ber of available data (26 loci) is undoubtedly insufficient to draw any strong conclusion, the result indicates that the distribution of heterozygosity deviates from that of neutrality and it is shifted toward a balanced selection. The result is presented in Fig. 1B (*8*). It seems quite certain that the heterozygote distributions of protein polymorphisms and of blood group polymorphisms are essentially different; see also (*4*).

TSUNEYUKI YAMAZAKI
TAKEO MARUYAMA
National Institute of Genetics, Mishima, Shizuoka-ken, 411, Japan

References and Notes

1. T. Yamazaki and T. Maruyama, *Science* **178**, 56 (1972).
2. M. Kimura and T. Ohta, *Nature* **229**, 467 (1971).
3. T. Maruyama, *Genet. Res.* **20**, 141 (1972).
4. J. F. Crow, *J. Hered.* **63**, 306 (1972).
5. M. Kimura, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
6. J. C. Avise and R. K. Selander, *Evolution* **26**, 1 (1972); F. J. Ayala, *Proc. Sixth Berkeley Symp. Math. Stat. Prob.* **5**, 211 (Univ. of California Press, Berkeley and Los Angeles, 1972); ———, J. R. Powell, Th. Dobzhansky, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2480 (1971); L. Bullini and M. Coluzzi, *Nature* **239**, 160 (1972); M. T. Clegg and R. W. Allard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1820 (1972); W. E. Johnson, R. K. Selander, M. H. Smith, Y. J. Kim, *Studies in Genetics VII, Univ. Texas Publ. 7213*, 297 (1972); R. K. Koehn and D. I. Rasmussen, *Biochem. Genet.* **1**, 131 (1967); K. Kojima, J. Gillespie, Y. N. Tobari, *ibid.* **4**, 627 (1970); C. B. Krimbas and S. Tsakas, *Evolution* **25**, 454 (1971); S. Lakovaara and A. Saura, *Hereditas* **69**, 77 (1971); *Genetics* **69**, 377 (1971); C. O. Mckinney, R. K. Selander, W. E. Johnson, S. Y. Yang, *Studies in Genetics VII, Univ. Texas Publ. 7213*, 307 (1972); S. Prakash, *Proc. Natl. Acad. Sci. U.S.A.* **62**, 778 (1969); ———, R. C. Lewontin, J. L. Hubby, *Genetics* **61**, 841 (1969); R. K. Selander, G. Hunt, S. Y. Yang, *Evolution* **23**, 379 (1969); R. K. Selander and W. E. Johnson, *Syst. Zool.* **20**, 377 (1971); R. K. Selander, M. H. Smith, S. Y. Yang, W. E. Johnson, J. B. Gentry, *Studies in Genetics VI, Univ. Texas Publ. 7103*, 49 (1971); R. K. Selander, S. Y. Yang, G. Hunt, *Studies in Genetics V, Univ. Texas Publ. 6918*, 272 (1969); R. K. Selander, S. Y. Yang, R. C. Lewontin, W. E. Johnson, *Evolution* **24**, 402 (1970); S. Y. Yang, L. L. Wheeler, I. Bock, *Studies in Genetics VII, Univ. Texas Publ. 7213*, 213 (1972).
7. L. L. Cavalli-Sforza and W. F. Bodmer, *The Genetics of Human Populations* (Freeman, San Francisco, 1971), pp. 724–731.
8. The scale in Fig. 1B is different from that of Fig. 1A because the amount of heterozygosity is normalized so that the total amount of heterozygosity is one. Only 10 points are plotted in Fig. 1B, while 20 points appear in Fig. 1A, because of a large difference in the number of loci examined (over 400 loci for Fig. 1A, but only 26 loci for Fig. 1B).
9. Contribution No. 962 from the National Institute of Genetics, Mishima, Shizuoka-ken 411, Japan. Aided in part by a grant-in-aid from the Ministry of Education, Japan. We are grateful to Dr. J. F. Crow for the suggestion of analyzing human blood group data separately and for many valuable discussions.

