# Effect of calcium and phosphorus salts on the utilization of iron by anaemic rats

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In a previous paper, Chapman & Campbell (1957) reported that the addition of relatively large amounts of bone meal to bread diets low in iron retarded haemoglobin regeneration in anaemic rats.

In view of the contradictory reports in the literature, mentioned by these authors, about the effect of calcium and phosphorus on the utilization of iron, it was considered desirable to extend this work to determine what effect other calcium salts might have on the utilization of iron when added to bread diets and also to determine the effect of two sodium phosphates. By so doing it was thought that it might be possible to determine whether the calcium or the phosphate portion of the bone meal had caused interference with the utilization of iron.

## EXPERIMENTAL

*Plan of experiment.* The analytical methods, basal diet, and type of rats used in this study were similar to those already described (Chapman & Campbell, 1957).

The project was carried out in two experiments. In Exp. 1, bone meal, calcium carbonate, disodium phosphate and commercial sodium hexametaphosphate were used as additives; in Exp. 2, calcium lactate and calcium chloride were used as sources of calcium. The levels of calcium added to these diets were similar to the highest levels used in the earlier work, i.e. equivalent to 7·5 g bone meal/lb. flour (Chapman & Campbell, 1957). Two levels of added ferrous sulphate were employed, 0 and 10 mg/lb. flour. The two phosphates were added in amounts sufficient to provide the same level of phosphorus as was contributed by the bone meal. Ten rats, five male and five female, were used on each treatment.

## RESULTS

The iron, calcium and phosphorus content of the diets, the ratios calcium:phosphorus and calcium:iron, the amount of diet consumed per rat in the 10-week period as well as the amounts of iron and calcium consumed per rat are shown in Table 1. It can be seen from the analyses of the diets that the flour used in Exp. 1 contained considerably more iron than that used in Exp. 2. The addition of calcium carbonate and calcium chloride to the diets resulted in a significant decrease in the amount of food consumed; addition of the other salts did not. This reduction in the amount of food consumed has

automatically resulted in a reduction in the intake of iron by the rats on these two diets. It is apparent particularly with diet 15: the total iron consumed in the 10-week period amounted to 9·5 mg, compared to 15·4 mg for the control rats.

Table 1. *Composition and analysis of diets, and food consumption of rats*

| Diet no.* | No. of rats | Additive: $FeSO_4$ (mg/lb. flour) | Additive: Other salts (g/lb. flour) | Analytical results: Fe (mg/100 g) | Analytical results: Ca (mg/100 g) | Analytical results: P (mg/100 g) | Ratio, Ca:P | Ratio, Ca:Fe | Diet consumed in 10 weeks (g/rat) | Fe consumed per rat (mg) | Ca consumed per rat (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Experiment 1 | | | | | | | |
| 1 | 10 | 0 | 0 | 3·62 | 320 | 167 | 1·9 | 88 | 698 | 25·3 | 2·23 |
| 2 | 10 | 10 | 0 | 4·08 | 314 | 157 | 2·0 | 77 | 722 | 29·4 | 2·27 |
| 3 | 10 | 0 | Bone meal, 7·50 | 3·71 | 723 | 289 | 2·5 | 195 | 679 | 25·2 | 4·91 |
| 4 | 10 | 10 | Bone meal, 7·50 | 4·33 | 723 | 321 | 2·3 | 167 | 671 | 29·0 | 4·85 |
| 5 | 10 | 0 | Calcium carbonate, 7·26 | 3·51 | 792 | 153 | 5·2 | 226 | 624 | 21·9 | 4·94 |
| 6 | 10 | 10 | Calcium carbonate, 7·26 | 4·33 | 787 | 158 | 5·0 | 182 | 626 | 27·1 | 4·93 |
| 7 | 10 | 0 | Disodium phosphate, 6·87 | 3·55 | 310 | 364 | 0·85 | 87 | 765 | 27·2 | 2·37 |
| 8 | 10 | 10 | Disodium phosphate, 6·87 | 3·94 | 312 | 360 | 0·87 | 79 | 768 | 30·3 | 2·40 |
| 9 | 10 | 0 | Sodium hexametaphosphate, 4·93 | 3·39 | 316 | 362 | 0·87 | 93 | 690 | 23·4 | 2·18 |
| 10 | 10 | 10 | Sodium hexametaphosphate, 4·93 | 4·06 | 315 | 360 | 0·87 | 77 | 672 | 27·3 | 2·12 |
| | | | | Experiment 2 | | | | | | | |
| 11 | 10 | 0 | 0 | 2·64 | 321 | 155 | 2·1 | 122 | 585 | 15·4 | 1·88 |
| 12 | 10 | 10 | 0 | 3·11 | 315 | 150 | 2·1 | 101 | 644 | 20·0 | 2·03 |
| 13 | 10 | 0 | Calcium lactate, 19·04 | 2·53 | 742 | 144 | 5·2 | 293 | 616 | 15·6 | 4·57 |
| 14 | 10 | 10 | Calcium lactate, 19·04 | 2·98 | 733 | 142 | 5·2 | 246 | 545 | 16·2 | 3·99 |
| 15 | 10 | 0 | Calcium chloride, 6·85 | 2·12 | 728 | 151 | 4·8 | 343 | 446 | 9·5 | 3·25 |
| 16 | 10 | 10 | Calcium chloride, 6·85 | 2·96 | 730 | 149 | 4·9 | 247 | 553 | 16·4 | 4·04 |

