A significant improvement in the ratios of peak to background is expected when the field of view of the detector is properly limited. Once extraneous radiation is eliminated, we should be able to obtain semiquantitative results, for example, Ca/P ratios, and detect a larger number of biologically important minerals.

Figure 3 shows spectra obtained from a single granule and from the matrix of a mitochondrion on a day several weeks after those in Fig. 2 were obtained. During that interval the microscope was used extensively to study integrated circuits, and the Si contamination was noticeable. From these spectra, one might suspect the presence of Si in the granule; however, the occurrence of a Si peak is spurious and apparently depends on the nature of the material being analyzed and the history of the instrument. It is possible that contamination of this sort may account for the reported presence of Si in mammalian tissues (*10*). However, multiple wavelength interferences, such as that found between first-order Si_K radiation and fifth-order Zn_K radiation, may also be responsible for the Si reportedly present when crystal spectrometers are used.

These experiments demonstrate that electron probe microanalysis can be used to determine the chemical composition of ultrastructural features of cells. They also demonstrate several pitfalls regarding contamination and sample preparations that must be avoided. The demonstration that Ca and P are present in the granules confirms the opinions of others that these elements are present in granules observed by means of the electron microscope (*1*, *2*). In the absence of data necessary to establish them as features of a living cell and not as artifacts, we feel that a definite role in Ca metabolism cannot be assigned to them at present.

LLOYD V. SUTFIN
MARIJKE E. HOLTROP
Children's Hospital Medical Center, Boston, Massachusetts 02115
ROBERT E. OGILVIE
Massachusetts Institute of Technology, Cambridge 02139

References and Notes

1. A. L. Lehninger, *Biochem. J.* **119**, 129 (1970).
2. J. H. Martin and J. L. Matthews, *Clin. Orthop. Relat. Res.* **68**, 273 (1970).
3. E. C. Weinbach and T. Von Brand, *Biochim. Biophys. Acta* **148**, 256 (1967).
4. R. S. Thomas and J. W. Greenawalt, *J. Cell Biol.* **39**, 55 (1968).
5. M. E. Holtrop, *Calcif. Tissue Res.*, in press.
6. T. Hall, in *Quantitative Electron Probe Microanalysis*, K. F. J. Heinrich, Ed. (National Bureau of Standards Special Publication 298, Washington, D.C., 1968), p. 269; L. V. Sutfin and R. E. Ogilvie, *Proc. Annu. Scanning Electron Microsc. Symp. 3rd* (1970), p. 27; in *Energy Dispersion X-Ray Analysis*, J. C. Russ, Ed. (American Society for Testing and Materials Special Technical Publication 481, Philadelphia, 1971), p. 197.
7. C. M. Taylor, A. J. Tousimis, J. A. Nicolino, Proc. Nat. Conf. on Electron Probe Analysis, 6th, Pittsburgh, 1971, paper No. 33.
8. E. L. Thurston and J. C. Russ, *Proc. Annu. Scanning Electron Microsc. Symp. 4th* (1971), p. 513.
9. P. Gaylarde and I. Sarkany, *Science* **161**, 1157 (1968).
10. P. W. Schafer and J. A. Chandler, *ibid.* **170**, 1204 (1970); E. M. Carlisle, *ibid.* **167**, 279 (1970).
11. One of us (L.V.S.) thanks Profs. S. A. Miller and R. S. Harris, present and past directors, respectively, of the Oral Science Training Program at the Massachusetts Institute of Technology for their encouragement and support. This work was supported by NIH training grant DE-105, Office of Naval Research contract NR 105-094, NIH grant AM 06375-09, and the John A. Hartford Foundation. We thank K. D. Altmann for specimen preparation and C. J. MacQuarrie for assistance with the photography.