26 November 1973

Calcium Absorption and Calcium-Binding Protein Synthesis: Solanum malacoxylon Reverses Strontium Inhibition

Abstract. *The ingestion of diets containing high concentrations of stable strontium inhibits calcium absorption and intestinal calcium-binding protein synthesis and, as shown by others, does so by inhibiting the conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, the active form of vitamin D. The addition of the South American plant* Solanum malacoxylon *to strontium-containing diets counteracts the inhibitory action of dietary strontium, thereby indicating that the plant contains a factor which can mimic the action of 1,25-dihydroxycholecalciferol and representing the first such factor identified in a botanical source.*

A disease of cattle in the Argentine known as "Enteque seco" is characterized by extensive soft tissue calcification and is due to the ingestion of the plant *Solanum malacoxylon* by the grazing animal (*1*). Controlled studies with different species, including cattle (*2*), rabbits (*3*), sheep (*4*), and guinea pigs (*4*), verified the etiology of the disease and showed that the plant or an extract of the plant caused an increased absorption of calcium and phosphorus. Analogy between the active principle in *S. malacoxylon* and vitamin D has been made, and it was shown that the unknown factor seems to act faster and fade faster than a massive dose of vitamin D (*3*) and appeared to cause an increase in calcium-binding activity in rabbit ileum (*5*). The similarity between the activity of vitamin D and *S. malacoxylon* was confirmed by the report by O'Donnell and Smith (*6*). However, from the reported symptoms and signs of Enteque seco, it was more likely that the active principle in *S. malacoxylon* might have biological properties similar to the active form of vitamin D, 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$]. 1,25-$(OH)_2D_3$ is formed in the kidney by the 1-hydroxylation of the precursor metabolite, 25-hydroxycholecalciferol (25-OHD_3), the latter resulting from the 25-hydroxylation of vitamin D_3 (cholecalciferol) in the liver (*7*).

To test the hypothesis that the *S. malacoxylon* factor had activity similar to that of 1,25-$(OH)_2D_3$, use was made of the established observation that a high stable strontium intake by chicks blocks the conversion of 25-OHD_3 to 1,25-$(OH)_2D_3$ by the kidney enzymes (*8*). The lack of 1,25-$(OH)_2D_3$ production in the presence of strontium results in a depressed absorption of calcium and the inhibition of synthesis of the intestinal vitamin D–dependent calcium-binding protein (*9*). The inhibitory action of strontium, as would be predicted, can be overcome by the administration of 1,25-$(OH)_2D_3$ per se but not by 25-OHD_3 or cholecalciferol (*8*), and we now report here that the inhibitory effect of dietary strontium can also be reversed by *S. malacoxylon*—evidence that the *S. malacoxylon* factor does, indeed, have activity similar to that of the active metabolite of vitamin D_3, 1,25-$(OH)_2D_3$.

Day-old White Leghorn cockerels were fed a commercial chick diet (Agway) for 23 days; at this time (designated day 0), they were placed on either a control diet containing 1.3 percent Ca, 0.65 percent P, and 1200 international units (I.U.) of vitamin D_3 per kilogram or a diet containing 2.5 percent Sr, 0.2 percent Ca, 0.65 percent P, and 1200 I.U. of vitamin D_3 per kilogram. On day 4, some of the chicks on the high strontium diet were continued on this diet, and the others were fed the same high strontium diet without vitamin D_3 but supplemented with either 0.2 or 0.5 percent of *S. malacoxylon* powder. The basal diet is the rachitogenic diet (*9*). On days 0, 4, and 7, six chicks from the appropriate groups were anesthetized with ether, and the degree of absorption of ^{47}Ca from a ligated duodenal segment in situ was determined (*10*). The 1-ml test dose contained NaCl, 150 m*M*; $CaCl_2$, 25 m*M*; and ^{47}Ca, 0.05 μc/ml, *p*H 7.2. After an absorption period of 15 minutes, the chicks were bled by cardiac puncture and killed with an overdose of sodium pentabarbitol. The duodenum was excised and the radioactivity therein was counted immediately in a well-type scintillation detector against reference ^{47}Ca. The ^{47}Sc contribution was excluded by the use of a single channel analyzer. The segment, after counting, was thoroughly rinsed and slit open lengthwise; the mucosa was removed from the underlying muscle coats (*11*). The duodenal mucosa was further processed and the supernatant

from the mucosal homogenate was assayed for the vitamin D–dependent calcium-binding protein (CaBP) by radioimmunoassay (*12*). Protein was measured by the method of Lowry *et al.* (*13*). Absorption data are expressed as the percentage of the administered dose of ^{47}Ca, and CaBP as micrograms per milligram of total protein in the supernatant of the mucosal homogenate.

