inert tracers from single tissue types. This would suggest that diffusion is making a more significant contribution to limiting the rate of blood-tissue exchange than is generally accepted.

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Vitamins D_2 and D_3 in New World Primates: Influence on Calcium Absorption

Abstract. *In* Cebus albifrons *monkeys it was demonstrated that vitamin D_3 promotes the intestinal absorption of calcium-47 and that vitamin D_2 does not increase absorption above that seen in monkeys deficient in vitamin D. These data support previous observations that vitamin D_2 is not effective in preventing metabolic bone disease in this species.*

The relative ineffectiveness of vitamin D_2 in the chick has been well established. It was demonstrated in 1930 that in this species vitamin D_2 has 10 percent (or less) of the activity of vitamin D_3 in the prevention and cure of rickets, promotion of body growth, egg production, and other parameters (*1, 2*). Approximately 30 years ago several reports presented clinical evidence indicating a similar but quantitatively smaller difference in the activity of vitamin D_2 and D_3 in human infants (*1*). These reports have been questioned, and whether or not a significant difference in activity does exist in man has been unresolved.

We have reported evidence (*3, 4*) which indicates that vitamin D_2 is not utilized or is significantly less effective than vitamin D_3 in the prevention of rickets, osteomalacia, and osteodystrophia fibrosa in several species of South American primates. These data were based on the development of bone disease in *Cebus albifrons* and several species of tamarins (*Saguinus oedipus, S. nigricollis, S. mystax*) maintained on a purified diet containing vitamin D_2 and the reversal of the disease process by substituting vitamin D_3 for D_2. We now report the influence of vitamin D_2 and vitamin D_3 on calcium absorption, which provides additional evidence that vitamin D_3 is more active than D_2 in the Cebus monkey.

Six *Cebus albifrons* monkeys were maintained on a purified diet containing 2000 international units (IU) of vitamin D_2 per kilogram of diet for 24 months (*3*). The diet was composed of (in percentages): sucrose, 62.00; casein, 25.00; corn oil, 8.00; salt mix number IV (Hegsted *et al.*, 1941), 4.00; choline chloride, 0.50; dry vitamin mix, 0.40; and inositol, 0.10. The dry vitamin mix contained (in percentages): thiamine, 0.50; riboflavin, 0.50; pyridoxine 0.50; niacin, 2.45; calcium pantothenate, 1.50; folic acid, 0.05; biotin, 0.01; and dextrose, 94.44. Vitamin A acetate and the vitamin D_2 in absolute alcohol were added to the diet in amounts of 12,500 IU/kg and 2000 IU/kg, respectively. Ascorbic acid in dilute alcohol was added to the ration daily to supply 25 mg per monkey per day.

After the development of severe osteodystrophia fibrosa, vitamin D_3 (2000 IU/kg of diet) was substituted for vitamin D_2 for a period of 5 months. After observations on the reversal of the disease process, all vitamin D was removed from the diet. After the monkeys had been on the diet lacking vitamin D for 12 months, the clinical findings, roentgenograms, and serum alkaline phosphatase signified the exacerbation of osteodystrophia fibrosa. At this point two animals (Nos. 201 and 202) were provided a daily oral supplement of 500 IU of vitamin D_3; two (Nos. 209 and 214) with 500 IU of vitamin D_2; and two (Nos. 221 and 222) were left untreated. Twelve days after the supplements were initiated radio-calcium uptake studies were conducted on each animal. After the monkeys were fasted for 24 hours, 7 μc of calcium-47 were administered via stomach tube and the animals were provided with 50 g of purified diet. Serum samples were obtained at 2, 4, 6, and 24 hours, and urine and feces were collected at 24-hour intervals for 5 and 7 days, respectively. The skull was monitored at 48 hours by holding the head beneath the probe of a scintillation counter. One milliliter of serum was counted for each time interval, in a gamma counter. The urine was heated for 1 hour with 10 ml of HCl, cooled, and made up to 200 ml with distilled water, and 2-ml aliquots were counted. The feces were mixed with a small amount of water and heated with 25 ml of HCl, cooled, and brought up to 200 ml with water, and 2-ml aliquots were counted.

To determine whether, in the Cebus monkey, vitamin D has any direct influence on the mineralization of bone, or on urinary excretion of calcium, the animals were maintained on the oral supplement of vitamin D for an additional 42 days and then injected intravenously with 3 μc of Ca^{47} (experiment 2). Serum samples were collected at 5, 10, 30, 60, 180, and 360 minutes. Urine and feces were collected and processed as in the first study and the skull was monitored at 48 hours. After the monkeys had been an additional 70 days on the vitamin D supplements, a second oral uptake study was performed (experiment 3), essentially as described above. However, a dose of 5 rather than 7 μc of Ca^{47} was given, and the animals were not fed for 6 hours.