6 July 1971; revised 7 September 1971

Strontium Induced Rickets: Metabolic Basis

Abstract. *Dietary strontium inhibits both the synthesis of 1,25-dihydroxycholecalciferol and intestinal calcium absorption in vitamin D_3–repleted chicks. 1,25-Dihydroxycholecalciferol restores calcium absorption to normal, while 25-hydroxycholecalciferol is without effect in the strontium-fed chick. It is suggested that strontium induces rickets by blocking the biosynthesis of 1,25-dihydroxycholecalciferol, the metabolically active form of vitamin D in the intestine.*

Ingestion of radioactive strontium (for example, ^{90}Sr) and the biological damage which may result has been of concern for several years. A dietary supplementation of stable strontium is reported to inhibit the intestinal absorption of ^{90}Sr, thereby serving as a deterrent to ingestion of radioactive strontium (*1*). However, prudence must be used in such treatment since replacement of dietary calcium with strontium results in diminished growth, improper bone mineralization (*2*), and an inhibition of intestinal calcium absorption (*1*). These physiological changes parallel very closely those seen in animals deficient in vitamin D and, therefore, the disease is referred to as "strontium rickets." Treatment with high doses of vitamin D is ineffective in curing strontium rickets (*3*), whereas feeding of a normal calcium diet reverses the lesions (*4*). Strontium is believed to act antagonistically to calcium in the processes of bone mineralization (*4*) and intestinal absorption (*1*).

Cholecalciferol (that is, vitamin D_3) is metabolized to hydroxy derivatives prior to its action at the cellular level (*5*). Cholecalciferol is first hydroxylated to 25-hydroxycholecalciferol (25-HCC) in the liver (*6*). The 25-HCC travels via blood to the kidney where it is hydroxylated to 1,25-dihydroxycholecalciferol (1,25-DHCC) and is then sequestered by the intestine, where it effects the assembly of the calcium transport system (*7*, *8*). Not only does 1,25-DHCC act more rapidly than 25-HCC, it stimulates intestinal calcium transport in nephrectomized (*9*) and actinomycin D–treated rats (*10*) whereas 25-HCC does not. This, together with metabolism studies of 1,25-[26,27-^{3}H]-DHCC (*10*), establishes 1,25-DHCC as the metabolically active form of vitamin D in the intestine. Hence, the rachitogenic action of strontium may possibly result from its inhibition of intestinal calcium transport because of a block in the metabolism of vitamin D to its functional hydroxylated derivative. We now show that dietary strontium inhibits 1,25-DHCC production from 25-HCC and that 1,25-DHCC but not 25-HCC can reverse strontium inhibition of intestinal calcium absorption. A concurrent study has also revealed a simi-

Table 1. The effect of dietary strontium on intestinal calcium absorption after cholecalciferol, 25-HCC, or 1,25-DHCC repletion of vitamin D–deficient chicks.

Treatment	Strontium diet	Daily dose* (pmole)	Calcium absorbed† (micrograms of calcium per 20 minutes)
Cholecalciferol	−	260	47 ± 2‡
Cholecalciferol	+	260	17 ± 2
25-HCC	−	130	52 ± 4‡
25-HCC	+	130	14 ± 1
1,25-DHCC	−	130	61 ± 2‡
1,25-DHCC	+	130	52 ± 6‡
Ethanol	−		14 ± 1
Ethanol	+		13 ± 1

* Dose was given orally in 0.2 ml of cottonseed oil (Wesson Oil). Chicks were given four doses; experimentation was conducted 24 hours after the last dose. † Values are presented as mean ± standard error of three to six observations. ‡ Significantly different from respective control (ethanol) group ($P < .01$, Student's t-test).

