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Vitamin D Metabolism and Expression in Rats Fed on Low-Calcium and Low-Phosphorus Diets

By SAMUEL EDELSTEIN,*§ DINA NOFF,* LEVANA SINAI,* ARIE HARELL,* JULES B. PUSCHETT,† ELLIS E. GOLUB‡ and FELIX BRONNER‡||

** Department of Endocrinology, Municipal-Governmental Medical Center, Ichilov Hospital and Sackler School of Medicine, University of Tel Aviv, Tel-Aviv-Jaffa, Israel, †Renal Electrolyte Division, Allegheny General Hospital and University of Pittsburgh School of Medicine, Pittsburgh, PA 15212, U.S.A., and ‡Department of Oral Biology, University of Connecticut Health Center, Farmington, CT 06032, U.S.A.*

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1. Cholecalciferol, radioactively labelled with both ^{14}C and ^{3}H, was administered weekly for 7 weeks to rats that had been depleted of vitamin D for 4 weeks before repletion with the radioactive vitamin. This permitted measurement of the steady-state effect on vitamin D metabolism of low-calcium and low-phosphorus regimens, as compared with a normal mineral intake. These dietary manoeuvres were carried out during the last 3 weeks of repletion. Cholecalciferol, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol were determined in plasma, intestine, kidney and bone. Ca^{2+}-binding-protein content was measured in intestine and kidneys of comparable animals. 2. In rats on the low-calcium diets, 1,25-dihydroxycholecalciferol concentration was elevated in plasma, bone, kidney and intestine, and intestinal Ca^{2+}-binding protein was increased to over twice the concentration found in the control animals. 3. The low-phosphorus regimens led to a decrease in plasma phosphate and 1,25-dihydroxycholecalciferol in all tissues studied, for the latter to the point where it was undetectable in plasma and bone. Intestinal and renal concentrations of Ca^{2+}-binding protein were unchanged in the low-phosphate-intake group and decreased in the very-low-phosphate-intake group. 4. It is concluded that in the rat, unlike in the chick, hypophosphataemia is not associated with a stimulation of the production of 1,25-dihydroxycholecalciferol or its expression in the synthesis of Ca^{2+}-binding protein. Therefore the plasma phosphate concentration does not appear to be directly involved in the regulation of the functional metabolism of vitamin D.

Intestine, kidney and bone are thought to be target organs of vitamin D action. Plasma calcium homoeostasis results from the interactions of these three tissues (Bronner, 1973), with hypocalcaemia being a characteristic sign of simple vitamin D deficiency (Hurwitz *et al.*, 1969). The functional form of the vitamin in these sites is believed to be the dihydroxylated derivative 1,25-dihydroxycholecalciferol. This compound is produced from cholecalciferol (vitamin D_3) by C-25 hydroxylation in the liver, followed by C-1 hydroxylation in the kidney. It is the last step that is thought to be rate-limiting and therefore subject to biological regulation (DeLuca, 1973; Fraser & Kodicek, 1973; Norman & Henry, 1974).

§ To whom reprint requests should be addressed. Present address: Department of Biochemistry, The Weizmann Institute of Science, Rehovot, Israel.

|| On sabbatical leave, 1976–1977, Visiting Professor, Sackler School of Medicine, University of Tel-Aviv, and Visiting Scientist, The Weizmann Institute of Science, Rehovot, Israel.

Since hypocalcaemia is characteristic of vitamin D deficiency, it is reasonable to suppose that the enzymic formation of 1,25-dihydroxycholecalciferol is accelerated in conditions of low serum calcium by negative feedback. An inverse steady-state correlation betweev plasma calcium and plasma 1,25-dihydroxycholecalciferol concentrations has been reported in hypocalcaemia which resulted from low calcium intake (Boyle *et al.*, 1972).

When vitamin D deficiency is superimposed on phosphate deficiency, the plasma phosphate concentration in the dual-deficiency state is lower than in phosphate deficiency alone (Hurwitz *et al.*, 1969). Since vitamin D is thought to act also on phosphate absorption (Chen *et al.*, 1974), renal handling (Puschett *et al.*, 1972*a*,*b*) and bone turnover (Castillo *et al.*, 1975), it has been suggested (DeLuca, 1976) that hypophosphataemia also results in increased formation of 1,25-dihydroxycholecalciferol.