* Diet contained 80 % dried bread, 12 % casein, 3 % U.S.P. salt mixture (*Pharmacopoeia of the United States of America, Fourteenth Revision*, 1950), 3 % maize oil, 2 % Alphacel (a non-nutritive cellulose supplied by Nutritional Biochemicals Incorporated, Cleveland, Ohio, U.S.A.) and a complete vitamin mixture.

The effect of these additives on gains in body-weight, iron content of liver and heart weight is shown in Table 2. The presence of calcium carbonate, calcium lactate and calcium chloride in the diets resulted in a significant decrease in the gains in weight shown by these rats. As shown in Table 1, calcium carbonate and calcium chloride in the diet led to a reduction in the amount of food consumed, but the presence of the lactate did not significantly reduce food consumption.

In Exp. 1, the presence of either bone meal or calcium carbonate in the flour led to a significant decrease in the iron content of the liver. In Exp. 2, in which calcium lactate and calcium chloride were used as sources of calcium, their presence also appeared to result in decreased liver iron content.

All forms of calcium used in Exps. 1 and 2 led to significant increases in the heart weights expressed in g/100 g body-weight.

The effect of adding bone meal, calcium carbonate, calcium lactate and calcium chloride on haemoglobin regeneration is shown in Fig. 1. For simplicity of presenta-

Table 2. *Effect of additives in the diet on gain in weight, iron content of liver and heart weight of rats*

(Mean values with their standard errors for groups of five male or five female rats)

| Diet no. | Additive: $FeSO_4$ (mg/lb. flour) | Additive: Other salts (g/lb. flour) | Mean gain in body-weight with its standard error (g): Males | Mean gain in body-weight with its standard error (g): Females | Mean iron content of liver with its standard error (mg/100 g): Males | Mean iron content of liver with its standard error (mg/100 g): Females | Mean heart weight with its standard error (g/100 g body-weight): Males | Mean heart weight with its standard error (g/100 g body-weight): Females |
|---|---|---|---|---|---|---|---|---|
| | | | Experiment 1 | | | | | |
| 1 | 0 | 0 | 177±17 | 121±1 | 11·6±2·8 | 7·7±0·9 | 0·353±0·015 | 0·381±0·013 |
| 2 | 10 | 0 | 185±19 | 124±2 | 11·2±2·0 | 12·5±2·1 | 0·341±0·006 | 0·396±0·010 |
| 3 | 0 | Bone meal, 7·50 | 155±13 | 111±5 | 5·7±0·2 | 7·7±1·3 | 0·397±0·025 | 0·436±0·016 |
| 4 | 10 | Bone meal, 7·50 | 166±18 | 116±4 | 6·9±0·4 | 11·2±1·9 | 0·365±0·013 | 0·406±0·007 |
| 5 | 0 | Calcium carbonate, 7·26 | 119± 7 | 83±8 | 5·8±1·0 | 6·0±0·5 | 0·458±0·020 | 0·498±0·038 |
| 6 | 10 | Calcium carbonate, 7·26 | 132±11 | 81±2 | 6·1±1·1 | 5·9±1·0 | 0·451±0·026 | 0·485±0·013 |
| 7 | 0 | Disodium phosphate, 6·87 | 194±14 | 119±6 | 8·0±1·6 | 8·5±0·9 | 0·348±0·014 | 0·425±0·017 |
| 8 | 10 | Disodium phosphate, 6·87 | 177±14 | 119±2 | 10·4±0·9 | 15·4±0·9 | 0·365±0·010 | 0·409±0·007 |
| 9 | 0 | Sodium hexametaphosphate, 4·93 | 169±15 | 115±6 | 7·5±1·6 | 10·8±2·8 | 0·352±0·008 | 0·394±0·013 |
| 10 | 10 | Sodium hexametaphosphate, 4·93 | 174±20 | 119±2 | 7·3±1·3 | 11·7±3·0 | 0·346±0·009 | 0·399±0·013 |
| | | | Experiment 2 | | | | | |
| 11 | 0 | 0 | 148± 9 | 97±6 | 10·3±1·0 | 9·6±1·5 | 0·376±0·005 | 0·456±0·010 |
| 12 | 10 | 0 | 173±14 | 97±7 | 18·4±2·5 | 16·1±3·1 | 0·367±0·026 | 0·441±0·024 |
| 13 | 0 | Calcium lactate, 19·04 | 73± 8 | 52±6 | 10·5±2·1 | 9·2±1·8 | 0·556±0·021 | 0·593±0·024 |
| 14 | 10 | Calcium lactate, 19·04 | 96± 9 | 62±4 | 6·0±1·3 | 9·5±1·7 | 0·468±0·016 | 0·530±0·030 |
| 15 | 0 | Calcium chloride, 6·85 | 41± 4 | 44±2 | 6·5±0·5 | 9·2±1·2 | 0·775±0·127 | 0·632±0·031 |
| 16 | 10 | Calcium chloride, 6·85 | 84± 3 | 55±4 | 8·3±0·3 | 8·5±2·0 | 0·500±0·008 | 0·547±0·014 |