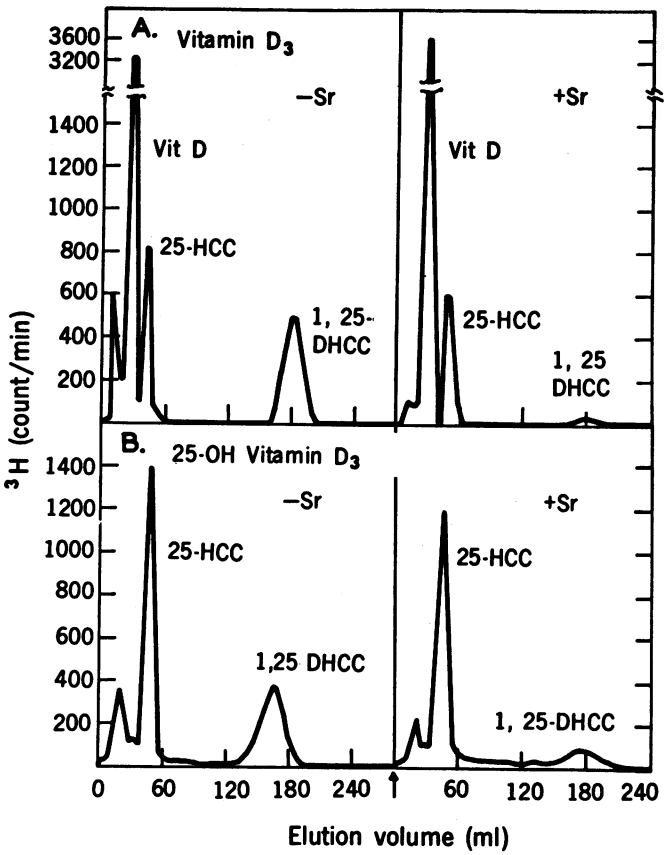


Fig. 1. Chicks were fed a +Sr or −Sr diet and given 260 pmole of cholecalciferol daily for 4 days. (A) Four chicks were injected with 650 pmole of [1,2-^{3}H]cholecalciferol 3½ hours before they were killed. The intestinal metabolites were separated on a column (1 by 30 cm) containing 18 g of Sephadex LH-20 which was developed with a mixture of chloroform and Skellysolve B (65 : 35;. (B) Three checks were injected with 325 pmole of 25-[26,27-^{3}H]HCC 2 hours before they were killed. The intestinal metabolites were separated on a column (1.9 by 30 cm) containing 20 g of Sephadex LH-20 which was developed with a mixture of chloroform and Skellysolve B (65 : 35).

lar inhibition of 1,25-DHCC synthesis by dietary and serum calcium (*11*).

Day-old white Leghorn cockerels were fed a purified rachitogenic diet (*12*) until they were 2 weeks old. Chicks were then shifted to a plus (+Sr) or minus (−Sr) strontium diet (+Sr diet: 0.08 percent Ca, 2.35 percent Sr; −Sr diet: 0.08 percent Ca, no Sr) for 3 days, at the end of which a daily oral supplementation of vitamin D_3, 25-HCC, or 1,25-DHCC was initiated and continued for 4 days. Vitamin metabolism and calcium absorption studies were conducted 24 hours after the last supplementation.

Calcium absorption was estimated in situ (*12*), with the absorption time shortened to 20 minutes. Results were expresed as micrograms of calcium absorbed per 20 minutes.

Metabolism of [1,2-^{3}H]vitamin D_3 (0.6 c/mmole) (*13*) and 25-[26,27-^{3}H]-HCC (1.3 c/mmole) (*14*) was studied in −Sr and +Sr chicks repleted with a low level of vitamin D_3 (260 pmole daily). [^{3}H]Vitamin D_3 and 25-[^{3}H]HCC was dissolved in 95 percent ethanol and given to the chicks intravenously; the animals were killed 3½ and 2 hours later, respectively. Intestinal mucosa was collected and extracted with choloroform-methanol (*15*). Chromatographic separation of [1,2-^{3}H]vitamin D_3 and 25-[26,27-^{3}H]HCC metabolites was effected with columns containing Sephadex LH-20 which were equilibrated and developed with 65 percent chloroform: 35 percent Skellysolve B (*16*). The radioactivity of ^{45}Ca and ^{3}H was estimated using a Tri-Carb model 3375 liquid scintillation counter (Packard) (*12, 13*).

