CESTRUM DIURNUM: A DOMESTIC PLANT WITH 1,25-DIHYDROXYCHOLECALCIFEROL-LIKE ACTIVITY[1]

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Calcinosis in grazing animals, some associated with toxic plants, has been described in various parts of the world. In Argentina and Brazil, these diseases are known as "Enteque seco" and "Espichamento", respectively, and both were shown to be due to the ingestion of the shrub, Solanum malacoxylon[2] (1-3). Similar diseases have been reported in Jamaica (4), Hawaii (5), the Alpine region of Germany (6), and India (7). In Germany, the calcinotic disease is due to the plant, Trisetum flavescens (6) but, in the other areas, the involvement of a specific plant species has not yet been demonstrated conclusively.

There has been a recent outbreak of calcinosis in the Miami, Florida, area and the occurrence of the plant, Cestrum diurnum, in sites accessible to the affected animals suggested it to be the cause (8). Part of the

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2. Abbreviations: 25-(OH)D_3 = 25-hydroxycholecalciferol; 1,25-$(OH)_2D_3$ = 1α, 25-dihydroxycholecalciferol; CaBP = vitamin D-induced calcium-binding protein; S.m. = Solanum malacoxylon; C.d. = Cestrum diurnum; cAMP = cyclic adenosine - 3′:5′ - monophosphate.

proof that Cestrum diurnum (C.d.) is the toxic plant in Florida is given in this report and this is done by making analogy between the biological properties of C.d. and S.m., the plant shown to cause calcinosis in South America. We had previously reported that S.m. overcame the inhibitory effect of high stable strontium diets on calcium absorption and the synthesis of the vitamin D-dependent calcium-binding protein (CaBP) (9). Since the high strontium diet blocks the conversion of 25-$(OH)D_3$ to 1,25-$(OH)_2D_3$ (9,10), this provided evidence that S.m. contained a substance that mimics the action of 1,25-$(OH)_2D_3$ (10). This was confirmed by Walling and Kimberg (private communication; Beth Israel Hospital, Boston) who demonstrated that S.m. was effective in stimulating calcium transport in the anephric rat.

In the present report, it is documented that C.d. can also reverse the inhibitory effect of stable strontium diet in chicks and thereby demonstrating that the active principle in the Cestrum species has biological properties similar to 1,25-$(OH)_2D_3$ and to that in S.m.

Day old White Leghorn cockerels were raised to 3 wks. of age on a commercial chick starter diet (Agway, Inc.). At this time, the chicks, in groups of six, were either placed on a control diet (Group I) containing 1.14% Ca, 0.775% P and 1200 International Units (IU) of vitamin D_3 per kilogram (cf. ref. 11 for basal diet), a diet containing 2.56% Sr, 0.12% Ca, 0.775% P and 1200 IU of vitamin D_3 per kilogram (Group II), or the same high strontium diet except that vitamin D_3 was omitted but contained either 1.5% (Group III) or 3% Cestrum diurnum (Group IV) dried leaf powder. After a 4 day feeding period, the chicks were anesthetized with ether and the degree of absorption of ^{47}Ca from a ligated duodenal segment in situ was determined (12). The 1 ml test dose contained sodium chloride (150 mM), calcium chloride (25 mM) and ^{47}Ca (0.05 μc/ml), pH 7.2. After an absorption period of 25 min., the chicks were bled by cardiac puncture and then killed with an overdose of sodium pentobarbital.

The duodenal segment was excised intact and the radioactivity therein counted immediately in a well-type scintillation detector against reference ^{47}Ca. The ^{47}Sc contribution to the ^{47}Ca radioactivity was excluded by the use of a single channel analyzer. After counting, the segment was slit open lengthwise and the mucosa, after thorough rinsing with cold 0.9% NaCl, was removed from the underlying muscle coats. The duodenal mucosa was homogenized, centrifuged, and the vitamin D-dependent calcium-binding protein content of the supernatant from the homogenate was determined by radialimmunoassay (13,14). Protein was measured by the method of Lowry et al (15). Also immediately after counting, a duodenal segment adjacent to the ligated segment was taken and the cAMP content of the mucosa was determined by the method previously described (16). The absorption data are expressed as % of administered dose of ^{47}Ca, the CaBP as μg/mg of total protein in the supernatant fluid of the mucosal homogenate, and cAMP as pmoles/mg DNA.