Calcium and strontium in the plasma were determined by atomic absorption spectrometry, and plasma phosphate was determined by a modification of the procedure of Fiske and Subbarow (*14*), with the use of an autoanalyzer (Technicon). The *S. malacoxylon* plant was made available by Dr. H. R. Camberos, Ministry of Agriculture, Buenos Aires, Argentina, and Dr. G. K. Davis, University of Florida.

The experimental data on the response of strontium-fed chicks to *S. malacoxylon* powder are presented in Fig. 1. At days 4 and 7, the strontium diet significantly ($P < .001$) inhibited ^{47}Ca absorption (Fig. 1A) and reduced duodenal CaBP levels to nearly zero (Fig. 1B). Those chicks placed on the diets free of vitamin D_3 but containing *S. malacoxylon* recovered absorption and CaBP capacities (Fig. 1, A and B) in a dose-related fashion, and the correlation coefficient between ^{47}Ca absorption and CaBP was .90. This reversal of strontium inhibition by the plant was replicated in two other studies.

Clearly, the South American plant does have vitamin D–like activity as demonstrated by the induction of the vitamin D–dependent protein in our study and in vitamin D–deficient, rachitic chicks (*15*). The fact that *S. malacoxylon* can overcome the inhibitory effect of dietary strontium indicates that the factor in the plant mimics the action of $1,25\text{-}(OH)_2D_3$ since, as previously noted, convincing evidence is available showing that, under similar conditions, the conversion of $25\text{-}OHD_3$ to $1,25\text{-}(OH)_2D_3$ is blocked (*8*). This effect cannot be due to the calciferol-like activity of the plant alone, which, by bioassay, would contribute about 600 I.U. of vitamin D_3 equivalents per kilogram of diet. The high strontium control diet already contained 1200 I.U. of vitamin D_3 per kilogram, and certainly the inhibitory effect of strontium was evident in the presence of this and greater quantities (*9*) of vitamin D_3 (Fig. 1).

It is conceivable that the *S. malacoxylon* factor is a potent stimulator of the kidney 1-hydroxylase system and allows the $25\text{-}OHD_3$ hydroxylation to proceed even in the presence of strontium. This possibility seems unlikely as indicated from the following experiment. *Solanum malacoxylon* powder was extensively extracted with a mixture of methanol and chloroform to remove bona fide vitamin D. An aqueous extract of the residue was prepared and found effective in stimulating calcium absorption and inducing CaBP synthesis in the vitamin D–deficient chick. Since the only source of vitamin D–like potency was the extract, this indicated that the factor was not acting by stimulating 1-hydroxylation of $25\text{-}OHD_3$ that preexisted in the animal. This same aqueous extract also reversed the inhibitory effect of high levels of dietary strontium. However, an unequivocal approach to the above proposition will be the determination of the effectiveness of *S. malacoxylon* in rachitic nephrectomized animals or in an isolated intestinal organ culture system (*16*), or both.

Table 1. Plasma calcium, phosphate, and strontium concentrations, together with terminal body weights of chicks on the experimental diets. The strontium diet alone decreased growth ($P < .001$) and plasma Ca ($P < .001$). The values given are for experimental day 7 only and represent the mean ± S.E.M. of six chicks per group. In any given column, the different superscript letters indicate that the values differ at $P < .05$, the comparisons being made between groups. SM, *Solanum malacoxylon* powder.