Vitamin D–repleted chicks fed a strontium diet demonstrated an impaired ability to produce 1,25-DHCC when injected with either [1,2-^{3}H]vitamin D_3 or 25-[26,27-^{3}H]HCC. Sephadex LH-20 chromatography of intestinal chloroform extracts from −Sr chicks injected with either [1,2-^{3}H]vitamin D_3 or 25-[26,27-^{3}H]HCC showed that 13 and 31 percent of the applied radioactivity was 1,25-DHCC, respectively (Fig. 1). However, with a similar protocol only 2.5 and 8 percent of the chromatographed radioactivity was present as 1,25-DHCC in +Sr chicks. Strontium treatment had no apparent influence on the appearance of 25-HCC in [1,2-^{3}H]-vitamin D_3–injected animals (Fig. 1). Therefore, strontium seemed to effect its action by inhibiting the conversion of 25-HCC to 1,25-DHCC, a reaction that takes place in the kidney.

Vitamin D–deficient nephrectomized rats, which are unable to synthesize 1,25-DHCC, show no stimulation of calcium transport in response to physiological doses of 25-HCC (*8*). A similar picture is obtained in chicks fed strontium owing to the inhibition of 1,25-DHCC synthesis. Both vitamin D_3 and 25-HCC are ineffective in overcoming the strontium inhibition of intestinal calcium absorption (Table 1). In contrast, +Sr chicks given 1,25-DHCC were able to maintain their calcium absorption at a nearly normal level.

From our results, the characteristic rachitic lesions and inhibition of intestinal calcium absorption observed in animals fed strontium can be attributed to the low intestinal concentration of 1,25-DHCC, the metabolically active form of vitamin D in the intestine (*12, 17*). In at least one respect, strontium-treated animals may serve as a model for the study of vitamin D–resistant rickets because of the observed metabolic lesion. Although such animals are given vitamin D daily, their intestines are in effect "vitamin D–deficient" because of a metabolic block in the synthesis of 1,25-DHCC. Kidney mitochondria from strontium-fed chicks show a low rate of 1,25-DHCC synthesis (*18*). Thus, strontium may act like high serum calcium in the control of 1,25-DHCC synthesis and thereby block this calcium-sensitive reaction. Such an action would effectively turn off intestinal calcium transport.

J. L. Omdahl
H. F. DeLuca
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison 53706

References and Notes

1. R. A. Corradino and R. H. Wasserman, *Proc. Soc. Exp. Biol. Med.* **133**, 960 (1970).
2. J. C. Bartley and E. F. Reber, *J. Dairy Sci.* **44**, 1754 (1961).
3. A. E. Sobel, A. R. Goldfarb, B. Kramer, *Proc. Soc. Exp. Biol. Med.* **31**, 869 (1934).
4. A. E. Sobel, J. Cohen, B. Kramer, *Biochem. J.* **29**, 2640 (1935).
5. H. F. DeLuca, *Fed. Proc.* **28**, 1678 (1969).
6. G. Ponchon and H. F. DeLuca, *J. Clin. Invest.* **48**, 1273 (1969).
7. D. R. Fraser and E. Kodicek, *Nature* **228**, 764 (1970); R. W. Gray, I. T. Boyle, H. F. DeLuca, *Science* **172**, 1232 (1971).
8. I. T. Boyle, L. Miravet, R. W. Gray, M. F. Holick, H. F. DeLuca, *Endocrinology*, in press.
9. Y. Tanaka, H. F. DeLuca, J. L. Omdahl, M. F. Holick, *Proc. Nat. Acad. Sci. U.S.* **68**, 1286 (1971).
10. C. A. Frolik and H. F. DeLuca, *Arch. Biochem. Biophys.*, in press.
11. I. T. Boyle, R. W. Gray, H. F. DeLuca. *Proc. Nat. Acad. Sci. U.S.* **68**, 2131 (1971)
12. J. L. Omdahl, M. F. Holick, T. Suda, Y. Tanaka, H. F. DeLuca, *Biochemistry* **10**, 2935 (1971).
13. P. F. Neville and H. F. DeLuca, *ibid.* **5**, 2201 (1966).

