4 h after beginning the morning meal on the 11th day the pigs were slaughtered with a captive-bolt humane killer. The gastro-intestinal tracts were removed as rapidly and carefully as possible and divided into the sections described by Moore & Tyler (1955*a*). The contents of each section were removed, weighed and, after thorough mixing in a Waring Blendor, samples of the fresh gastro-intestinal contents were taken for determination of pH, dry matter and soluble Ca and P. Total Ca and P and phytate phosphorus were determined on the remainder of the contents after drying at 100–105° and grinding. The methods of analysis were those used by Moore & Tyler (1955*a*) in previous experiments.

RESULTS

General. Pigs nos. 15 and 16 of the present experiment were fed on the same diet as pigs nos. 3 and 4 of the earlier experiment (Moore & Tyler, 1955*a*), except that pigs nos. 3 and 4 received no beryllium carbonate. They were also slaughtered at the same time after feeding (i.e. 4 h). Thus the results obtained from the analysis of the gastro-intestinal contents of pigs nos. 15 and 16 are directly comparable with those obtained for pigs nos. 3 and 4, and in the tables presented below the mean values for pigs nos. 3 and 4 are given in addition to individual results for pigs nos. 15 and 16. The basal ration fed to pigs nos. 15 and 16 was of a different consignment from that fed to pigs nos. 3 and 4, which accounts for slight differences in the chemical composition of the feeds.

A general discussion on the interpretation of the results of the analysis of gastro-intestinal contents has been given by Moore & Tyler (1955*a*).

pH. The pH values of the feed and of the gastro-intestinal contents are given in Table 1. The inclusion of beryllium carbonate in the diet did not seem to affect the pH of the gastro-intestinal contents to any great extent. The relatively higher pH of the contents of the first section of the stomach of pig no. 15 was in all probability due to an individual variation in the production of acid gastric juice.

Phosphorus. The total weight of P in each section and the percentage of P in the dry matter of the feed and gastro-intestinal contents are given in Table 2. A striking difference was observed between the percentage of P in the dry matter of the stomach contents of the pigs given beryllium carbonate and of those receiving the normal diet. Moore & Tyler (1955*a, b*) had noted that during the first 4 h of digestion P was removed from the stomach of pigs receiving a normal diet more rapidly than the bulk of the dry matter. Such a preferential removal of P from the stomach of pigs nos. 15 and 16 was not apparent. In fact, the percentage of P in the contents of the second section of the stomach was greater than in the feed, showing that in pigs nos. 15 and 16 dry matter tended to be removed from the stomach at a greater rate than P. Therefore there was not the large increase in percentage of P in the contents on passing from the second section of the stomach to the first section of the small intestine of pigs nos. 15 and 16 that was observed with pigs nos. 3 and 4, but only a small increase

which may be attributed to the secretion of endogenous P into the upper small intestine.

For reasons discussed by Moore & Tyler (1955*a*) it was difficult to place any interpretation on the percentage P values obtained for the contents of the small intestine.

Table 1. *pH of the feed and gastro-intestinal contents of pigs nos. 15 and 16 and pigs nos. 3 and 4*

	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*
Feed	6.14	6.14	6.26
Stomach: section 1	5.88	5.02	5.02
section 2	3.96	4.50	3.51
Small intestine: section 1	5.93	5.44	6.15
section 2	6.94	6.75	6.96
section 3	7.74	7.18	7.46
section 4	7.64	7.40	7.26
section 5	7.45	7.33	6.91
Caecum: section 1	5.89	5.77	6.00
section 2	5.82	5.84	5.93
Colon: section 1	5.88	5.90	5.92
section 2	6.92	6.72	6.39
Rectum	7.92	7.66	6.95

* See Moore & Tyler (1955*a*).

Table 2. *Total weight of phosphorus and percentage of phosphorus in the dry matter of the feed and gastro-intestinal contents of pigs nos. 15 and 16 and pigs nos. 3 and 4*

	Weight of P in section (g)			P content in dry matter (%)		
	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*
Feed				0.84	0.84	0.78
Stomach: section 1	0.84	0.63	0.69	0.83	0.82	0.61
section 2	0.32	0.17	0.18	0.94	0.88	0.33
Small intestine: section 1	0.09	0.09	0.13	1.09	1.15	1.32
section 2	0.16	0.07	0.16	1.22	1.05	1.46
section 3	0.10	0.24	0.35	1.28	1.27	1.39
section 4	0.13	0.24	0.43	1.29	1.23	1.35
section 5	0.24	0.22	0.33	1.51	1.39	1.25
Caecum: section 1	0.27	0.43	0.37	1.56	1.78	1.17
section 2	0.44	0.63	0.58	1.64	1.87	1.16
Colon: section 1	0.36	0.62	0.73	1.56	1.86	1.10
section 2	0.54	0.64	0.96	1.73	2.05	1.20
Rectum	0.70	0.88	0.41	1.85	2.15	1.47

* See Moore & Tyler (1955*a*).

