Intestinal Calcium Absorption and Calcium-Binding Protein: Influence of Dietary Calcium (36279)

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Regulation of absorption at the intestinal level represents a basic yet powerful means of homeostatic control. Such a control system for calcium has been known for over 30 years (1). Low body stores of calcium promote increased absorption (*i.e.*, facultative effect), while high stores result in decreased absorption (1–3). This compensatory action for the maintenance of calcium is detectable within a few days following its imbalance (4) and is dependent on vitamin D (2).

Studies of late have been directed towards an explanation of the calcium adaptation mechanism at the intestinal level. Rats regulated to a low-calcium diet demonstrate an increased capacity to actively transport this cation (5, 6). Additionally, an increased concentration of an intestinal vitamin D-inducible calcium-binding protein (CaBP) has been observed following low-calcium feeding (7–10). Results from kinetic studies (11) have also suggested an increased affinity for calcium by this transport system in low-calcium adapted animals. Regardless, the question remains, "What controls the calcium adaptive process?"

Generally, the regulator of this process has been considered to be a hormone. However, hypophysectomy, adrenalectomy, gonadectomy and thymectomy failed to block the adaptive mechanism (2, 5, 6). Additionally, neither parathyroid hormone (PTH) nor thyrocalcitonin (TCT) appears to be required, although these results are somewhat equivocal (5, 9, 12). Nicolaysen (2) originally coined the term "endogenous factor" with regard to an unknown bone hormonal factor which he considered to be the effector of the adaptive process. Investigators of late (10, 13) have failed to demonstrate such a factor but used an "endogenous" type of factor to explain their results. The current study is an attempt to demonstrate the regulatory properties of calcium regarding the control of its absorption.

Materials and Methods. One-day-old White Leghorn cockerels (DeKalb) were given vitamin D-deficient feed (14) and distilled water *ad libitum*. Vitamin D_3 was supplemented in the diet or given orally to the chick, as described in the text.

Calcium absorption studies were performed *in situ* using the duodenal loop. The chicks were fasted for 15 hr, anesthetized with chloral hydrate (35 mg/100 g body wt, im) and the duodenal loop exposed. Next, 0.2 ml of ^{45}Ca phosphate-free bicarbonate solution (0.1 μCi $^{45}CaCl_2$, 120 m*M* NaCl, 4.9 m*M* KCl, 9.2 m*M* $NaHCO_3$ and 6 m*M* $CaCl_2$; pH 7.0) was introduced into the isolated duodenum through the distal ligature. The ligature was secured and the loop returned to the peritoneal cavity for a 20-min period. Following this, the loop was excised, ashed at 600°, the ash dissolved in 2 *N* HCl and the percent of ^{45}Ca absorption determined. CaBP activity was assayed in mucosal supernatant fractions which were pretreated with a cation exchange resin[3] (2.5 ml supernatant to 0.25 ml resin) in order to normalize the endogenous calcium concentration (15). The assay consisted of the uniform and rapid mixing of 1 ml supernatant with 0.1 ml chelex

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[3] 200-400 Mesh Chelex-100 (Bio-Rad Laboratories, Richmond, CA).

TABLE I. The Influence of Dietary Calcium on Transient Changes in Calcium Absorption and CaBP Activity in the Chick.

Exp.	Dietary treatment[a]	Calcium absorption (% ^{45}Ca absorbed)	CaBP activity (% ^{45}Ca pool/mg protein)
1[b]	low Ca	90.2 ± 0.7[d]	4.60 ± 0.14
	normal Ca	74.6 ± 2.9[e]	3.81 ± 0.13[e]
2[c]	low Ca	69.6 ± 6.7	5.68 ± 0.09
	high Ca	86.6 ± 3.0[e]	7.47 ± 0.27[e]

[a] See Fig. 1 for diagrammatic representation.

[b] Chicks were fed normal (1% Ca) diet until 14 days of age, at which time one-half of the chicks were shifted to a low-calcium (0.08%) diet. Chicks were killed at 20 days of age (i.e., day 6 in Fig. 1). Vitamin D_3 was given im (100 IU/wk).

[c] Protocol same as Exp. 1 except all chicks received a low-calcium diet for 6 days beginning on day 14. On day 20 one-half of the low-calcium chicks were shifted to a high-calcium diet (2% Ca), with all chicks killed at 23 days of age (i.e., day 9 in Fig. 1). Vitamin D was included in the diet (30 IU/100 g).

[d] Results are given as mean ± SE for 5 observations.