Table 1. Thousands of counts per minute over the skull of monkeys at 48 hours.

Vitamin supplement	Thousands of counts		
	Exp. 1 (oral)	Exp. 2 (i.v.)	Exp. 3 (oral)
Vitamin D_3			
Monkey 201	17.1	6.8	12.2
Monkey 202	23.2	7.0	15.3
Vitamin D_2			
Monkey 209	2.5	6.8	9.1
Monkey 214	1.5	5.2	9.1
None			
Monkey 221	2.0	7.1	6.8
Monkey 222	0.7	5.9	6.6

The statistical comparison (*3*) of the means of the various determinations for the three groups was made with one-way analysis of variance. For analysis purposes the original data were converted as follows: serum peak specific activity to log values; total urinary and fecal percent excretion to 2 arc sin $\sqrt{X}$; and counts per minute (cpm) over the skull to $\sqrt{}$ cpm. These transformations were used to obtain the homogeneity of variance necessary for comparison of means by analysis of variance. The resulting analysis of variance compared the three means simultaneously. In addition, *t*-tests were calculated to compare the means of the three groups.

The results of the first study are presented in Figs. 1 and 2. The specific activity of the serum (Fig. 1) clearly indicates a significantly greater absorption in the two animals given vitamin D_3 supplements ($P < .01$). There is no significant difference between the D_2 group and the animals given no supplement. The difference in absorption is reflected in fecal excretion (Fig. 2), which is significantly lower in the group given vitamin D_3 supplement ($P < .01$). The animals given vitamin D_2 supplement excreted more Ca^{47} than the nonsupplemented group although the difference was of lower significance ($P < .05$). There were no significant differences between the three groups in urinary excretion. The skull counts were significantly higher in the D_3 animals ($P < .01$) and without significant difference between the two other groups (Table 1).

Following intravenous injection of Ca^{47} the serum cleared at approximately the same rate in all six animals; however, differences were present in the pattern of excretion. The monkeys given vitamin D_3 supplement excreted significantly less Ca^{47} in the feces (Fig. 3) than the other two groups ($P < .01$). There was no significant difference between the D_2-supplemented and the nonsupplemented animals. Also in the urine (Fig. 4) significantly less Ca^{47} was excreted by the D_3 group ($P < .01$), but in addition the D_2 monkeys excreted significantly less than the nonsupplemented group ($P < .01$). Based on percent of original dose, combined excretion in the urine and feces in the three groups at 96 hours was 1.81 percent in the D_3 group, 8.88 percent in the D_2 group, and 15.31 percent in the nonsupplemented group. Most of this loss was in the feces. This resulted in a retention at 96 hours of 98.19 percent, 91.12 percent, and 84.69 percent, respectively, in the three groups. There were no significant differences in the head counts between the groups (Table 1).

The results of the second study of oral uptake were similar to those of the first study. The serum uptake was significantly higher in the monkeys given vitamin D_3 supplement ($P < .01$), with no difference between the D_2 and the nonsupplemented groups. Fecal excretion was significantly lower in the D_3 group ($P < .05$), with no significant difference between the two other groups. There was no significant difference in urinary excretion of Ca^{47} between the three groups. The head counts (Table 1) of the D_3 group were significantly higher ($P < .01$) than in the two other groups, and with no significant difference between the D_2 and nonsupplemented animals.

The results of the two oral uptake studies indicate that in the Cebus monkey vitamin D_3 promotes the absorption of Ca^{47} and that vitamin D_2 at the levels employed does not significantly increase absorption of Ca^{47} in comparison with animals deficient in vitamin D. These facts are reflected in greater deposition of Ca^{47} in bone and decreased fecal loss of Ca^{47} in the D_3-supplemented animals.