That beryllium carbonate decreases P absorption was clearly shown by the higher percentage of P in the dry matter of the contents of the large intestine of pigs nos. 15 and 16 when compared with the corresponding values for pigs nos. 3 and 4. It has been shown by Moore & Tyler (1955*a, b*) that P present in the large intestine of the

pig is almost entirely unabsorbed exogenous P of previous meals and not P that has been excreted through the wall of the large intestine.

The percentage solubility of P and percentage of soluble P in the dry matter of the feed and gastro-intestinal contents are given in Table 3. The solubility of P in the first section of the stomach of pigs nos. 15 and 16 was not very different from that observed in the corresponding sections of pigs nos. 3 and 4, but in the second section of the stomach the solubility of P in the contents was much lower in the pigs given beryllium carbonate. It is in this section of the gastro-intestinal tract alone that beryllium appears to be of any consequence in depressing the solubility of P, for, surprisingly enough, the solubility in the contents of the small intestine of pigs nos. 15 and 16 was actually greater than in the corresponding sections of pigs nos. 3 and 4.

Table 3. *Percentage of soluble phosphorus in the dry matter and solubility of phosphorus of the feed and gastro-intestinal contents of pigs nos. 15 and 16 and pigs nos. 3 and 4*

	Soluble P content of dry matter (%)			Solubility of P (%)		
	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*
Feed	0.14	0.14	0.12	16.2	16.2	15.8
Stomach: section 1	0.13	0.17	0.11	15.6	20.8	18.9
section 2	0.14	0.17	0.15	15.1	19.2	45.4
Small intestine: section 1	0.51	0.62	0.49	46.5	54.1	35.7
section 2	0.35	0.63	0.28	28.5	59.7	18.9
section 3	0.32	0.33	0.16	25.4	26.0	11.7
section 4	0.40	0.35	0.17	31.1	29.0	12.6
section 5	0.33	0.26	0.19	21.5	18.6	14.8
Caecum: section 1	0.28	0.24	0.18	17.7	13.6	15.6
section 2	0.27	0.24	0.19	16.7	12.7	16.1
Colon: section 1	0.24	0.22	0.18	15.1	11.9	16.2
section 2	0.27	0.21	0.16	15.8	10.1	12.4
Rectum	0.32	0.20	0.14	17.4	9.3	9.8

* See Moore & Tyler (1955*a*).

The percentages of phytate and non-phytate P in the dry matter of the feed and gastro-intestinal contents are given in Table 4. The higher percentage of phytate P in the stomach contents of the pigs given beryllium carbonate suggested a decreased hydrolysis of phytate in the stomach. This suggestion was borne out by a consideration of the percentage values for non-phytate P which were lower in the contents of the first section of the stomach of pigs nos. 15 and 16 than in the feed. The percentage of non-phytate P in the contents of the first section of the stomach of pigs nos. 3 and 4, however, was greater than in the food, and Moore & Tyler (1955*a, b*) had considered this finding to be evidence of phytate hydrolysis in this section. The high value for the percentage of non-phytate P in the contents of the first section of the small intestine of pigs nos. 3 and 4 was due to the preferential removal of non-phytate P from the stomach to the small intestine and to the secretion of endogenous P into the upper small intestine (Moore & Tyler, 1955*a, b*). The corresponding values for pigs nos. 15 and 16 were noticeably smaller and were probably entirely due to the secretion of P

into the first section of the small intestine, since non-phytate P would appear to be precipitated in the second section of the stomach of the pigs given beryllium carbonate.

Indication that the absorption of P was reduced by adding beryllium carbonate to the diet was also provided by a comparison of the percentage values for non-phytate P of the contents of the small intestine of pigs nos. 3 and 4 with those of pigs nos. 15 and 16. Since it may be assumed that only non-phytate P is absorbed from the intestine, absorption of P should result in a decrease in the percentage of non-phytate P in the contents as these pass along the small intestine. This decrease was very evident with pigs nos. 3 and 4 but not with pigs nos. 15 and 16. The higher percentage of non-phytate P in the contents of the large intestine of pigs nos. 15 and 16 showed that the absorption of non-phytate P derived from previous meals had been reduced by beryllium carbonate. A decreased phytate hydrolysis was again apparent from the higher percentage of phytate P in the contents of the large intestine of pigs nos. 15 and 16.