When 1,25-dihydroxycholecalciferol interacts with the intestinal cell, a specific Ca^{2+}-binding pro-

tein is formed (Emtage *et al.*, 1973; Wasserman *et al.*, 1974; Freund & Bronner, 1975*a*). Indeed, the intestinal Ca^{2+}-binding protein can now be considered as the most direct molecular expression of vitamin D status (Bar & Wasserman, 1974; Bronner & Freund, 1975).

In order to study the regulatory effect of hypocalcaemia and hypophosphataemia on the metabolism of vitamin D and its molecular expression, it was decided to measure the concentrations of cholecalciferol metabolites in target tissues and to relate these to concentrations of the Ca^{2+}-binding proteins. Such studies in the chick have shown that both hypocalcaemia and hypophosphataemia lead to increased concentrations of 1,25-dihydroxycholecalciferol in target cells and to increased production of the Ca^{2+}-binding protein (Edelstein *et al.*, 1975). However, in the rat, the situation is surrounded by controversy. Thus, whereas DeLuca and collaborators (Tanaka *et al.*, 1973; Ribowich & DeLuca, 1975) have reported that hypophosphataemia in rats is associated with increased calcium absorption and Haussler and collaborators (Hughes *et al.*, 1975; Haussler *et al.*, 1977) have reported increased plasma concentrations of 1,25-dihydroxycholecalciferol in rats on low-phosphate diets, Hurwitz *et al.* (1969) found that their hypophosphataemic vitamin D-replete rats absorbed less calcium than normophosphataemic controls. Moreover, Freund & Bronner (1975*b*) found no increase in Ca^{2+}-binding protein in hypophosphataemic vitamin D-replete rats compared with normophosphataemic controls, whereas Thomasset *et al.* (1976) reported an increase in duodenal Ca^{2+}-binding protein during phosphate deficiency.

Accordingly, the effect of hypophosphataemia on the concentration of 1,25-dihydroxycholecalciferol in plasma and in target tissues was studied and Ca^{2+}-binding protein was measured in the duodenum under these conditions. As will be shown below, hypophosphataemia in rats, resulting from low phosphate intake, was not associated with an increase in 1,25-dihydroxycholecalciferol in intestine, kidney, bone or plasma, nor with increased Ca^{2+}-binding protein. On the other hand, low calcium intake led to the expected increase in the target-tissue concentration of 1,25-dihydroxycholecalciferol and in its expression in intestinal mucosa.

Experimental

Materials

[4-^{14}C]Cholecalciferol (sp. radioactivity 32.3 Ci/mol) and [1,2-^{3}H]cholecalciferol (sp. radioactivity 12.6 Ci/mmol) were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). [1,2-^{3}H,4-^{14}C]Cholecalciferol was obtained by mixing the two above labelled preparations so as to give an $^{3}H/^{14}C$ ratio of approx. 5.0.

Animals and diets

Male weanling rats (weighing 25–30g, Wistar) were maintained for 4 weeks on a partially synthetic vitamin D-deficient diet (TD no. 72081; Teklad Mills, Madison, WI, U.S.A.) that contained 0.4% calcium and 0.3% phosphorus. At the end of this period, repletion with radiolabelled vitamin D was begun. Every 5–7 days during the repletion period, each animal received by subcutaneous injection 0.75 μg of [1,2-^{3}H,4-^{14}C]cholecalciferol dissolved in 0.1 ml of propylene glycol. After 4 weeks of repletion, the rats were divided into groups and placed on three regimens: (*a*) continuation of the normal mineral diet (0.4% Ca, 0.3% P); (*b*) low-calcium diet (0.004% Ca, 0.3% P, TD no. 96296 with added phosphorus. Teklad Mills); (*c*) low-phosphorus diet (0.4% Ca, 0.13% P, TD no. 96296 with added Ca; Teklad Mills). The diets were vitamin D-deficient, the animals continuing to receive [^{3}H,^{14}C]cholecalciferol by injection. On the 20th day the rats were killed, and intestinal mucosa, kidney, bone and plasma were analysed for cholecalciferol metabolites. Plasma calcium and phosphorus were also measured.