The results following intravenous injection of Ca^{47} indicated similar clearance from the serum with retention of 98.2 percent, 91.1 percent, and 84.7 percent of the original dose at 96 hours in the D_3, D_2, and nonsupplemented animals, respectively. These differences in retention are principally accounted

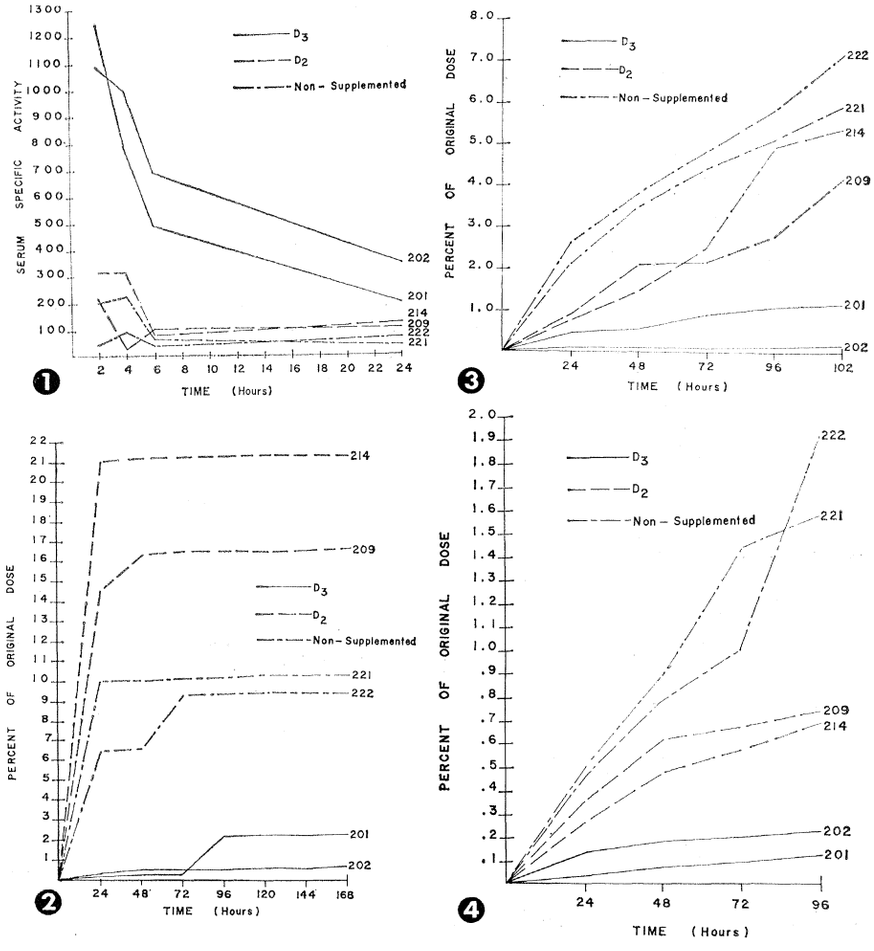

Fig. 1. Specific activity of the serum (counts per minute per milligram serum calcium) following an oral dose of 7 μc of Ca^{47} in monkeys deficient in vitamin D and monkeys given D_2 and D_3 supplements, respectively. Fig. 2. Cumulative excretion of Ca^{47} in the feces, expressed as percent of original dose following an oral dose of 7 μc in monkeys deficient in vitamin D and monkeys given D_2 and D_3 supplements, respectively. Fig. 3. Cumulative excretion of Ca^{47} in the feces, expressed as percent of original dose following an intravenous dose of 3 μc in vitamin-D deficient and D_2- and D_3-supplemented monkeys. Fig. 4. Cumulative excretion of Ca^{47} in the urine, expressed as percent of original dose following an intravenous dose of 3 μc in vitamin-D deficient and D_2- and D_3-supplemented monkeys.

for by the greater fecal loss in the D_2 and nonsupplemented animals. This is most likely the result of the failure to reabsorb calcium excreted via the intestine in these two groups. Urinary loss of Ca^{47} was significantly different in the three groups but the levels were so low in all three groups (0.31 percent in the D_3, 1.38 percent in the D_2, and 3.41 percent in the nonsupplemented) that it appears that if this reflects any action of vitamin D_3 or D_2 or both at the level of the kidney it is not of great importance in maintaining calcium balance. The differences in retention clearly indicate differences in deposition in the bone. However, neither the estimation of activity of the serum nor the external counting over the head are sufficiently accurate to demonstrate the differences in the degree of retention observed after intravenous injection. Whether vitamin D_2 or D_3 has an active role at the level of the bone was not demonstrated by this study and warrants additional experimentation.

The data for both oral and intravenous administration of Ca^{47} demonstrate a primary effect of vitamin D_3 on intestinal absorption of calcium, rather than at the level of the bone or kidney, and clearly indicate that vitamin D_3 is more active than vitamin D_2 in promoting calcium absorption in Cebus monkeys. Further investigation of the effectiveness of various forms of vitamin D in man is warranted in light of this evidence in a primate species.