Table 4. *Percentage of phytate and non-phytate phosphorus in the dry matter of the feed and gastro-intestinal contents of pigs nos. 15 and 16 and pigs nos. 3 and 4*

	Phytate P content of dry matter (%)			Non-phytate P content of dry matter (%)		
	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*
Feed	0.46	0.46	0.46	0.38	0.38	0.32
Stomach: section 1	0.57	0.56	0.23	0.27	0.26	0.38
section 2	0.46	0.53	0.13	0.49	0.36	0.20
Small intestine: section 1	0.40	0.41	0.22	0.69	0.74	1.10
section 2	0.69	0.40	0.47	0.54	0.65	0.99
section 3	0.67	0.59	0.68	0.61	0.68	0.72
section 4	0.76	0.60	0.78	0.53	0.63	0.57
section 5	0.95	0.84	0.73	0.57	0.55	0.52
Caecum: section 1	0.89	0.85	0.55	0.67	0.92	0.62
section 2	0.83	0.86	0.53	0.81	1.01	0.63
Colon: section 1	0.85	0.88	0.41	0.71	0.98	0.70
section 2	0.74	1.03	0.41	0.99	1.02	0.79
Rectum	1.06	1.26	0.65	0.79	0.90	0.82

* See Moore & Tyler (1955*a*).

Calcium. The total weight of Ca in each section and percentage of Ca in the dry matter of the feed and gastro-intestinal contents are given in Table 5, and the percentage solubility of Ca and percentage of soluble Ca in the dry matter of the feed and gastro-intestinal contents in Table 6.

Although Ca was removed preferentially from the stomach of pig no. 16 this was not so in the first section of the stomach of pig no. 15. The explanation of this difference is to be found very probably in the difference in solubility of Ca, which in turn is a reflexion of the difference in pH in the contents of the first section of the stomach of pigs nos. 15 and 16. *In vitro* experiments by Hill & Tyler (1954*a*) have shown that a pH range of approx. 5.00–5.80 is very critical for the solution of Ca added as calcium carbonate to bran, wheat or oats. The mixtures described by these workers can be

regarded as similar to the contents of the stomach of the pigs of the present experiment. In fact, if the contents of the first section of the stomach are considered, a pH of 5.88 corresponding to a Ca solubility of 5.61% in pig no. 15 and a pH of 5.02 corresponding to a calcium solubility of 40.2% in pig no. 16 agree fairly well with the results presented by Hill & Tyler (1954a).

Table 5. *Total weight of calcium and percentage of calcium in the dry matter of the feed and gastro-intestinal contents of pigs nos. 15 and 16 and pigs nos. 3 and 4*

	Weight of Ca in section (g)			Ca content in dry matter (%)		
	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*
Feed	—	—	—	1.64	1.64	1.67
Stomach: section 1	1.94	0.99	1.79	1.92	1.29	1.57
section 2	0.20	0.19	0.36	0.59	0.99	0.67
Small intestine: section 1	0.09	0.14	0.21	1.15	1.73	1.93
section 2	0.27	0.12	0.33	2.03	1.85	3.03
section 3	0.20	0.43	0.76	2.49	2.33	2.96
section 4	0.28	0.52	0.91	2.88	2.73	2.85
section 5	0.55	0.55	0.70	3.51	3.46	2.60
Caecum: section 1	0.54	0.64	0.80	2.08	2.67	2.44
section 2	0.85	1.13	1.29	3.18	3.38	2.55
Colon: section 1	0.68	1.02	1.59	2.95	3.08	2.43
section 2	0.80	0.93	2.03	2.57	2.97	2.51
Rectum	1.20	1.20	0.85	3.10	2.93	3.05

* See Moore & Tyler (1955a).

Table 6. *Percentage of soluble calcium in the dry matter and solubility of calcium in the feed and gastro-intestinal contents of pigs nos. 15 and 16 and pigs nos. 3 and 4*