To measure mucosal Ca^{2+}-binding protein under conditions of high, low and very low phosphorus intake and low calcium intake, 120g male Sprague–Dawley rats were divided into groups and placed on partially synthetic diets, 1.5% Ca and 1.5% P (TD no. 67207, Teklad Mills), 1.5% Ca and 0.2% P (TD no. 70389, Teklad Mills), 1.5% Ca and 0.3% P (TD no. 75087, Teklad Mills) and 0.06% Ca and 0.02% P (TD67205A, Teklad Mills) respectively. After 7 days (14 days for the animals on the low-calcium diet) the rats were decapitated, their duodena and kidneys removed and analysed for Ca^{2+}-binding-protein content. Plasma calcium and phosphorus were determined on tail blood during the 7-day period and at the time of death.

Lipid extraction and chromatography

After the animals were killed, the scraped mucosa from the proximal 12cm of the small intestine, kidneys and bones from six rats from each group were weighed, minced and pooled, and the lipids were extracted with chloroform/methanol (2:1, v/v) (Bligh & Dyer, 1959). Pooled plasma samples from six animals were also extracted with chloroform/methanol. The lipid extracts obtained from the intestine and the bones were analysed for cholecalciferol metabolites by t.l.c. (Lawson *et al.*, 1971). The lipid extracts obtained from the kidneys and plasma were analysed by column chromatography on Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden), equilibrated and developed in chloroform/light petroleum (b.p. 40–60°C)/methanol

(75:23:2, by vol.) and chloroform/light petroleum (b.p. 40–60°C) (1:1, v/v) respectively, as previously described (Weisman *et al.*, 1976). This procedure permits efficient separation of the dihydroxylated metabolites of cholecalciferol, but does not allow satisfactory determination of the plasma 25-hydroxycholecalciferol concentrations.

Ca^{2+}-binding-protein assay

Mucosal tissue was harvested and processed and the supernatant chromatographed as previously described (Freund & Bronner, 1975*b*), except that the elution buffer contained approx. 0.005mM-Ca^{2+} and 0.03μCi of ^{45}Ca/ml. This made it possible to determine binding by the Hummel & Dreyer (1962) procedure, i.e. to measure the areas of peak B ($V_e/V_0 \simeq 1.7$) of the elution profile. Results obtained by this procedure were comparable with those obtained by the semi-micro competitive-binding assay utilizing Chelex resin (Freund & Bronner, 1975*b*). The binding activity found in peak B was expressed as ng-atoms of Ca^{2+} bound and referred to the weight of mucosal tissue. For better comparison, the amount of protein loaded on to the column was kept similar in each diet and the same chromatographic column was used throughout.

For kidney, Ca^{2+}-binding protein was analysed as above, except that values were expressed as ng-atoms of Ca^{2+} bound/mg of protein recovered in peak B. Protein loads were kept the same and the same chromatographic column (Sephadex G-75) was used in all runs with kidney material (cf. Hermsdorf & Bronner, 1975).

Calcium and phosphorus assays

Plasma calcium was determined on blood plasma obtained by cardiac puncture or on tail-vein samples collected in heparinized capillary tubes, which were centrifuged (1000*g* for 10min). The resulting plasma was placed in tared tubes, diluted with 0.1% $LaCl_3$, and analysed by atomic-absorption spectrophotometry (Perkin–Elmer model 290 or model 303: Freund & Bronner, 1975*b*).

Phosphorus analysis, by the method of Ames (1966), was done on the supernatant obtained by centrifugation (10000*g* for 10min) after precipitation of the plasma with 12.5% (w/v) trichloroacetic acid. Typically 20μl of supernatant was used for phosphorus analysis.

Measurements of radioactivity

Radioactivity measurements were carried out either in a Nuclear–Chicago Unilux or in a Packard Tri-Carb automatic liquid-scintillation spectrometer no. 3380. Lipid samples were dried and counted for radioactivity in a solution of 100mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene and 4.0g of 2,5-diphenyloxazole/litre of toluene. Quenching was corrected for by using the automatic external standardization and correlation curves for combined counting of ^{14}C and ^{3}H. ^{45}Ca was counted in Bray's (1960) scintillator at an efficiency of about 50%, with the aid of internal standards.