Group	Diet	Body weight (g)	Plasma Ca (mg/100 ml)	Plasma P_i (mg/100 ml)	Plasma Sr (mg/100 ml)
1	Control	319 ± 5^a	11.5 ± 0.04^a	5.2 ± 0.3^a	
2	High Sr	285 ± 3^b	9.7 ± 0.1^b	$4.5 \pm 0.1^{b,c}$	6.0 ± 0.3^a
3	High Sr + 0.2% SM	260 ± 19^b	$10.0 \pm 0.2^{b,c}$	4.7 ± 0.2^b	6.0 ± 0.5^a
4	High Sr + 0.5% SM	255 ± 21^b	10.4 ± 0.3^c	4.2 ± 0.1^c	7.7 ± 0.3^b

It is also unlikely that *S. malacoxylon* operates directly on the intestinal calcium transport system, similar to the antibiotic filipin (*17*), since the direct incorporation of *S. malacoxylon* aqueous extract into the ^{47}Ca-labeled dosing solution was ineffective in stimulating ^{47}Ca absorption, and such a factor would not likely cause the synthesis of the vitamin D–induced calcium-binding protein.

The effect of the various treatments on plasma Ca, Sr, and inorganic phosphate concentrations, and on body weights, are shown in Table 1.

The present results bear on the fundamental nature of the soft-tissue calcification disease Enteque seco (*18*). In the normal animal, the calcium absorption mechanism is reasonably well controlled, with the efficiency of absorption changing in response to such variables as dietary calcium concentration, age, pregnancy, and growth (*19*).

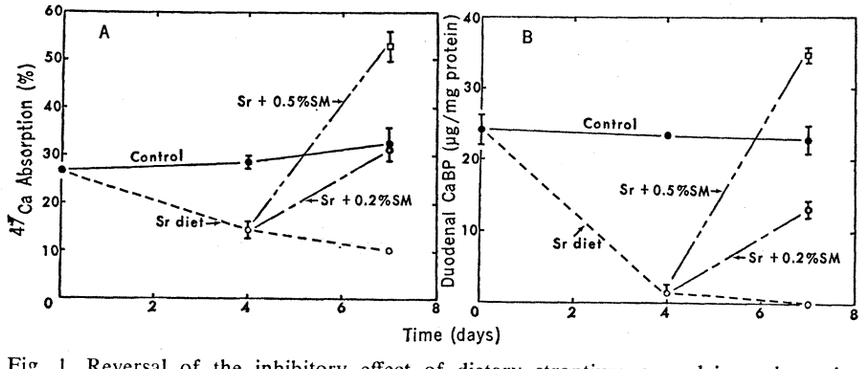

Fig. 1. Reversal of the inhibitory effect of dietary strontium on calcium absorption and net synthesis of the vitamin D–dependent calcium-binding protein (CaBP) by *S. malacoxylon* (*SM*). Chicks at 3½ weeks of age were fed a normal diet for 7 days or a diet containing strontium (2.5 percent) for 4 days. On day 4, some of the strontium-fed chicks were continued on that diet or given the same diet without vitamin D_3, but with the addition of *S. malacoxylon* powder (either 0.2 or 0.5 percent) for 3 days more. The degree of duodenal absorption of a test dose of ^{47}Ca is shown in (A) and net synthesis of CaBP in (B). Each point represents the mean of six chicks. The standard error of means rest within the confines of the symbol or are as designated by the range lines.

The modifiable reaction appears to be the degree of conversion of 25-OHD_3 to 1,25-$(OH)_2D_3$ by kidney enzymes (*20*); the more 1,25-$(OH)_2D_3$ produced, the greater the efficiency of calcium absorption. Since the *S. malacoxylon* factor effectively bypasses (or conceivably stimulates) the controlling point, that is, the kidney hydroxylase step, calcium absorption (and probably other aspects of calcium metabolism) occurs at a high rate and essentially out of control, thus accounting for the pathological symptoms of the disease. This is supported by the finding that *S. malacoxylon* elicits no gross toxicity in chicks on a low calcium diet but is deleterious when a normal calcium diet is fed (*15*), suggesting no direct action of the plant factor alone on the soft tissues.