Calcium in the plasma of the experimental animals was determined by atomic absorption spectrometry and plasma phosphate was determined by a modification of the procedure of Fiske and Subbarow (17) with the use of an autoanalyzer (Technicon). The Cestrum diurnum plant was harvested in Florida, with the cooperation of Dr. Julia Morton of the University of Miami, Florida, and processed in Ithaca. The plant was air dried at room temperature and then ground to a fine powder.

The experimental data on the response of the strontium fed chicks to C. diurnum powder are presented in Fig. 1. As can be seen the strontium diet alone, in confirmation of our previous experience (9,11), significantly inhibited calcium absorption and greatly suppressed the concentration of CaBP in the duodenal mucosa. In addition, duodenal cAMP concentration, previously shown to correlate with both CaBP concentration and calcium absorptive capacity of embryonic chick duodenum maintained in organ culture (16), was also decreased by dietary strontium.

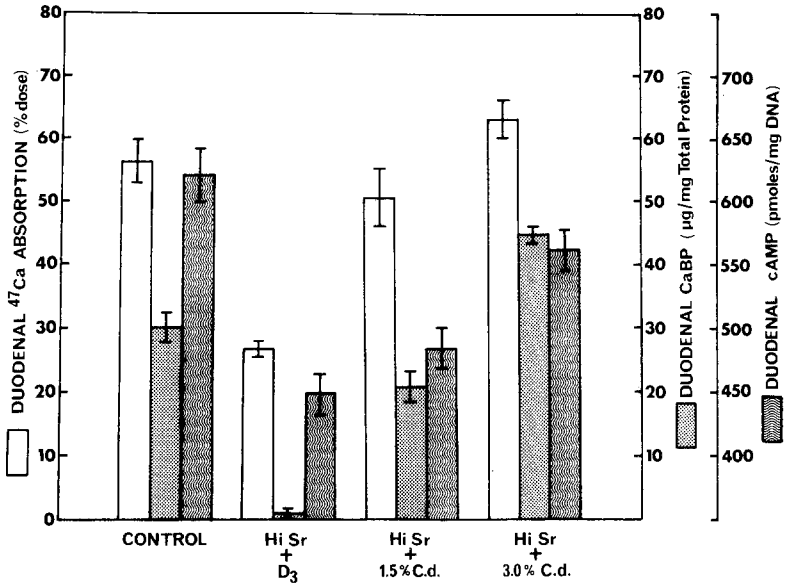

Fig. 1. Prevention of the inhibitory effect of dietary stable strontium on calcium absorption, the synthesis of the vitamin D-induced calcium-binding protein (CaBP), and the synthesis of cAMP by Cestrum diurnum (C.d.). Values represent the mean ± S.E.M. of 6 chicks per group.

Those chicks receiving 1.5% or 3.0% C. diurnum powder in the diet reversed the inhibitory effect of stable strontium on CaBP, ^{47}Ca absorption and cAMP in a dose related fashion.

The effect of the various treatments on plasma Ca and phosphate concentrations, and on body weights, are shown in Table 1. It may be noted that the strontium diet alone decreased body weight with an additional depression on growth at the higher level of C. diurnum. The plant significantly increased plasma calcium levels as compared to the group receiving stable strontium plus vitamin D_3 alone and, at the same time, there was some reduction in plasma phosphorus levels.