[e] Significantly different ($p < .01$).

resin and 0.1 μCi ^{45}Ca for 30 sec, followed by rapid centrifugation (*i.e.*, 3000*g*) (15, 16). Near equilibrium was obtained in the partitioning of ^{45}Ca between the soluble CaBP molecules and the chelex resin. More of the ^{45}Ca pool remained in the supernatant with increasing CaBP concentrations. The results were expressed as the % ^{45}Ca pool/mg protein. Radioactivity of ^{45}Ca was measured using a gas flow detector (Nuclear Chicago, Model 447), protein by the Lowry procedure (17), and calcium with an atomic absorption spectrophotometer (Instrumentation Laboratory, Model 153). The mean ± SE was calculated for each group with Student's *t* test employed to assess significance.

Results. Chicks adapted to a low-calcium intake exhibited both increased intestinal calcium absorption and CaBP concentration (Table I, Exp. 1) compared to chicks fed a normal calcium diet.[4] The adaptation process was vitamin D-dependent, wherein deficient chicks gave low values for calcium absorption and CaBP activity (*i.e.*, 25% ^{45}Ca absorption and 0.9% ^{45}Ca pool/mg protein, respectively) regardless of dietary calcium intake. In a time course study of the adaptation process, chicks fed a normal calcium diet demonstrated declining CaBP activity with age (Fig. 1). In contrast, low-calcium-adapted chicks sustained the initial level of CaBP activity with highly significant difference ($p < .01$) between the two groups from days 5 through 13 (Fig. 1). Feeding a high-calcium diet to chicks previously regulated to a low-calcium regimen was associated with a transitory rise in CaBP activity. This change was most noticeable 3 days following the low-to-high calcium feeding change (Fig. 1, day 9) wherein the group shifted to a high-calcium diet showed significantly greater CaBP activity than chicks maintained on the lower calcium level. However, the CaBP activity decreased sharply thereafter and was comparable to normal-calcium-fed chicks and significantly less than the continuously low-calcium-fed animals at 13 days.

These results prompted additional efforts to determine whether the transitory stimulation of CaBP activity in the high-calcium group coincided with an increased intestinal calcium absorption. Subsequent results (Table I, Exp. 2) demonstrated that the initial response to the high-calcium diet by low-calcium-adapted chicks was increased CaBP as well as an enhancement of intestinal absorption, with changes being highly significant ($p < .01$).

Vitamin D_3 was found to be essential for development of the calcium facultative effect in chicks, similar to previous reports (2, 5). This permissive action of vitamin D_3, however, was observed to be sensitive to the dietary calcium intake. Chicks maintained on a normal calcium diet showed no change in CaBP activity due to varying vitamin D dosages. In contrast, chicks fed a low-calcium diet demonstrated increased CaBP activity in response to an increase in vitamin D dosage (Fig. 2). A minimal vitamin D level (10 IU/2 days) was sufficient to demonstrate the facultative effect in low-calcium-fed chicks,

[4] The terms low, normal and high-calcium diets refer to diets containing 0.08, 1.0 and 2.0% calcium, respectively.

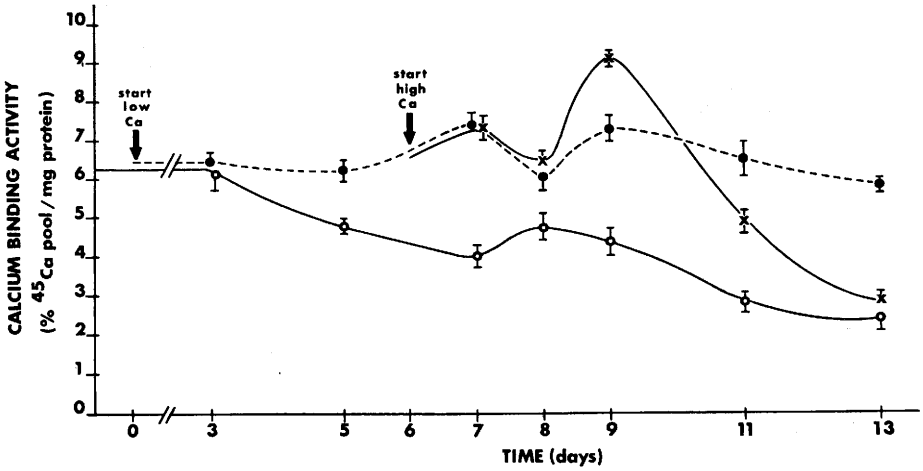

FIG. 1. Time course of CaBP activity in chicks fed varying calcium regimens. O—O Chicks fed normal diet (1% Ca) from date of hatch until 14 days of age (zero time). ●- - -● Normal-calcium chicks shifted to a low-calcium diet (0.08% Ca) at 14 days of age (zero time). X—X Chicks adapted to a low-calcium diet shifted to a high-calcium diet on day 6 (20 days of age). Results are expressed as mean ± SE for 5 observations.