The *S. malacoxylon* factor is water soluble and readily extracted from the plant by aqueous or other highly polar solvents (*21*) and this has been confirmed at our laboratory. This important property tends to eliminate the factor as being vitamin D or even 1,25-$(OH)_2D_3$ per se. It could, however, be a derivative of the metabolite containing a highly polar moiety, thereby accounting for its solubility properties. Required is the isolation and characterization of the factor in order to understand the relation of the factor to the calciferol series, if any, and to aid in elucidating its mode of action. Its therapeutic potential in certain human and animal disease states remains to be determined.

R. H. WASSERMAN

Department of Physical Biology,
New York State Veterinary College,
Cornell University,
Ithaca, New York 14850

References and Notes

1. B. J. Carrillo and N. A. Worker, *Rev. Invest. Agropecu. INTA* **4**, 9 (1967).
2. B. F. Sansom, M. J. Vagg, J. Döbereiner, *Res. Vet. Sci.* **12**, 604 (1971).
3. C. A. Mautalen, *Endocrinology* **90**, 563 (1972).
4. H. R. Camberos, G. K. Davis, M. I. Djafor, in *Trace Element Metabolism in Animals*, C. F. Mills, Ed. (Livingstone, Edinburgh, 1970), pp. 369–373.
5. A. W. Wase, *Fed. Proc.* **31**, 708 (1972).
6. J. M. O'Donnell and M. W. Smith, *Nature (Lond.)* **244**, 357 (1973).
7. G. Ponchon and H. F. DeLuca, *J. Clin. Invest.* **48**, 1273 (1969); D. R. Fraser and E. Kodicek, *Nature (Lond.)* **228**, 764 (1970).
8. J. L. Omdahl and H. F. DeLuca, *J. Biol. Chem.* **247**, 5520 (197 → *Science* **174**, 949 (1971).
9. R. A. Corradino and R. H. Wasserman, *Proc. Soc. Exp. Biol. Med.* **133**, 960 (1970); R. A. Corradino, J. G. Ebel, P. H. Craig, A. N. Taylor, R. H. Wasserman, *Calcif. Tissue Res.* **7**, 81 (1971).
10. R. H. Wasserman, *J. Nutr.* **77**, 69 (1962).
11. ——— and A. N. Taylor, *Science* **152**, 791 (1966).
12. R. A. Corradino and R. H. Wasserman, *ibid.* **172**, 731 (1971).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
14. C. H. Fiske and Y. Subbarow, *J. Biol. Chem.* **66**, 375 (1925).
15. R. H. Wasserman and A. Bar, in preparation.
16. R. A. Corradino, *J. Cell Biol.* **58**, 64 (1973).
17. T. H. Adams, R. G. Wong, A. W. Norman, *J. Biol. Chem.* **245**, 4432 (1970); R. G. Wong, T. H. Adams, P. A. Roberts, A. W. Norman, *Biochim. Biophys. Acta* **219**, 61 (1970); A. W. Norman, personal communication.
18. The same disease is known in Brazil as "Espichamento," and is caused by *S. malacoxylon* [N. A. Worker and B. J. Carrillo, *Nature (Lond.)* **215**, 72 (1967); J. Döbereiner, C. H. Tokarnia, J. B. D. da Costa, M. deS. Dayrell, *Pesq. Agropecu. Bras. Ser. Vet.* **6**, 91 (1971)]. In Hawaii, a similar condition termed Naalehu disease [F. T. Lynd, E. H. Willers, L. A. Weight, P. W. Gebauer, *Am. J. Vet. Res.* **26**, 1344 (1965)] might be caused by a related plant, *Solanum sodomaeum*, which is toxic to Japanese quail and White Leghorn cockerels in high dietary concentrations [E. Ross and H. H. Furumoto, *Poult. Sci.* **49**, 13 (1970)].
19. R. H. Wasserman, A. N. Taylor, C. F. Fullmer, in *Metabolism and Function of Vitamin D*, D. Fraser, Ed. (Biochemical Society Special Publication No. 3, London, in press); R. H. Wasserman and R. A. Corradino, *Vitamins and Hormones* (Academic Press, New York, in press).
20. I. T. Boyle, R. W. Gray, H. F. DeLuca, *Proc. Natl. Acad. Sci. U.S.A.* **63**, 2131 (1971); M. Garabedian, M. F. Holick, H. F. DeLuca, I. T. Boyle, *ibid.* **69**, 1673 (1972); H. Rasmusen, M. Wong, D. Bikle, D. B. P. Goodman, *J. Clin. Invest.* **51**, 2502 (1972); D. R. Fraser and E. Kodicek, *Nat. New Biol.* **241**, 163 (1973); L. Galante, S. T. MacAuley, K. W. Colston, I. MacIntyre, *Lancet* **1972-I**, 985 (1972); L. Galante, K. W. Colston, S. T. MacAuley, I. MacIntyre, *Nature (Lond.)* **238**, 271 (1972); Y. Tanaka, H. Frank, H. F. DeLuca, *Science* **181**, 566 (1973); L. Galante, K. W. Colston, I. M. A. Evans, P. G. H. Byfield, E. W. Matthews, I. MacIntyre, *Nature (Lond.)* **244**, 438 (1973).
21. N. A. Worker and B. J. Carrillo, *Nature (Lond.)* **215**, 72 (1967); H. R. Camberos, G. K. Davis, M. I. Djafor, C. F. Simpson, *Am. J. Vet. Res.* **31**, 685 (1970).
22. Supported by PHS grant AM-04652 and AEC contract AT(11-1)-3167. The technical assistance of F. C. Davis, M. Brindak, and K. Ni and the technical advice of Dr. R. A. Corradino, Dr. E. N. Taylor, and Dr. A. Bar are acknowledged.

