RELATIONSHIP OF CALCIUM UPTAKE AND CALCIUM-BINDING PROTEIN SYNTHESIS IN CHICK AND RAT INTESTINE IN RESPONSE TO *SOLANUM MALACOXYLON*

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1. Introduction

It now seems clear that one of the ways by which vitamin D affects calcium absorption is by the stimulation of the de novo synthesis of calcium-binding protein [1–3]. How calcium-binding protein acts to promote calcium absorption is still a matter for discussion, but the correlation between vitamin D-stimulated calcium absorption and the production of calcium-binding protein is such that the protein must play an intimate part in this process [3,4]. To determine whether calcium-binding protein plays an obligatory role in calcium absorption the effect has been studied of other substances capable of changing calcium absorption, to see if there is always a corresponding change in the level of calcium-binding protein. Experiments of this nature have so far produced conflicting results. Nicarbazin for instance has been shown to inhibit both calcium-binding protein levels and egg formation in the hen [5] but cortisone, given with vitamin D to rachitic chicks, inhibits the action of vitamin D on calcium absorption without affecting the vitamin D-dependent synthesis of calcium-binding protein [6].

Another agent, capable of changing calcium metabolism in different animals, has recently been found in the leaves of the shrub *Solanum malacoxylon*. Used in the dried form or as an extract, active principles in the leaf act to raise plasma calcium and phosphate levels and to increase calcium absorption [7–10]. It has been claimed that the effect of the leaf extract on raising blood phosphate levels is more rapid than vitamin D but there is so far no report on the mechanism by which the active principle in the leaf brings about these vitamin D-like actions. It is not known whether the stimulation in calcium absorption is due to an increase in the level of intestinal calcium-binding protein or whether another mechanism is involved.

2. Methods

2.1. *Calcium uptake*

Rachitic rats and chicks were dosed orally with either an aqueous suspension of dried *S. malacoxylon* leaf or with 12.5 μg of cholecalciferol in propylene glycol. Small discs of intestine, four from each animal, were incubated and the mucosal uptake of ^{45}Ca from the medium measured after 1, 3 and 5 min [11].

2.2. *Assay of calcium-binding protein*

1) Chelex assay was carried out as previously described [12], the principle of which is the competition for ^{45}Ca between the calcium-binding protein and the resin, Chelex; 2) The chick calcium-binding protein was measured by immunoelectrophoresis [13] and by immunoprecipitation. In the latter the intestinal supernatant fractions were incubated with ^{45}Ca and then an immunoprecipitate formed by addition of the antibody. The ^{45}Ca in the precipitate was proportion-

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al to the amount of calcium-binding protein in the fraction. The two immunological techniques gave values in close agreement.

3. Results

The rate of calcium uptake by the intestine of rachitic chicks in response to 200 mg of the dried *S. malacoxylon* leaf was measured at time intervals up to 72 hr after dosing. The calcium uptake levels were unaffected up to 36 hr after dosing but a significant increase was observed at 48 hr with a further rise at 60 hr by which time the effect was maximal (fig. 1). The 'Chelex' assay showed the supernatant of the mucosa of the rachitic rats to contain some calcium-binding protein activity, 0.73% ^{45}Ca in the supernatant/mg of protein, which was increased to 0.88% by vitamin D after 48 hr while the leaf after 48 hr gave a value of 1.5%. These supernatant fractions were also chromatographed on Sephadex G–100 equilibrated with 13.7 mM Tris–HCl, pH 7.4, 120 mM NaCl, 4.74 mM KCl, 1 mM mercaptoethanol to test whether the increase in Chelex binding was specific for calcium-binding protein. The chromatographic fractions were tested by the 'Chelex' assay for calcium-binding protein, and a peak of activity was detected in fractions eluted at 2.5 void vol from the chromatographies of the mucosal supernatants prepared from leaf and vitamin D-dosed rats. This compares favourably with the findings of Drescher and DeLuca [12] who reported that rat intestinal calcium-binding protein was eluted at 2.7 void vol. The low activity detected in whole mucosal supernatants prepared from rats on a normal diet was similar to that reported previously [14].

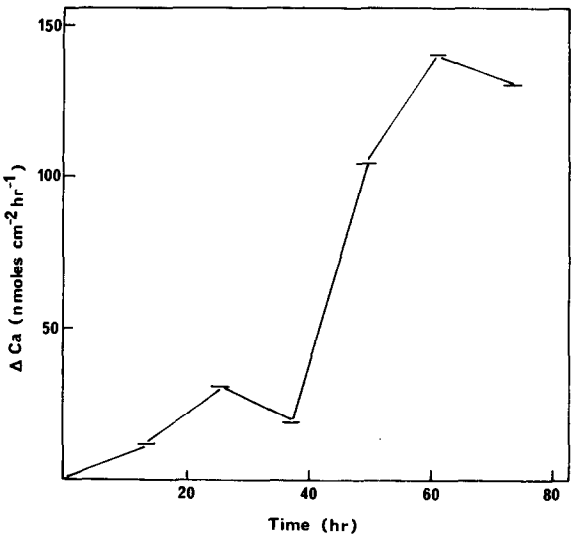

Fig. 1. Groups of five rachitic rats were given 200 mg of the dried leaf per rat, killed at the times indicated and Ca^{2+} uptake measured, the length of the bar indicating the time taken to complete the experiment. Values give the increases above corresponding control values, the mean control uptake determined from 1 to 3 min being 190.4 ± 20.4 nmoles cm^{-2} hr^{-1} (20 observations).