	Soluble Ca content of dry matter (%)			Solubility of Ca (%)		
	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*	Pig no. 15	Pig no. 16	Pigs nos. 3 and 4 (mean)*
Feed	0.06	0.06	0.07	3.9	3.9	4.1
Stomach: section 1	0.11	0.52	0.65	5.6	40.2	41.5
section 2	0.34	0.56	0.45	57.2	56.7	67.2
Small intestine: section 1	0.44	0.85	0.85	38.4	48.9	44.9
section 2	0.57	0.64	0.93	28.2	34.7	30.6
section 3	0.40	0.31	0.61	16.1	13.1	20.8
section 4	0.67	0.43	0.49	23.2	15.7	17.4
section 5	0.39	0.26	0.25	11.2	7.5	9.5
Caecum: section 1	0.62	0.70	0.35	19.9	26.2	14.2
section 2	0.76	0.74	0.40	23.9	22.0	15.6
Colon: section 1	0.66	0.62	0.45	22.5	20.3	18.5
section 2	0.46	0.57	0.43	17.8	19.3	17.4
Rectum	0.46	0.56	0.37	14.9	19.0	13.0

* See Moore & Tyler (1955a).

Comparison of the Ca results for the contents of the remaining sections of the gastro-intestinal tract of the pigs fed on a normal diet with those of pigs fed on a diet supplemented with beryllium carbonate did not reveal the substantial differences noted for P. Nevertheless, there was a general tendency for the percentage of Ca in the dry matter to be higher in the contents of the large intestine of pigs nos. 15 and 16 than in those of pigs nos. 3 and 4, suggesting that Ca absorption was reduced to a certain extent by inclusion of beryllium carbonate in the diet.

DISCUSSION

Guyatt *et al.* (1933) considered that rickets induced in rats by the feeding of beryllium carbonate at levels of 0.12–3.00% of a normal diet was primarily due to deficient absorption of P from the intestine, although *in vitro* experiments by Sobel, Goldfarb & Kramer (1935) showed that there was also a local disturbance of calcification in beryllium rickets. Guyatt *et al.* (1933) found that beryllium phosphate is precipitated at pH values as low as 2.6, and therefore formed the opinion that deficient absorption of P from the intestine was in fact due to a precipitation of phosphate in the gastro-intestinal contents. As phosphate was liberated from phosphoric-acid esters present in the food by enzymic hydrolysis in the gastro-intestinal tract, it would be precipitated by beryllium ions resulting from the solution of the beryllium carbonate by the gastric juice. That P absorption is decreased on the addition of beryllium carbonate to the diet of pigs is clearly shown by the present experiment, and that it is due in part to the precipitation of phosphate by the beryllium ions liberated in the second section of the stomach is also shown. However, it would appear that the major factor contributing to the decreased absorption of P was a substantial reduction in phytate hydrolysis.

Moore & Tyler (1955*a, b*) have shown that phytate is hydrolysed in the stomach of the pig on a normal diet, in particular in the cardiac section, where the pH of the contents, in the early stages of digestion at least, does not attain a value as low as 2.2, a pH at which Hill & Tyler (1954*b*) have shown cereal phytase to be irreversibly inactivated. From the present work it would appear that the hydrolysis of phytate in the stomach of the pigs is considerably reduced when beryllium carbonate is added to the diet. The solubility of P in the contents of the first section of the stomach of the pigs receiving the normal diet was about the same as in the contents of the corresponding section of the stomach of the animals receiving the diet containing beryllium carbonate. Hill & Tyler (1954*b*) have shown cereal phytase to be active at pH values found in the contents of the first section of the stomach of the pigs of the present experiment with and without beryllium carbonate included in the diet. This finding suggests that phytate hydrolysis is reduced as a result of the inhibition of cereal phytase by the small concentration of beryllium ions that would undoubtedly be present in the contents of the first section of the stomach. Although the inhibition of cereal phytase by beryllium has never been reported and the subject has not been fully investigated by the present authors, it is of interest that Klemperer, Miller & Hill (1949) and Grier, Hood & Hoagland (1949) have found that beryllium is a strong inhibitor of alkaline phosphatases.

In addition to the probable inhibition of cereal phytase, the observed decrease in phytate hydrolysis must also be associated with a precipitation of beryllium-phytate compounds in the second section of the stomach where the concentration of beryllium ions must be considerable. Comparison of the solubility of P in the contents of the second section of the stomach of pigs nos. 3 and 4 (mean 45%) with the corresponding values for pigs nos. 15 and 16 (mean 17%) points to a precipitation of phytate P in this section of the stomach when beryllium carbonate is included in the diet. Qualitative tests *in vitro* have shown that beryllium phytate compounds are insoluble at pH values of 3.0 or less.

