A comparison of the effects of dietary calcium and phosphorus deficiency on the in vitro and in vivo metabolism of 25-hydroxycholecalciferol in the chick

BY BARBARA A. SOMMERVILLE, R. SWAMINATHAN* AND A. D. CARE

Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT and
**Department of Chemical Pathology, Leeds General Infirmary, Leeds* 1

(*Received* 20 *October* 1977 – *Accepted* 11 *November* 1977)

1. Young chicks fed a diet deficient in calcium showed an eightfold increase in the in vitro renal production of 1,25-dihydroxycholecalciferol (1,25-DHCC) and those fed a diet deficient in phosphorus showed a threefold increase when compared to chicks on a normal diet.

2. The in vivo accumulation of 1,25-DHCC in the gut mucosa was doubled in both low-Ca and low-P groups as was the rate of Ca absorption from the duodenum and the Ca-binding protein activity. The accumulation of 1,25-DHCC in bone increased threefold in the low-Ca group but showed no change in the low-P group.

3. It was concluded that the increased rate of Ca absorption found in dietary P deficiency depends rather upon the capacity of the gut mucosa to accumulate larger amounts of 1,25-DHCC than upon an increased renal production of this metabolite. The mechanism by which this is achieved is unknown, but it unlikely to be a general increase in availability of 1,25-DHCC since no rise occurred in bone 1,25-DHCC levels.

Cholecalciferol (CC) is hydroxylated in the liver to form 25-hydroxycholecalciferol (25-HCC) and then this metabolite undergoes further hydroxylation in the kidney to form either 1,25-dihydroxycholecalciferol (1,25-DHCC) or 24,25-dihydroxycholecalciferol (24,25-DHCC). The activity of renal 25-hydroxycholecalciferol-1-hydroxylase (25-HCC-1-hydroxylase) measured by incubating kidney homogenates in vitro with labelled 25-HCC, has been shown to increase when the dietary calcium level is low (Omdahl, Gray, Boyle, Knutson & DeLuca, 1972; Swaminathan, Sommerville & Care, 1977*a*). In vivo studies have shown that dietary Ca deficiency results in an increased accumulation of 1, 25-DHCC in body tissues, particularly the mucosa of the small intestine (Edelstein, Harell, Bar & Hurwitz, 1975). A low-phosphorus diet also results in an increased accumulation of 1,25-DHCC in the gut mucosa (Tanaka, Frank & DeLuca, 1973; Edelstein *et al.* 1975; Friedlander, Henry & Norman, 1977), accompanied by an increase in the rate of Ca absorption from the small intestine. However, there are contradictory reports concerning the effect of low levels of dietary P on the renal 25-HCC-1-hydroxylase activity, some workers reporting that there is an increase (Baxter & DeLuca, 1976; Friedlander *et al.* 1977; Swaminathan, Sommerville & Care, 1977*b*) and some that there is no effect (Henry, Midgett & Norman, 1974; Montecuccoli, Bar, Risenfeld & Hurwitz, 1977).

The present experiment was designed to compare the effects of a low-Ca diet and a low-P diet on the in vitro and in vivo metabolism of 25-HCC, Ca absorption from the duodenum and duodenal calcium-binding protein (CaBP) activity in an effort to elucidate the different factors involved in adaptation to dietary Ca and P deprivation in the CC-replete chick.

MATERIALS AND METHODS

Ten-day-old male 909 chicks (Thornbers, Hebden Bridge) were given one of three diets which analysis showed to contain: low calcium 0·9 g Ca/kg, 5·0 g P/kg; low phosphorus 9·5 g Ca/kg, 2·0 g P/kg; normal 9·3 g Ca/kg, 7·4 g P/kg. All diets contained 65 nmol CC/kg. The composition of the diet was as described by Morrissey & Wasserman (1971). When 20 d old, the chicks were fasted for 4 h and duodenal Ca absorption was measured using the in vivo ligated-loop technique described by Swaminathan & Care (1975). A blood sample was taken for plasma Ca and inorganic phosphate (PO_4) determination and immediately after death the kidneys were removed and a 10% homogenate was prepared in 15 mmol Tris acetate buffer/l (pH 7·4) containing 1·9 mmol $MgCl_2$/l, 5 mmol sodium succinate/l and 200 mmol sucrose/l. A 3 ml portion of each homogenate was incubated at 39° with 500 pmol of 25-[26,27-^{3}H]HCC (16 Ci/mol; The Radiochemical Centre, Amersham, Bucks.). Lipid extracts of the incubation mixture were prepared and chromatographed on Sephadex LH20 (Pharmacia Ltd, Uppsala, Sweden) (0·55 m × 0·014 m glass column, flow rate 0·7 ml/min) with chloroform–hexane (65:35, v/v) eluent as described by Swaminathan *et al.* (1977*a*). The results were expressed as the amount of 25-HCC converted to 1,25-DHCC or 24,25-DHCC/g kidney tissue per min. In order to study the in vivo metabolism of 25-HCC, four chicks from each dietary group were injected intraperitoneally with 2 μCi of 25-[26,27-^{3}H]HCC in 0·1 ml 20% ethyl alcohol 15 h before killing. At death, the small intestine, the humerus, femur and tibia were removed for extraction and chromatography as described above. Plasma Ca concentration was measured by the method of Gitelman (1967) and plasma PO_4 concentration was determined by an automated method (Technicon Instruments Co. Ltd, 1966).