Attempts to correlate in time the changes in the calcium uptake by mucosa effected by the dried leaf with the calcium-binding activity of the supernatant fraction of the homogenates of the same intestines showed that there was no close correlation between onset of the effect on calcium uptake and an increase in calcium-binding protein levels. However, the failure to detect a correlation may have been due to the insensitivity of the Chelex assay. Accordingly, similar measurements were made on rachitic chick intestine in which the calcium-binding protein was measured more specifically using antibody to chick intestinal calcium-binding protein [15].

The mucosa of the rachitic birds responsed to vitamin D in the same manner as the rat mucosa in

Table 1
Calcium uptake by intestinal mucosa from rachitic chicks treated with *S. malacoxylon* leaf

Group	Ca^{2+} uptake		Rate of Ca^{2+} uptake
	1 min	3 min	
1A	3.52 ± 0.28	5.42 ± 0.70	0.95 ± 0.26
1B	4.00 ± 0.32*	5.49 ± 0.38*	0.74 ± 0.16*
2A	3.22 ± 0.19	3.92 ± 0.30	0.38 ± 0.21
2B	4.04 ± 0.33*	6.71 ± 0.73‡	1.34 ± 0.24†

Groups of five birds received either (1B) 200 mg of the dried leaf/bird 48 hr before killing, or (2B) 400 mg of leaf/bird 72 hr before killing. Groups 1A and 2A were appropriate controls. Results are either mean calcium uptake (± S.E.) expressed as nmoles Ca^{2+} cm^{-2} mucosal surface or the rate of calcium uptake expressed as nmoles Ca^{2+} cm^{-2} min^{-1}. Statistical significance of difference from appropriate control, N.S.*, $p < 0.01$‡, $p < 0.02$†.

Table 2
Calcium-binding protein levels in intestinal mucosa of birds treated with *S. malacoxylon* leaf

Expt. 1		Expt. 2		Expt. 3	
Leaf	Vitamin D	Leaf	Vitamin D	Leaf	Vitamin D
0.10	0.43	0.14	1.1*	0.29	1.76
0.16	0.52	0.03	–	0.24	1.29
0.09	0.64	0.04	–	0.08	
0.06	0.40	0.06	–	0.06	
	0.71	0.03	–		

In Expt. 1 and 3 the chicks each received a dose of 200 mg of the dried leaf and were killed after 48 hr and 96 hr respectively. In Expt. 2 each chick was dosed with 400 mg of the leaf and was killed after 72 hr. The results are expressed as mg/g of tissue.
* Determined on sample of mucosa pooled from five chicks.

that the ^{45}Ca uptake values measured 48 hr after the injection of 12.5 μg vitamin D, increased significantly (5.26 to 6.23 nmoles Ca^{2+} cm^{-2}). Table 1 shows ^{45}Ca uptake by the mucosa of rachitic birds dosed 48 hr previously with the dried leaf (200 mg/bird) to remain unchanged compared to the uptake by the rachitic controls but stimulation of ^{45}Ca uptake was seen when the dose was increased to 400 mg of leaf/bird and the uptake measured 72 hr later.

The level of calcium-binding protein was increased in the intestine of all chicks treated with the leaf (table 2). The wide variation in the calcium-binding protein levels of the leaf-treated chicks in contrast to the vitamin D-dosed animals may be a consequence of a variable degree of digestion of the leaf. Undoubtedly calcium-binding proteins levels were raised in all cases, since the protein could not be detected in the intestine of the rachitic controls (< 5 μg/g of tissue). Again, as in the rat, both Ca^{2+} uptake and calcium-binding protein levels could be shown to increase following administration of leaf, but the first detection of induced calcium-binding protein (at 48 hr) preceded any effect on calcium uptake (first detected at 72 hr).

4. Discussion

Recently it has been shown that vitamin D allows the de novo synthesis of calcium-binding protein [3] and the active principle in the leaf must be doing likewise, but the stimulation in calcium uptake in rachitic animals by *S. malacoxylon* seems to involve other components of the calcium absorption mechanism as well. Thus in contrast to the situation after dosing with vitamin D small amounts of calcium-binding protein could be detected before an increase in mucosal uptake of calcium and the uptake of calcium in any individual bird could not be related to the concentration of calcium-binding protein. Studies of the metabolism of vitamin D have shown that the active form is the hormonal metabolite 1,25-dihydroxycholecalciferol, formed from 25-hydroxycholecalciferol in the kidney [16]. The general similarity in effect of dried leaf and of vitamin D on Ca^{2+} uptake and calcium-binding protein, taken together with the evidence showing some differences in their mode of action, suggests it might now be worthwhile investigating whether the leaf principle could be used as a substitute for vitamin D in conditions where there is restricted metabolism of vitamin D to its active metabolites. The chemical identity of the active principle of the leaf is of obvious importance considering the clinical value of 1,25-dihydroxycholecalciferol and experiments are currently being undertaken to isolate and identify the active principle involved. As estimated from the best response in Expt 3 (table 2) the activity in the leaf is equivalent to 2.5 μg of vitamin D/g of dried leaf making the leaf a potent source of vitamin D activity.

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