The relative amounts of 1,25-dihydroxycholecalciferol in the peaks eluted from the columns and the t.l.c. plates were calculated from the $^{3}H/^{14}C$ specific-radioactivity ratios (Weber *et al.*, 1971). Care was taken to differentiate between 1,25-dihydroxycholecalciferol and other polar cholecalciferol derivatives that co-chromatograph with the dihydroxylated compound.

Determination of protein

Protein was determined spectrophotometrically by the method of Layne (1957).

Errors in determination of cholecalciferol metabolites

Double-labelled radioactive cholecalciferol, obtained by mixing ^{14}C- and ^{3}H-labelled molecules of cholecalciferol, was injected continuously into the rats as the only source of vitamin D. To be able to measure chromatographically the various metabolized derivatives of the injected vitamin, especially the dihydroxylated ones, sufficient radioactivity had to be obtained. As the specific radioactivity of the presently available [^{14}C]cholecalciferol is low, and, as the detection of 1,25-dihydroxycholecalciferol is based on a change in the $^{3}H/^{14}C$ ratio, lipid extraction had to be performed on pooled tissues. To ensure accuracy and precision in the determination of cholecalciferol metabolites, tissues were re-extracted twice; recoveries were corrected for after each step, and each vial was counted for radioactivity for 20min after temperature equilibration. The limit of detection of the assay is 0.015pmol of cholecalciferol metabolite, with maximal error of 5.0%.

Results

Cholecalciferol metabolites in plasma and intestinal mucosa

Table 1 shows that dietary restriction of either calcium or phosphorus resulted in a decrease in circulating 24,25-dihydroxycholecalciferol and an increase in a more polar peak, which was co-eluted with 1,25-dihydroxycholecalciferol. Loss of ^{3}H, indicating the presence of 1,25-dihydroxycholecalciferol, was observed only in the polar peak eluted from the column on which the lipid extract from the plasma of rats fed on the low-calcium diet was chromatographed.

Another dihydroxylated derivative of cholecalciferol, 25,26-dihydroxycholecalciferol, was present in blood and co-chromatographed with 1,25-dihydroxychole calciferol. This prevented an accurate deter-

mination of loss of ^3H from the plasma peak. This compound was not found in other tissues.

As shown in Fig. 1, lipid extracts prepared from intestinal mucosa of animals fed on the low-calcium diet showed increased content of 1,25-dihydroxycholecalciferol, whereas similar extracts from the intestinal mucosa of animals fed on the low-phosphorus diet contained virtually no 1,25-dihydroxycholecalciferol. Thus a low-phosphorus regimen led to virtual suppression of the intestinal accumulation of 1,25-dihydroxycholecalciferol.

Intestinal Ca^{2+}-binding protein

The findings set out in Table 2 indicate that progressive lowering of the dietary phosphate was associated with a progressive fall in plasma phosphate concentrations, and a smaller, but significant and progressive, rise in plasma Ca^{2+} concentration. The content of Ca^{2+}-binding protein in the mucosa was unaffected when plasma phosphate decreased to 6.4mg/100ml, but decreased to 40% of the control value in animals on the very-low-phosphorus intake. On the other hand, when comparable animals were fed on the 0.2%-phosphorus diet but with only 0.06% Ca, the intestinal Ca^{2+}-binding protein content was much higher than when their calcium intake was 1.5%. Indeed, the Ca^{2+}-binding-protein content of these animals was, as previously reported

Table 1. *Concentration of the dihydroxylated metabolites of cholecalciferol in plasma of rats fed on normal-mineral low-calcium and low-phosphorus diets*

The lipid extracts prepared from plasma were analysed for cholecalciferol metabolites by Sephadex LH-20 column chromatography as described in the Experimental section. The polar peak is eluted at V_e of 1,25-dihydroxycholecalciferol. 1,25-Dihydroxycholecalciferol content in this polar peak was calculated from ^3H loss, as described in the Experimental section. The results are means±S.E.M. for six determinations.