yet a higher vitamin D level (60 IU/2 days) increased the response. Correspondingly, the high-calcium potentiation of the facultative effect was also significantly increased ($p < .01$) by the additional vitamin D (Fig. 2). Both levels of vitamin D supported an expected growth rate and bone ash values in normal-calcium-fed chicks.

The calcium potentiative effect could not be demonstrated when strontium was substituted for dietary calcium on an equal molar basis (Table II), moreover, low-calcium-adapted chicks appeared unable to maintain their previous level of CaBP activity when

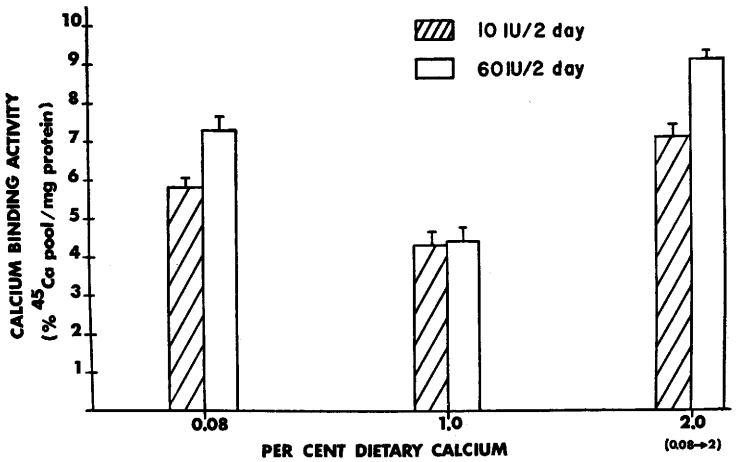

FIG. 2. Three groups of chicks were pre-fed specific calcium regimens as outlined in figure 1 with experimentation conducted at day 9 (23 days of age). Dietary calcium regimentation was maintained as shown in Fig. 1, with the vitamin D supplementation varied at 10 IU and 60 IU/2 day, given im in 95% ethanol. The 0.08% Ca group illustrates the adaptation response, while the 2% Ca group shows the potentiative effect. The 1% Ca group experienced no dietary change. It should be emphasized that the potentiative effect is transitory and disappears with time, as shown in Fig. 1. Results are expressed as mean ± SE for 5 observations.

TABLE II. Effect of Actinomycin D and Dietary Strontium on the Transient Dietary-Calcium Potentiation of CaBP Activity.

Dietary treatment	CaBP activity (% ^{45}Ca pool/mg protein)	Plasma calcium (mg/100 ml)
low Ca	2.23 ± 0.32	8.02 ± 0.40
low Ca + Act. D	1.70 ± 0.33	7.62 ± 0.38
high Ca	4.60 ± 0.24[d,f]	9.73 ± 0.11[f]
high Ca + Act. D[a]	2.08 ± 0.28	7.76 ± 0.38
high Sr[b]	1.36 ± 0.17[e]	6.59 ± 0.20[e,e]

[a] Actinomycin D was given ip at time of dietary change (i.e., 72 hr before sacrifice) (10 μg/100 g body wt). Dietary treatment was as shown for the 9-day time point in Fig. 2.

[b] $SrCO_3$ replaced $CaCO_3$ on an equal molar basis.

[c] Plasma strontium was 10.53 ± 0.82 mg/100 ml. Vitamin D_3 was included in the diet (30 IU/100 g feed).

[d] Results are given as mean ± SE of 5–9 observations.

[e] Significantly different from low-Ca group ($p < .05$).

[f] Significantly different from low-Ca group ($p < .01$).

shifted to the strontium diet. Similar suppressive effects were also noted in low-calcium animals treated with actinomycin D and then shifted to the high-calcium diet (Table II). Neither CaBP activity nor plasma calcium levels changed in low-adapted animals treated with the antibiotic and shifted to a high-calcium intake.