14. T. Suda, H. F. DeLuca, R. B. Hallick, *Anal. Biochem.* **43**, 139 (1971).
15. J. Lund and H. F. DeLuca, *J. Lipid Res.* **7**, 739 (1966).
16. M. F. Holick and H. F. DeLuca, *ibid.* **12**, 460 (1971).
17. M. R. Haussler, D. W. Boyce, E. T. Littledike, H. Rasmussen, *Proc. Nat. Acad. Sci. U.S.* **68**, 177 (1971).
18. J. L. Omdahl and H. F. DeLuca, in preparation.
19. Supported by PHS grant AM-14881 and AEC contract AT-(11-1)-1668. J.L.O. is supported by PHS postdoctoral fellowship 5-FO2-AM43354-02. We thank Yvonne Celmerowski for technical assistance.

12 July 1971; revised 13 August 1971

6β-Hydroxy-Δ¹-Tetrahydrocannabinol

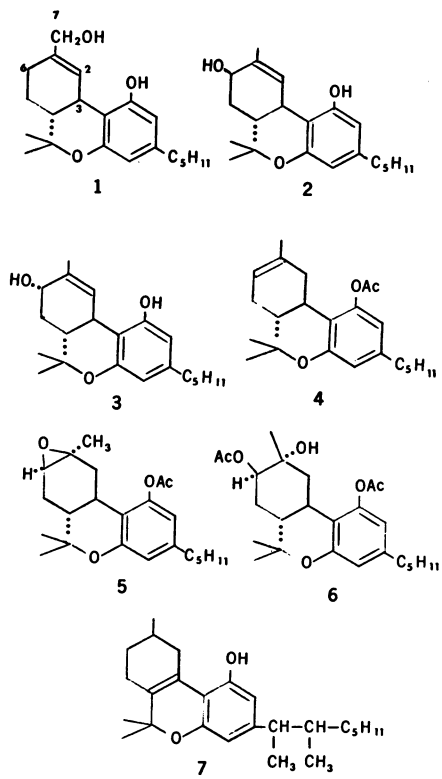
Synthesis and Biological Activity

Abstract. *6β-Hydroxy-Δ¹-tetrahydrocannabinol, a metabolite of Δ¹-tetrahydrocannabinol has been synthesized from Δ⁶-tetrahydrocannabinol. It shows high tetrahydrocannabinol-type activity in rhesus monkeys. The implications of this finding are discussed.*

The metabolism of Δ¹-tetrahydrocannabinol (Δ¹THC), the predominant active principle of marihuana (*1*), and of Δ⁶THC, a minor active component (*2*), has been extensively investigated (*3–5*). The major pathway so far identified is through hydroxylation of the allylic C-7 position of the terpenoid moiety. The metabolites 7-hydroxy-Δ¹-THC (**1**) and 7-hydroxy-Δ⁶THC (*6*), are biologically active, and it has been suggested (*7*) that these compounds are indeed the active forms of the respective THC's on the molecular level. Wall (*8*) has isolated a new metabolite, 6β-hydroxy-Δ¹THC (**2**), from an incubation of Δ¹THC with rabbit liver homogenate. The biological activity of **2** was not determined, apparently because of the difficulty of securing sufficient quantities for testing.

In continuation of our study (*9*) on structure and activity in the THC series we had already prepared both 6β-hydroxy-Δ¹THC (**2**) and 6α-hydroxy-Δ¹THC (**3**) when the identification of **2** as a metabolite was announced by Wall. For the synthesis of **2** we used Δ⁶THC acetate (**4**) as starting material. Epoxidation with *m*-chloroperbenzoic acid led to 1β,6β-epoxyhexahydrocannabinol acetate (**5**) in a yield of 84 percent. The molecular weight (372, determined from the mass spectrum) showed the addition of a single oxygen atom. The stereochemistry of the epoxy ring was determined from the nuclear magnetic resonance (NMR) spectrum: the signal of the equatorial C-6 proton at δ 2.87 appears as a doublet with a splitting constant of 4.5 hz. In the alternative axial position a large splitting constant of about 10 to 12 hz would have been expected.