Diet group	Plasma Ca (mg/dl)	Plasma P (mg/dl)	24,25-Dihydroxycholecalciferol (pmol/ml)	Polar peak (pmol/ml)	1,25-Dihydroxycholecalciferol (pmol/ml)
Normal-mineral (0.4% Ca, 0.3% P)	10.0±0.7	6.8±0.4	15.3	0.4	Not detectable
Low-calcium (0.004% Ca, 0.3% P)	8.1±0.9	5.5±0.9	5.9	0.8	0.2
Low-phosphorus (0.4% Ca, 0.13% P)	9.1±1.0	4.5±0.9	7.7	0.6	Not detectable

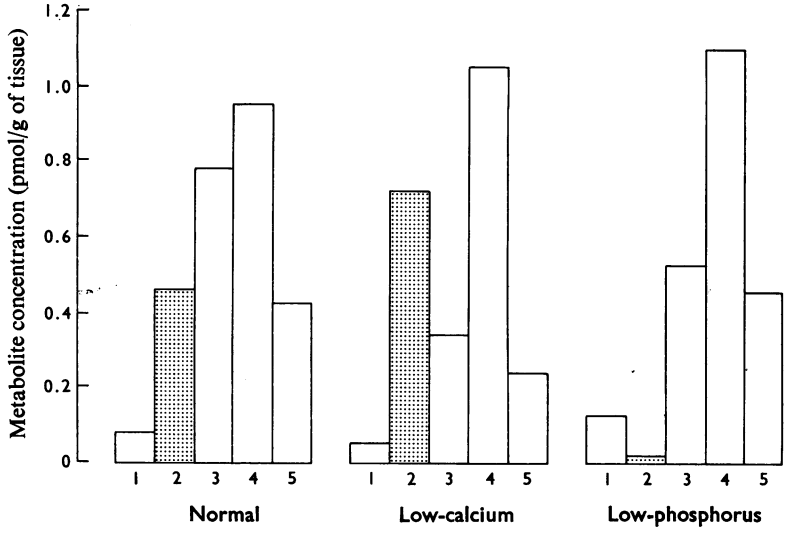

Fig. 1. *Distribution of cholecalciferol metabolites in the intestinal-mucosal tissue of rats fed on normal-mineral, low-calcium and low-phosphorus diets*

The lipid extracts prepared from the intestinal-mucosal tissue were analysed for cholecalciferol metabolites by t.l.c. as described in the Experimental section. 1, Polar metabolites; 2, 1,25-dihydroxycholecalciferol; 3, 24,25-dihydroxycholecalciferol; 4, 25-hydroxycholecalciferol; 5, cholecalciferol.

(Freund & Bronner, 1975*b*), the highest of the four groups studied. Thus lowering of dietary phosphorus to 0.2% had no effect on Ca^{2+}-binding protein, whereas lowering the calcium intake to 0.06% more than doubled the mucosal content of Ca^{2+}-binding protein.

Cholecalciferol metabolites and Ca^{2+}-binding protein in kidney

Table 3 shows that the kidney content of most cholecalciferol metabolites was much higher than the intestinal content (Fig. 1). The sum of all metabolites was 30–40 pmol/g in kidney, and only about 7–8 pmol/g in bone and intestine combined.

The kidneys of the animals on the low-phosphorus diet had the highest 25-hydroxycholecalciferol concentration (Table 3), though their cholecalciferol and cholecalciferol ester concentrations were lower than those of the other two diet groups. This is consistent with the suggestion (Puschett *et al.*, 1975) that 25-hydroxycholecalciferol may play a specific role in the hypophosphataemic euparathyroid mammal.

Whereas low-calcium intake was associated with a rise in the concentration of renal 1,25-dihydroxycholecalciferol (Table 3), low-phosphorus intake and hypophosphataemia were not. Rather, the tissue concentration of this metabolite was decreased. The renal Ca^{2+}-binding protein was also decreased in the animals on the very-low-phosphorus diets (Table 2). However, as previously noted (Hermsdorf & Bronner, 1975), renal Ca^{2+}-binding protein in the rat was not very sensitive to variations in mineral intake.