Bearing in mind the views of Guyatt *et al.* (1933) on the action of beryllium carbonate in decreasing absorption of P, and the fact that Moore & Tyler (1955*b*) have found that the absorption of P 4 h after feeding was most active from the proximal half of the small intestine of the pig, we were surprised to find that the solubility of P in the contents of the upper small intestine of the pigs given beryllium carbonate was in fact considerably greater than in the corresponding sections in the pigs on the normal diet. Thus, in terms of solubility alone, there would seem to be no reason why P absorption should be impaired in the pigs given beryllium carbonate, but, whereas the concentration of soluble P in the contents of the first two sections of the small intestine of pigs nos. 15 and 16 was greater than in pigs nos. 3 and 4, the concentration of non-phytate P in the contents of these sections was notably lower in pigs nos. 15 and 16. It seems likely, therefore, that although there were larger amounts of soluble P in the contents of the upper small intestine of the pigs receiving beryllium carbonate, this soluble P was largely phytate P that had escaped precipitation in the stomach, and which, as far as is known, cannot be absorbed.

Further information on the mode of action of beryllium carbonate in decreasing P absorption may be obtained from a consideration of the results obtained for the contents of the large intestine. If the large intestine is taken as a whole (i.e. caecum, colon, rectum), it may be calculated that the percentage of P in the dry matter in the contents in pigs nos. 15 and 16 was 1.84 (mean) and in pigs nos. 3 and 4, 1.21 (mean). This difference represents an increase of 0.63% total P in the dry matter of the contents of the large intestine when beryllium carbonate was given, and it may also be calculated that 74% of this increase was, in fact, phytate P.

The present experiment also indicates that Ca absorption was decreased to a certain extent by inclusion of beryllium carbonate in the diet of the pigs. Indeed, this decrease might have been expected in view of the reduced phytate hydrolysis, for it has been known for some time that increasing the phytate content of the diet results in a decreased Ca absorption (Young, Gregory & Vere-Jones, 1935). The reduced Ca absorption observed with increasing phytate intake has usually been explained by assuming that Ca is precipitated in the gastro-intestinal tract as calcium phytate, but there is no evidence that this was so in pigs nos. 15 and 16. It must also be pointed out that Duncan & Miller (1936) could find no decrease in plasma Ca on feeding beryllium carbonate to dogs.

SUMMARY

1. The mode of action of dietary beryllium carbonate in decreasing the intestinal absorption of phosphorus in the pig has been investigated.

2. Beryllium carbonate was included in the diet of two pigs at a level of 2.4% for a period of 10 days. On the 11th day the pigs were slaughtered 4 h after feeding and the pH, total Ca and P, solubility of Ca and P and phytate P were determined in the contents of the gastro-intestinal tract. The results were compared with those obtained for pigs fed on an identical diet but with no added beryllium carbonate.

3. P absorption from the intestine was reduced by beryllium carbonate mainly as a result of a decreased phytate hydrolysis.

4. Beryllium carbonate appeared to act as an inhibitor of cereal phytase in addition to precipitating beryllium-phytate compounds.

5. There were indications that the inclusion of beryllium carbonate in the diet also reduced Ca absorption but this reduction was probably due to the reduced phytate hydrolysis.

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REFERENCES

- Bergeim, O. (1926). *J. biol. Chem.* **70**, 29.
Branion, H. D., Guyatt, B. L. & Kay, H. D. (1931). *J. biol. Chem.* **92**, xi.
Duncan, C. W. & Miller, E. J. (1936). *J. Nutr.* **11**, 371.
Guyatt, B. L., Kay, H. D. & Branion, H. D. (1933). *J. Nutr.* **6**, 313.
Grier, R. S., Hood, M. B. & Hoagland, M. B. (1949). *J. biol. Chem.* **180**, 289.
Hill, R. & Tyler, C. (1954a). *J. agric. Sci.* **44**, 293.
Hill, R. & Tyler, C. (1954b). *J. agric. Sci.* **44**, 306.
Jacobson, S. A. (1933). *Arch. Path. (Lab. Med.)*, **15**, 8.
Kay, H. D. & Skill, D. I. (1934). *Biochem. J.* **28**, 1222.
Klemperer, F. W., Miller, J. M. & Hill, C. J. (1949). *J. biol. Chem.* **180**, 281.
Lowe, J. T. & Steenbock, H. (1936). *Biochem. J.* **30**, 1991.
Moore, J. H. & Tyler, C. (1955a). *Brit. J. Nutr.* **9**, 63.
Moore, J. H. & Tyler, C. (1955b). *Brit. J. Nutr.* **9**, 81.
Sobel, A. E., Goldfarb, A. R. & Kramer, B. (1935). *J. biol. Chem.* **108**, 395.
Young, L., Gregory, R. A. & Vere-Jones, N. W. (1935). *J. Soc. chem. Ind., Lond.*, **54**, 270.