Discussion. Feeding a low-calcium diet to chicks effected a facultative action as previously discussed (2, 5). A new finding, however, was an additive stimulation of both CaBP activity and intestinal absorption capacity in such low-calcium adapted chicks suddenly given a high-calcium diet. The response was transitory, becoming evident at 72 hr following exposure to the high-calcium diet and declining rapidly thereafter (Fig. 1). The facultative effect in the low-calcium fed chicks, as well as the later potentiative properties of the high-calcium diet were dependent on vitamin D_3. Vitamin D_3 seemed to act permissively, wherein neither response could be demonstrated in vitamin D_3-deficient chicks.

Vitamin D is purported to act via a nuclear process (18), while the ubiquitous calcium ion is implicated in numerous biochemical processes, most of which are non-nuclear in action (19, 20). Results herein showed that the calcium-potentiative effect was actinomycin D-sensitive. Whether calcium ions *per se* were acting at the genome level was not determined, although maintenance of mRNA activity appeared necessary for demonstration of the calcium-potentiative effect.

Strontium has been shown to inhibit both calcium absorption and appearance of the CaBP (21). Here strontium was unable to effect the calcium-potentiated increase of CaBP activity (Table II), most likely due to its inhibition of CaBP synthesis.

Chicks apparently adapt to a low-calcium intake by forming and maintaining an improved system for intestinal calcium transport. How a sudden influx of calcium augments such a system is not readily explained. However, certain suggestions can be made. For example, calcium concentrations of 1–5 m*M* were reported to inhibit ribonuclease (22). Such activity could prolong the RNA turnover rate. Calcium has also been linked to an increase in mitotic activty (23). Cellular calcium concentrations of 1-5 m*M* are common in the intestinal epithelial cell (24). Therefore it may be that the calcium acted on ribonuclease and/or mitotic activity, which presumably could have resulted in augmentation of the already highly active transport system.

The likelihood that calcium mediated its effect via other calcium-homeostatic factors (*e.g.*, PTH or TCT) seems unlikely (5, 9). An action for TCT in the low-adaptation, or PTH in the calcium-potentiation experiment seems incomplete in view of the respective low- and high-plasma calcium values. However, a possible role for PTH in the low-adaptative and TCT in the potentiation experiment cannot be ruled out from the results reported here.

The nebulous "endogenous" factor, hypothesized to emanate from bone in a reciprocal manner to bone mineralization, is frequently used to explain the calcium adapta-

tion process. Yet, the results of this study wherein low-calcium-adapted chicks exhibited elevated CaBP activity in response to increased vitamin D dosage are novel (Fig. 2). Chicks supplemented with two levels of vitamin D gave equivalent blood plasma calcium values[5] indicating similar bone mobilization activity. Such results would seemingly preclude a large differential in the degree of bone mineralization, which would in turn suggest an equivalent release of the bone endogenous factor. However, such animals showed increased CaBP activity in response to the elevated dose of vitamin D (Fig. 2, 0.08% Ca) suggesting that the adaptation to a low-calcium intake may not be explained solely by the endogenous factor. For instance, the inverse relationship between calcium absorption and intake could be explained by considering calcium's effect on vitamin D metabolism. In such a closely controlled system, a low-calcium state could effect an increase in the circulating concentration of the intestinal-active vitamin D metabolite (*e.g.*, 1,25-dihydroxy vitamin D_3). Accordingly, a high-calcium state would decrease the level of such metabolites. Metabolism studies using ^{3}H-vitamin D are required, however, before a definitive statement can be made concerning this possibility.

Summary. Chicks regulated to a low-calcium diet exhibited the expected facultative process regarding the intestinal absorption of calcium. Augmentation of the facultative process was noted when such low-calcium adapted chicks were given a high-calcium diet. This response was transitory, becoming evident 72 hr after initiation of the high-calcium feeding and declining rapidly thereafter. The potentiative response was actinomycin sensitive and could not be produced by the molar substitution of strontium for calcium. Vitamin D was essential for development of the facultative process in low-calcium fed chicks and the potentiative effect noted in such animals given a high-calcium diet. It is suggested that the additional calcium could have acted on the intestinal cellular processes to accentuate the facultative response by increasing mitotic activity or by inhibiting ribonuclease. It is also theorized that calcium could have effected the facultative response indirectly by controlling synthesis of an active vitamin D metabolite.

Note added in proof: An article by Boyle *et al.* (25), appearing after the submission of this paper, describes the suppression of 25-HCC metabolism to 1.25-DHCC in high-calcium-fed rats and suggests that 1.25-DHCC is the endogenous factor.

[5] Plasma calcium values were 8.39 and 8.33 mg/100 ml in chicks given 10 or 60 IU/2 days, respectively.

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