tion, the control groups in the two experiments have been combined. It can be clearly seen that all four forms of calcium tended to retard haemoglobin regeneration. Even when a small amount of iron was added to these diets the addition of any one of the four forms of calcium still led to a decrease in haemoglobin levels.

The effect of adding the two phosphates on the amount of food consumed is shown in Table 1, and their effect on gain in body-weight, iron content of liver and heart weight is shown in Table 2. Whereas the presence of disodium phosphate led to an increase in food consumption, the presence of sodium hexametaphosphate, on the other hand, had no effect on food consumption. In spite of the increase in food consumption in the presence of disodium phosphate, neither phosphate had any significant effect on the gains in weight of the rats or on the iron content of the liver. Sodium hexametaphosphate had no effect on the heart weights of the rats, but the presence of disodium phosphate did result in increases in heart weights that were just significant.

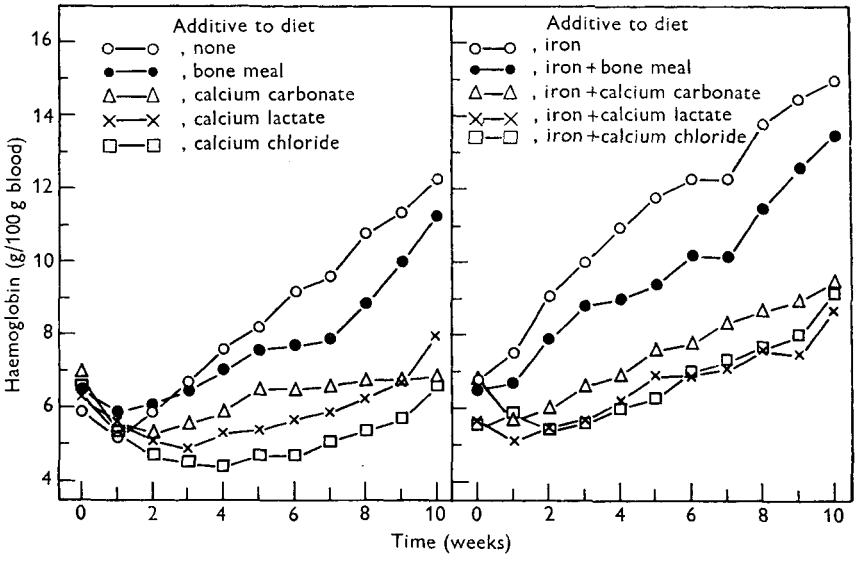


Fig. 1. Effect of calcium salts on haemoglobin regeneration in anaemic rats.