The mucosa from the distal segment of the duodenum was homogenized in Tris buffer (pH 7·4) and the activity of the CaBP was determined by the chelex-resin method as described by Wasserman, Corradino & Taylor (1968).

RESULTS

The values for the rate of Ca absorption, CaBP activity, and the plasma Ca and PO_4 concentrations of the three groups are given in Table 1. Duodenal Ca absorption in the low-Ca and the low-P groups were significantly higher than that of the normal-diet group. Duodenal CaBP was significantly increased in the low-P group. In the low-Ca group, CaBP was high but as there were only two observations statistical significance was not tested. Plasma Ca concentration was significantly higher in the low-P group and low in the low-Ca group. Plasma PO_4 on the other hand was low in both low-P and low-Ca groups.

Table 2 gives the results of the in vitro and in vivo metabolism of 25-HCC. The 25-HCC-1-hydroxylase activity in the low-Ca group was eight times as high as that in the normal diet group, whereas in the low-P group it was only three times as high as in the normal-diet group. The 25-HCC-24-hydroxylase in the low-Ca group was about one-sixth and in the low-P group it was about half that of the normal-diet group. This pattern of in vitro renal metabolism was not reflected in the in vivo tissue distribution of CC metabolites where the relative level of 1,25-DHCC in the small intestine was similar in the low-Ca and the low-P groups. The distribution of 24,25-DHCC in this tissue was similar in all three dietary groups. The bone contained smaller amounts of both dihydroxy-metabolites relative to 25-HCC and only the 1,25-DHCC level in the low-Ca group showed a marked difference, being two to three times as high as in the normal diet group.

Table 1. *The effect of low dietary calcium and phosphorus levels on plasma Ca and phosphate (PO_4) concentration, duodenal Ca absorption and calcium-binding protein (CaBP) activity*

(Mean values with their standard errors; number of chicks in parentheses.)

Composition of diet (g/kg)	Plasma concentration (mmol/l) Ca	Plasma concentration (mmol/l) PO_4	Duodenal Ca absorption (%)	Duodenal CaBP activity (net s:r/mg protein)†
Low Ca (0·9 Ca, 5·0 P)	1·77±0·02 (8) ***	1·54±0·11 (8) ***	83·3±3·2 (7) ***	1·3 (2)
Low P (9·5 Ca, 2·0 P)	2·82±0·10 (12) ***	0·85±0·10 (12) ***	89·8±2·4 (8) ***	0·93±0·10 (5) ***
Normal diet (9·3 Ca, 7·4 P)	2·30±0·03 (24)	2·03±0·05 (24)	46·1±2·2 (19)	0·42±0·03 (15)

Significance of difference from that of normal diet group by Student's *t*-test, ** $P < 0·005$; *** $P < 0·001$.
† CaBP activity was measured by the chelex ion-exchange method; s:r, total radioactivity in supernatant fraction:total radioactivity in resin.

Table 2. *The effect of low levels of dietary calcium and phosphorus on the* in vitro *and* in vivo *metabolism of 25-hydroxycholecalciferol (25-HCC)*

(Mean values with their standard errors; number of chicks in parentheses)

Composition of diet (%)	Activity of renal hydroxylase (pmol/min per g kidney) 25-HCC-1-hydroxylase	25-HCC-24-hydroxylase
Low Ca (0·10 Ca, 0·65 P)	40·7±5·6*** (3)	3·7±0·4***
Low P (0·20 P, 1·0 Ca)	16·7±2·4*** (5)	13·4±1·3**
Normal Diet (0·65 P, 1·0 Ca)	5·1±0·7 (9)	23·5±1·9

Composition of diet (%)	Tissue distribution of vitamin D metabolites (% total count) Small intestine 1,25-DHCC	Small intestine 24,25-DHCC	Bone 1,25-DHCC	Bone 24,25-DHCC
Low Ca (0·10 Ca, 0·65 P)	42·9±9·0*** (3)	8·1±1·3	15·1±2·9** (3)	7·7±1·6
Low P (0·20 P, 1·0 Ca)	37·0±3·0*** (4)	9·9±0·6	5·7±0·4 (3)	5·7±0·7
Normal Diet (0·65 P, 1·0 Ca)	19·7±2·5 (5)	9·4±2·0	5·9±0·9 (5)	7·8±0·8

Significance of difference from the normal diet group by Student's *t*-test: * $P < 0·05$; ** $P < 0·005$; *** $P < 0·001$.