Cholecalciferol metabolites in bone

In bones obtained from rats that were fed on the normal mineral diet, only trace amounts of 1,25-dihydroxycholecalciferol were detected (Table 4). In bones from rats fed on the low-calcium diet, 1,25-dihydroxycholecalciferol concentration was twice as high as in the controls, whereas no 1,25-dihydroxycholecalciferol was detected in the bones from the animals on the low-phosphorus regimens. The absolute amount of 1,25-dihydroxycholecalciferol found in bone was only about one-quarter that found in intestine (Table 4 and Fig. 1). This may reflect the higher content of extracellular material found in bone. Nevertheless, the amount of 1,25-dihydroxycholecalciferol in bone tissue was sufficiently high for Ca^{2+}-binding protein to be detected if it had been

Table 2. *Plasma calcium, plasma inorganic phosphorus, renal and intestinal Ca^{2+}-binding protein of rats fed on high-calcium high-phosphorus and low-calcium low-phosphorus diets*

Analysis was carried out as described in the Experimental section. Animals were maintained on the respective diets for 7 days. Results are means±S.E.M.

Diet group	Number of animals	Body weight (g)	Plasma Ca (mg/dl)	Plasma P (mg/dl)	Intestinal Ca^{2+}-binding protein (ng-atoms of Ca^{2+} bound/g of mucosa)	Renal Ca^{2+}-binding protein (ng-atoms of Ca^{2+} bound/mg of protein)
High-Ca, high-P (1.5% Ca, 1.5% P)	10	150±5	10.55±0.17	9.60±0.27	24.2±3.2	7.5±1.1
High-Ca, low-P (1.5% Ca, 0.2% P)	10	149±5	11.71±0.01	6.37±0.04	24.9±1.1	8.5±0.6
Low-Ca, low-P (0.06% Ca, 0.2% P)	3	133±6	10.02±0.38	Not done	53.3±1.0	Not done
High-Ca, very-low-P (1.5% Ca, 0.02% P)	10	130±2	12.69±0.38	4.59±0.06	10.1±2.5	5.7±0.2

Table 3. *Distribution of cholecalciferol metabolites in kidneys of rats fed on normal-mineral, low-calcium and low-phosphorus diets*

The lipid extracts prepared from the kidneys were analysed for cholecalciferol metabolites by Sephadex LH-20 column chromatography as described in the Experimental section.

Metabolite	Normal-mineral diet		Low-calcium diet		Low-phosphorus diet	
	%	pmol/g	%	pmol/g	%	pmol/g
Cholecalciferol esters	52	21.8	56	18.5	50	14.5
Cholecalciferol	31	13.0	26	8.6	22	6.4
25-Hydroxycholecalciferol	9	3.8	10	3.3	19	5.5
24,25-Dihydroxycholecalciferol	7	2.9	6	2.0	8	2.3
1,25-Dihydroxycholecalciferol	1	0.4	2	0.7	1	0.3

Table 4. *Distribution of cholecalciferol metabolites in bones of rats fed on normal-mineral, low-calcium and low-phosphorus diets*

The lipid extracts prepared from the bones were analysed for cholecalciferol metabolites on t.l.c. as described in the Experimental section.

	Normal-mineral diet		Low-calcium diet		Low-phosphorus diet	
Metabolite	%	pmol/g	%	pmol/g	%	pmol/g
Polar metabolites	4	0.17	4	0.23	1	0.06
1,25-Dihydroxycholecalciferol	2	0.08	3	0.17	Not detectable	
24,25-Dihydroxycholecalciferol	26	1.12	12	0.70	13	0.73
25-Hydroxycholecalciferol	32	1.38	31	1.80	38	2.12
Cholecalciferol	36	1.55	50	2.90	48	2.69

present. We have been unable to detect Ca^{2+}-binding protein in bone tissue (S. Edelstein, D. Noff, L. Sinai, A. Harell, J. B. Puschett, E. E. Golub & F. Bronner, unpublished work). This therefore raises the question whether 1,25-dihydroxycholecalciferol is a metabolite that is active in bone, and, if it is, what its molecular expression is.