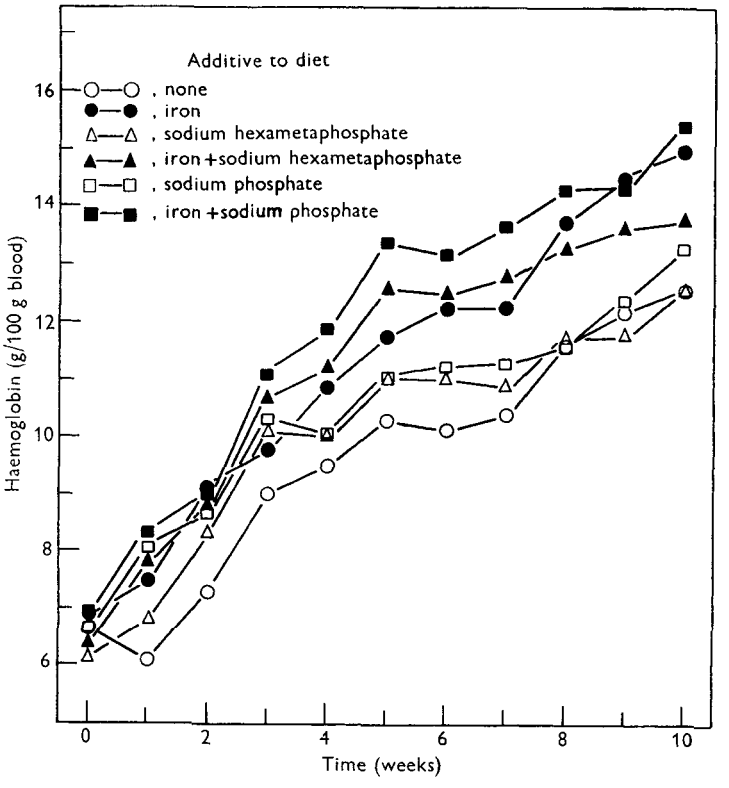


Fig. 2. Effect of two phosphates on haemoglobin regeneration in anaemic rats.

The effect of these two phosphates on haemoglobin regeneration is shown in Fig. 2. Statistical analysis of the results indicated that the presence of these two phosphates had no significant effect on haemoglobin levels.

## DISCUSSION

The results of the work reported suggest that adding relatively large amounts of calcium salts to bread diets adversely affects the utilization of iron by the anaemic rat, whereas adding phosphate salts does not. These results further suggest that the calcium rather than the phosphate portion of bone meal leads to an interference with the uptake of iron. They confirm the findings of Kletzien (1935, 1938, 1940) that several calcium salts reduced liver iron, though phosphates did not, but are not consistent with the views that dietary calcium will increase iron availability by precipitating the phosphorus as calcium phosphate. Kletzien (1940) reported that, under the conditions of his experiments, though 'calcium carbonate, citrate, acetate, tartrate, malate and chloride acted to diminish iron assimilation, calcium sulfate had no adverse effect'. Kletzien explains that 'the corrosive effects of calcium chloride are probably not due to its composition as such, but are related rather to its ready solubility and high degree of ionization, overcoming thereby the ionic antagonism of the sodium and potassium and interfering with the normal reactions involving chlorides, carbonates and phosphates. That absorption is a factor in making iron unavailable is not disproved; neither are changes in intestinal pH or the removal of iron by precipitation.' The diets used by Kletzien provided a daily intake of 0·5 mg iron, but there is no indication of the amounts of calcium or phosphorus consumed or contained in the diets.

It is perhaps worth mention that in our studies there was a direct relationship between the solubility of the calcium salt used and its effect on haemoglobin regeneration. Calcium chloride and calcium lactate, being the more soluble salts, retarded haemoglobin regeneration to the greater extent. The phosphate and carbonate had a smaller adverse effect on haemoglobin levels. The fact that calcium sulphate, according to Kletzien, does not affect the assimilation of iron cannot be explained on a solubility basis.

Though it might appear that the significant effects shown by the presence of bone meal in the diets on the liver iron content and heart weights are at variance with the results reported by Chapman & Campbell (1957), it should be pointed out that in the study now under consideration only the highest level of calcium and the lowest levels of iron were used, compared with the five levels of iron and three levels of bone meal used in the earlier work. With the same levels of iron and calcium the results of the two studies are in agreement.