1 October 1973; revised 26 November 1973 ■

Persistence of Cadmium-Induced Metabolic Changes in Liver and Kidney

Abstract. *Daily intraperitoneal injection of cadmium chloride (1 milligram per kilogram) for 45 days enhanced gluconeogenesis as evidenced by significant increases in the activities of liver and kidney cortex pyruvate carboxylase, phosphopyruvate carboxylase, hexosediphosphatase, and glucose-6-phosphatase, the quartet of key, rate-limiting enzymes involved in the biotransformation of noncarbohydrate precursors into glucose. Whereas cadmium treatment decreased the level of hepatic glycogen, the concentration of blood glucose and urea was significantly elevated by this heavy metal. Discontinuation of the heavy metal treatment for 28 days, in rats previously injected with cadmium for 45 days, failed to restore the observed biochemical alterations in hepatic and renal carbohydrate metabolism to control values. Evidence indicates that cadmium augments the glucose-synthesizing capacity of liver and kidney cortex and that various metabolic changes persist even after a 4-week period of withdrawal from exposure to the heavy metal.*

Prolonged exposure to environmental cadmium produces cellular alterations in a variety of body tissues. Testicular atrophy (*1*), hypertension (*2*), itai-itai disease (*3*), and permanent lung damage in the form of peribronchial fibrosis (*4*) are among the toxic symptoms attributed to the widespread usage of cadmium. In addition, this heavy metal produces histological and functional damage to kidney and liver, as evidenced by renal tubular atrophy, interstitial fibrosis, proteinuria, glycosuria (*5*, *6*), and cirrhosis (*7*). A similar relation between renal and hepatic dysfunction and an increase in glucose and protein in the urine has been noted in animals treated with either methylmercury or various chlorinated hydrocarbon insecticides (*8*, *9*). Since cadmium treatment affects overall carbohydrate metabolism (*3*, *10*), and the renal and hepatic abnormalities it produces closely resemble those seen in organomercurial-treated rats, we examined the influence of cadmium on renal and hepatic gluconeogenic enzymes, hepatic glycogen, blood glucose, and serum urea. We found that daily injection of cadmium chloride for 45 days (1 mg per kilogram of body weight) significantly elevated the concentration of blood glucose and urea; enhanced the activities of renal and hepatic pyruvate carboxylase (E.C. 6.4.1.1), phosphopyruvate carboxylase (E.C. 4.1.1.32), hexosediphosphatase (E.C. 3.1.3.11), and glucose-6-phosphatase (E.C. 3.1.3.9); and reduced the concentration of liver glycogen.

Experiments were carried out in male Wistar rats weighing approximately 100 g, housed in individual cages, and having free access to Master laboratory chow and water. Cadmium chloride was dissolved in physiological saline and administered daily for 45