Treatment of **5** with perchloric acid in acetone, followed by acetylation led to a mixture (*10*) which, on column chromatography over silica gel, gave, on elution with a 1 : 1 mixture of ether and petroleum ether, 1α-hydroxy-6β-acetoxyhexahydrocannabinol acetate (**6**) in 77 percent yield; the molecular weight was 432; $[\alpha]_D$ was −158° in ethanol; the ultraviolet spectrum showed a maximum λ_{max} in ethanol at 276 nm with molecular extinction, ε was 1770, and at 283 nm, ε was 1860. The NMR spectrum (*11*) gave evidence for six methyl groups [δ, 0.9 (t) 1.04, 1.12, 1.28, 2.00, 2.22 (singlets)], an equatorial proton in an α position to the acetoxyl group (δ, 4.76; bs, width at half height 4.5 hz), and two nonequivalent aromatic protons (δ, 6.21 and 6.40). Analysis of compound **6** showed



C, 69.49, H, 8.41; calculation for $C_{25}H_{36}O_6$ is C, 69.42; H, 9.15.

Dehydration of **6** by thionyl chloride in pyridine followed by reduction with lithium aluminum hydride led to a mixture (*10*) which was separated by preparative thin-layer chromatography giving 6β-hydroxy-Δ¹THC (**2**) in 24 percent yield. Compound **2** on analysis showed C, 76.09 and H, 9.03; calculation for $C_{21}H_{30}O_3$ indicates C, 76.33, H, 9.15; $[\alpha]_D$ was −133° in ethanol; ultraviolet spectrum, λ_{max} in ethanol, 276 nm (ε, 1510), 283 nm (ε, 1570); NMR in $CDCl_3$ (δ), 0.88 (t) side chain methyl; 1.07 (singlet), 1.38 (singlet) (methyl groups on C-8), 1.81 (singlet) (methyl group on double bond); 3.05 (bd, 10 hz) (C-3 proton); 4.05 (bd, 3.5 hz) (proton α to hydroxyl group); 6.06, 6.20 (aromatic protons); 6.65 (bs) (olefinic proton). The NMR spectrum is essentially identical to that reported by Wall (*8*) for the natural metabolite. Compound **2** is an unstable oil which, however, forms a stable 1 : 1 complex with dimethylformamide, m.p. 99°C. The NMR spectrum of this complex represents a summation of the spectra of **2** and dimethylformamide (in a ratio of 1 : 1) except for the aromatic protons which now appear at 6.17 and 6.21.

The NMR spectrum of 6α-hydroxy-Δ¹THC (**3**) (*4*) differs from that of the 6β isomer. Thus in **3** the C-6 proton appears at δ, 4.32 (bt, 11 hz).

6β-Hydroxy-Δ¹THC (**2**) was administered intravenously to rhesus monkeys in propylene glycol (0.1 ml per kilogram of body weight). The experimental conditions of this test have been described (*9*, *12*). At a dose of 1 mg/kg the monkeys were drowsy, showed significantly decreased motor activity, and occasional partial ptosis and head drop. These effects are identical to those observed with 0.25 mg of Δ⁶THC per kilogram of body weight in the same test. At a lower dose (0.5 mg/kg) 6β-hydroxy-Δ¹THC did not cause any observable effects in rhesus monkeys, while at a higher dose (2 mg/kg) we observed stupor, ataxia, suppression of motor activity, and full ptosis. The animals took up a typical crouched posture in which they remained for as long as 3 hours. The animals could, however, regain normal behavior for short periods of time if they were pinched or if they were presented with noise.

The isomeric 6α-hydroxy-Δ¹THC (**3**) is also active in rhesus monkeys at the same dose levels.