DISCUSSION

The results presented here demonstrate a significant increase in 25-HCC-1-hydroxylase activity associated with a diet of 0·2 %P. This confirms the findings of Baxter & DeLuca (1976) who used a diet containing 0·15 %P Friedlander *et al.* (1977) (diet level unspecified), and Swaminathan *et al.* (1977*b*) who used 0·25 %P diet. Henry *et al.* (1974) failed to show an increase in 25-HCC-1-hydroxylase activity on a diet claimed to contain no P, but Baxter

& DeLuca (1976) suggested that their diet could have contained as much as 0·3 %P. Montecuccoli *et al.* (1977) confirmed that no increase in 25-HCC-1-hydroxylase activity occurred on a diet containing 0·36 %P.

Paradoxically, although the 25-HCC-1-hydroxylase activity found in the low-Ca group of chicks was twice that found in the low-P group, the rate of duodenal Ca absorption was similar in both groups as was the accumulation of 1,25-DHCC in the intestinal mucosa and the CaBP activity. Edelstein *et al.* (1975) and Friedlander *et al.* (1977) demonstrated an increased accumulation of 1,25-DHCC in the gut mucosa in response to a low-P diet.

These results seem to indicate that the accumulation of 1,25-DHCC in the gut mucosa is influenced by different factors in the case of low-Ca and low-P diets. In the low P situation, either the 1,25-DHCC binding capacity of the mucosa must increase or the rate of breakdown of 1,25-DHCC must decrease. In the bone the level of this metabolite was low in the P-deficient chicks but high in the Ca-deficient chicks. This observation provides some evidence that the increased gut accumulation of 1,25-DHCC in P deficiency is not due to a general systemic fall in the rate of deactivation of the metabolite.

The work of Bar & Wasserman (1973), Bar, Hurwitz & Edelstein (1973) and Ribovich & DeLuca (1975) showed that bypassing the 1, hydroxylation step in the kidney by feeding dihydrotachysterol, 1α-hydroxycholecalciferol or 1,25-DHCC respectively, abolished the Ca absorption adaptive response to a low-Ca diet but not that to a low-P diet. These results provide further evidence that the adaptation to a low-P diet depends upon a change at the gut mucosa rather than at the kidney.

REFERENCES

Bar, A., Hurwitz, S. & Edelstein, S. (1975). *Biochim. biophys. Acta* **411**, 106.
Bar, A. & Wasserman, R. H. (1973). *Biochem. biophys. Res. Commun.* **54**, 191.
Baxter, L. A. & DeLuca, H. F. (1976). *J. biol. Chem.* **251**, 3158.
Edelstein, S., Harell, A., Bar, A. & Hurwitz, S. (1975). *Biochim. biophys. Acta* **385**, 483.
Friedlander, E. J., Henry, H. L. & Norman, A. W. (1977). *Fedn Proc. Fedn Am. Socs exp. Biol.* **36**, 1096.
Gitelman, H. J. (1967). *Analyt. Biochem.* **18**, 521.
Henry, H. L., Midgett, R. J. & Norman, A. W. (1974). *J. biol. Chem.* **249**, 7584.
Montecuccoli, G., Bar, A., Risenfeld, G. & Hurwitz, S. (1977). *Comp. Biochem. Physiol.* **57A**, 331.
Morrissey, R. L. & Wasserman, R. H. (1971). *Am. J. Physiol.* **220**, 1509.
Omdahl, J. L., Gray, R. W., Boyle, I. T., Knutson, J. & DeLuca, H. F. (1972). *Nature, New Biol.* **237**, 63
Ribovich, M. L. & DeLuca, H. F. (1975). *Arch. Biochem. Biophys.* **170**, 529.
Swaminathan, R. & Care, A. D. (1975). *Calcif. Tiss. Res.* **17**, 257.
Swaminathan, R., Sommerville, B. A. & Care, A. D. (1977*a*). *Br. J. Nutr.* **38**, 47.
Swaminathan, R., Sommerville, B. A. & Care, A. D. (1977*b*). *Clin. Sci Mol. Med.* (In the Press.)
Tanaka, Y., Frank, H. & DeLuca, H. F. (1973). *Science, N.Y.* **181**, 564.
Technicon Instruments Co. Ltd (1966). *Technicon Methodology Sheet*, N-4B. Basingstoke, Hants: Technicon Instruments Co. Ltd.
Wasserman, R. H., Corradino, R. A. & Taylor, A. N. (1968). *J. biol. Chem.* **243**, 3978.

Printed in Great Britain