The contradictory reports in the literature about the effect of calcium on the utilization of iron may be partly explained by the widely differing levels of calcium and iron used in the experimental diets. Generally speaking, when in animal studies the ratio calcium:iron has been relatively large, calcium has been found to have an adverse effect on the utilization of iron, whereas when the calcium:iron ratio was small, the presence of calcium was reported either to have no effect or actually to promote utilization of iron. It is appreciated that the calcium and iron contents of the diet are

only two of many factors influencing the utilization of iron; it is therefore of importance that analyses of the diets as fed be recorded.

With those diets in which the presence of a calcium salt influenced the iron content of the liver, haemoglobin regeneration or heart weights, consideration must be given to the effect the calcium had upon food consumption. If the presence of the added calcium has reduced food consumption, then the resulting effects might be due either to a reduction in the amount of iron consumed or to the presence of the calcium.

As has been noted earlier, the diets containing calcium carbonate and calcium chloride did lead to significant reduction in the amounts of food consumed. The presence of either of these salts led to lowered iron contents of the liver and increased heart weights. At the same time, however, it was noted that the presence of bone meal or calcium lactate, which also led to small liver iron values and increased heart weights, did not reduce food consumption. Thus this study, in which similar significant effects were produced by all calcium salts whether they resulted in reduced food consumption or not, lends support to the suggestion that the effect is a result of the increased calcium consumption and not of a reduced iron intake.

It was also noted that, though diet no. 11 contributed 15·4 mg iron to the rats consuming it, and diet no. 16 contributed 16·4 mg iron, the rats consuming diet no. 16 contained less iron in their livers and had greater heart weights than the rats on diet no. 11. This finding substantiates the suggestion that it was the increase in calcium in diet no. 16 that caused these effects.

It has been suggested that the iron requirement of the rat is approximately 0·25 mg/day (McCoy, 1949) or a total of 17·5 mg during the 10-week period. This would mean that diets nos. 11 and 13–16 were not meeting the total iron requirement of the rats during the test period. It might therefore be argued that lack of iron rather than the presence of calcium has retarded haemoglobin regeneration. However, diets nos. 3–6, which supplied ample amounts of iron, also led to retarded haemoglobin regeneration in the presence of calcium. If the rat requires 0·25 mg iron and 50 mg calcium/day, this would give a ratio Ca:Fe of 200:1. Only in diets nos. 13–16 does the ratio Ca:Fe appreciably exceed this value.

The fact that, under the conditions of the studies reported here, phosphate does not interfere with the utilization of iron, whereas calcium does, might suggest that the mucosal block (Granick, 1946) is in operation and prevents absorption of iron, rather than that the iron has been rendered unavailable by the formation of some insoluble iron salt. If this is so, it is an example of a condition in which the mucosal block is operating contrary to the iron needs of the body. One might perhaps then postulate that the mucosal cells have become saturated with calcium, setting up a block to the further absorption of iron.

## SUMMARY

1. Diets made up of 80% bread, to which was added bone meal, calcium carbonate, calcium lactate, calcium chloride, disodium phosphate or commercial sodium hexametaphosphate have been fed to groups of ten anaemic rats (five male and five female) for 10-week periods.

2. Each of the calcium salts was found to interfere with the utilization of iron as reflected by liver iron stores, haemoglobin regeneration and increase in heart weight.

3. The two phosphates had no significant effect on either the iron content of the liver or on the rate at which haemoglobin was regenerated.

4. The interference caused by the calcium salts is discussed on the basis that the 'mucosal block' may be operating to prevent the absorption of iron.

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## Effect of bone meal in enriched flour on the utilization of iron by anaemic and normal rats

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Chapman & Campbell (1957*a*, *b*) have shown that small amounts of edible bone meal added to patent unenriched flour, baked into bread and fed to anaemic rats have no effect on iron utilization. If, however, relatively large amounts of bone meal, calcium carbonate or other calcium salts were added to diets containing small amounts of iron, the utilization of iron was upset, as reflected by liver iron storage and haemoglobin regeneration. The amounts of calcium carbonate or edible bone meal added were approximately five times the amount permitted by the Canadian Regulations under the Food and Drugs Act for enriched bread, which require the addition of 'calcium carbonate or edible bone meal in an amount that will provide in one pound of enriched flour not less than 500 milligrams and not more than 650 milligrams of calcium' (*Office Consolidation of the Food and Drugs Act and of the Food and Drug Regulations*, 1954). Pett (1952) has reported that bone meal in enriched flour resulted in anaemia in children.