Discussion

Our experimental protocol enabled us to replace body stores of vitamin D with doubly labelled vitamin D and to measure the steady-state effects of dietary manoeuvres on vitamin D metabolism and action. Restriction of calcium intake led, as in the chick (Edelstein *et al.*, 1975), to increased accumulation of 1,25-dihydroxycholecalciferol in intestinal tissue and to the expected increase in intestinal Ca^{2+}-binding protein (Table 3; see also Wasserman *et al.*, 1974; Freund & Bronner, 1975*b*; Bronner & Freund, 1975). But, unlike the chick, and contrary to the report of increased plasma concentrations of 1,25-dihydroxycholecalciferol in the rat (Hughes *et al.*, 1975; Haussler *et al.*, 1977), our phosphorus-deficient animals exhibited no increase in 1,25-dihydroxycholecalciferol in plasma, intestine, kidney or bone. They also did not exhibit increased concentrations of Ca^{2+}-binding protein, in contrast with the report of Thomasset *et al.* (1976). Rather, low-phosphorus intakes were associated with decreased or undetectable concentrations of 1,25-dihydroxycholecalciferol in plasma and target tissues and with a decrease in Ca^{2+}-binding protein, the latter measured in different animals on different low-phosphorus regimens.

It should be noted that the measured 1,25-dihydroxycholecalciferol values in plasma, intestine, kidney and bone were consistent, i.e. all increased in the calcium-deprived animals and all decreased in the phosphate-deficient animals. Moreover, concentrations of Ca^{2+}-binding protein, measured in different animals under similar dietary conditions, varied in the same direction as the 1,25-dihydroxycholecalciferol concentration. In addition, in unpublished experiments conducted over the past 3 years with rats fed on a low-phosphorus diet with soya protein as a base we also were unable to observe an increase in 1,25-dihydroxycholecalciferol. We have no explanation why our results are at such variance with those of Hughes *et al.* (1975) and Haussler *et al.* (1977), nor can we explain why our measured plasma values are an order of magnitude below those reported by Haussler *et al.* (1977). Differences in analytical procedures, animal species and experimental protocol may be contributing factors.

It is well known that the phosphorus-deficient rat is hypercalcaemic and hypercalciuric (Hurwitz *et al.*, 1969; Cuisinier-Gleizes *et al.*, 1976). This situation has been interpreted as having resulted from the body's great need for phosphate and its willingness to compromise calcium homoeostasis in order to obtain scarce phosphorus from the only source, the skeleton (Bronner, 1976). Whatever the cause of the hypercalcaemia and hypercalciuria, an increase in calcium absorption would seem to be anti-homeostatic, i.e. it would compromise calcium homoeostasis even further. Apparently this situation occurs in the chick, but perhaps does not occur commonly in the rat, since, in our hands, phosphorus deficiency has not been associated with increased calcium absorption. Hurwitz *et al.* (1969) have shown, for example, that vitamin D-replete animals on a high-calcium low-phosphorus diet absorbed only 30% of a daily intake of 83mg of Ca, whereas control animals absorbed 55% of a daily intake of 83mg of Ca. Moreover, as reported here and suggested previously (Freund & Bonner, 1975*b*), a low phosphorus intake did not lead to an increase in the mucosal content of Ca^{2+}-binding protein in rats.

The data reported by us indicate a correlation between the concentration of intestinal 1,25-dihydroxycholecalciferol and that of Ca^{2+}-binding protein. However, Bar *et al.* (1975) have shown that in the chick the intestinal response to low-phosphorus treatment in terms of Ca^{2+}-binding protein and Ca^{2+} transport is only partially correlated with 1,25-dihydroxycholecalciferol metabolism. Moreover,

Freund & Bronner (1975*b*) have suggested that cellular Ca^{2+} concentrations regulate the expression of 1,25-dihydroxycholecalciferol in the enterocyte, a suggestion confirmed *in vitro* (Golub *et al.*, 1977).

What then emerges is that low-calcium intakes are associated in both chicks and rats with an apparent increase in the production of 1,25-dihydroxycholecalciferol and increased concentrations of this metabolite in the enterocyte. In turn, this is associated with a marked increase in Ca^{2+}-binding protein.

Hypophosphataemia, on the other hand, does not seem to lead to an increased production of 1,25-dihydroxycholecalciferol in the rat nor to its expression in the form of intestinal Ca^{2+}-binding protein. On the contrary, very low phosphorus intake and pronounced hypophosphataemia were associated with decreased 1,25-dihydroxycholecalciferol tissue concentrations and lowered Ca^{2+}-binding-protein content. Thus low plasma phosphate concentration does not appear to play a direct role in the regulation of 1,25-dihydroxylase activity nor in the expression of its product in kidney or intestine.

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