These results demonstrate that C. diurnum contains a substance that can overcome the inhibitory effect of the stable strontium diet with regards the duodenal absorption of ^{47}Ca, the synthesis of the vitamin D dependent calcium binding protein and the duodenal concentration of cAMP. Under similar conditions, Omdahl and DeLuca (10,18) showed

Table 1

Plasma Calcium and Phosphate Concentrations and Terminal Body Weights of Chicks on the Experimental Diets*

Group	Diet	Body weight (gm)	Plasma Ca (mg/100 ml)	Plasma P_i (mg/100 ml)
1.	Control	296 ± 9[a]	11.1 ± 0.2[a]	5.2 ± 0.1[a]
2.	High Sr	252 ± 11[b]	10.2 ± 0.2[b]	4.7 ± 0.3[a,b]
3.	High Sr + 1.5% C.d.**	233 ± 4[b,c]	11.2 ± 0.3[a]	3.9 ± 0.3[b,c]
4.	High Sr + 3.0% C.d.**	216 ± 9[c]	12.3 ± 0.2[c]	3.9 ± 0.2[c]

* Values represent the mean ± SEM of 6 chicks per group. In any given column, the different superscript letters indicate that those values differ at $p < .05$, the comparisons being made between groups.

** C.d. = *Cestrum diurnum* leaf powder.

that, in strontium fed chicks, there is a defect in the formation of 1,25-$(OH)_2D_3$ and that these animals respond to 1,25-$(OH)_2D_3$ but not to 25-$(OH)D_3$ or vitamin D_3 per se. Thus the active principle in C.d. can mimic the action of 1,25-$(OH)_2D_3$ and therefore contains a substance with properties similar to the active principle in the South American plant, *Solanum malacoxylon*.

Other studies on C.d. have been done and these will be reported elsewhere. Among the pertinent findings were that the *Cestrum* plant appears to contain about 30,000 IU of vitamin D_3 equivalents per kilogram of C.d. and that most of the activity is soluble in a methanol: chloroform mixture (2:1). The latter property differentiates the C.d. principle chemically from the S.m. factor, since the latter is water soluble and insoluble in the methanol:chloroform mixture (19). Since the factor in C.d. can induce the synthesis of the calcium binding protein, it is presumed that the molecule contains a structure similar

enough to 1,25-$(OH)_2D_3$ to interact with the cytosol and nuclear receptors that precedes the formation of a specific mRNA (20). This was also postulated for the active substance in S.m.

It has been shown that the S.m. factor can directly influence calcium transport and CaBP synthesis by the intestine since the active substance is effective in an intestinal tissue in organ culture (21). Thus, if transformation of the S.m. factor is required, the necessary reactions can be carried on by the intestinal tissue itself. Whether this also pertains to the C.d. factor remains to be determined.

Like the situation with S. malacoxylon, the 1,25-$(OH)_2D_3$-like activity of the active principle in C. diurnum would seem to bear on the etiology of the calcinotic disease in the affected horses and cattle in Florida. Considerable evidence indicates that the conversion of 25-$(OH)D_3$ to 1,25-$(OH)_2D_3$ by the 1α-hydroxylase enzyme in the kidney is a controllable reaction (22,23). Under circumstances of calcium stress or calcium deficiency, the formation of 1,25-$(OH)_2D_3$ is enhanced which, in turn, increases the intestinal absorption of calcium and the synthesis of CaBP. On the other hand, when the diet contains adequate or more than adequate levels of calcium and phosphorus (or the need for calcium is decreased as in maturity), the formation of 1,25-$(OH)_2D_3$ is decreased. The finding that the active principles in both S. malacoxylon and C. diurnum can mimic the action of 1,25-$(OH)_2D_3$ indicates that the controlling point in the intestinal phase of calcium homeostasis is by-passed and that calcium absorption occurs at a high rate and essentially out of control. The excessively absorbed calcium apparently cannot be accommodated by normal physiological mechanisms and the calcinotic condition develops.

The medical application of the factors in C.d. and S.m. in disease states involving abnormal calcium and bone metabolism might represent a useful off-shoot of the present investigations.

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Summary

The biological activity of Cestrum diurnum, in connection with the occurrence of calcinosis in grazing animals in Florida, was investigated. The inclusion of the plant material in a high strontium diet fed to chicks overcame the inhibitory effect of this regime on calcium (^{47}Ca) absorption, the synthesis of the intestinal vitamin D-dependent calcium-binding protein, and net formation of intestinal cyclic adenosine-3′:5′-monophosphate. Since the high strontium diet blocks the conversion of 25-hydroxycholecalciferol to 1α,25-dihydroxycholecalciferol, this provides evidence of a substance in Cestrum diurnum with 1α,25-dihyroxycholecalciferol-like activity.

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