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Ultrastructure of Thrombosthenin, the Contractile Protein of Human Blood Platelets

Abstract. *Partially purified thrombosthenin with adenosine triphosphatase activity similar to that of actomyosin was subjected to electron microscopy. More than 50 percent of the material consisted of fibrils 80 to 100 angstroms in width. Occasional fibrils suggested a periodic structure. The morphology of isolated thrombosthenin resembled that of microfibrils in the cytoplasm and pseudopods of intact platelets.*

It has been known for almost a century that blood platelets are contractile and that they play an essential role in the process of clot retraction. Although this phenomenon has been observed and studied in many laboratories, the underlying mechanism is still not completely understood. In 1959 Bettex-Galland and Lüscher (*1*) extracted a contractile protein, thrombosthenin, from human platelets. Like muscle actomyosin, the activity of this protein depended on the presence of adenosine triphosphate (ATP) and Ca^{++} ions. It was, therefore, postulated that its muscle-like adenosine triphosphatase activity might be responsible for platelet-dependent clot retraction. These observations were confirmed by others (*2*, *3*). Thrombosthenin has been partially purified (*3*), and thus analysis of its ultrastructure has been facilitated.

We now describe the electron-microscopic appearance of isolated thrombosthenin and the correlation of its morphology with that of subcellular structures observed in intact platelets. Platelets were collected from 5 to 10 liters of human blood, washed six times in a solution of 0.1*M* sodium citrate and 0.15*M* NaCl, and suspended in 0.6*M* KCl in 0.015*M* tris (hydroxymethyl)aminomethane (tris) buffer at *p*H 7.5. They were gently lysed with *n*-butanol according to the procedure of Grette (*2*). The debris was sedimented by centrifugation at 12,000*g* for 15 minutes, and the contractile protein contained in the supernatant was precipitated with 0.002*M* $MgSO_4$ at 4°C. Centrifugation at 8700*g* for 10 minutes yielded a stiff gel. This gel was dissolved with 0.6*M* KCl in tris buffer and reprecipitated with 0.002*M* $MgSO_4$ six times. It was then further purified by chromatography on Bio-Gel P-300.

The chemical and immunologic properties of this preparation have been described in detail (*3*) and will only be summarized here. The adenosine triphosphatase activity of isolated thrombosthenin was shown in the presence of $5 \times 10^{-4}M$ ATP; 1 mg of the protein released 3 μg of in-

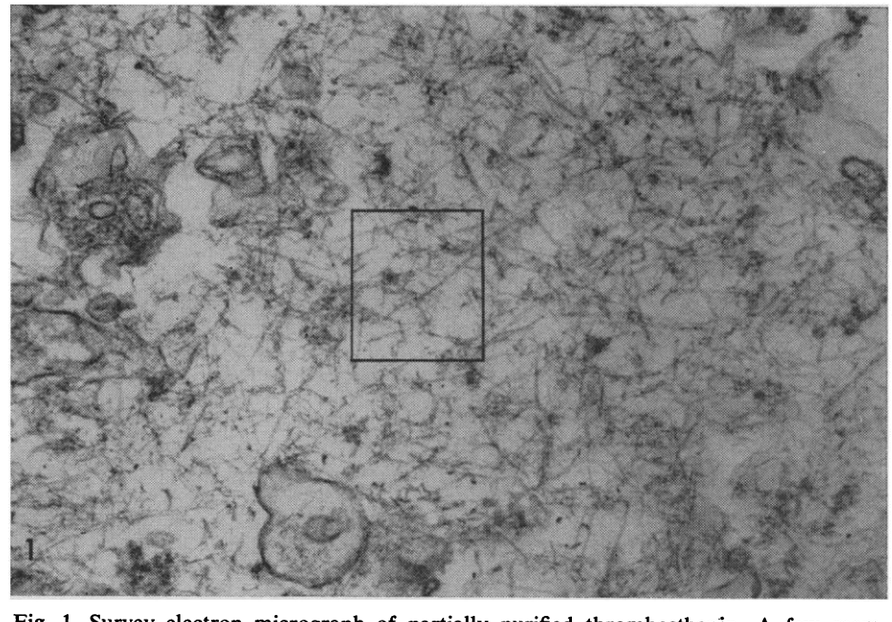

Fig. 1. Survey electron micrograph of partially purified thrombosthenin. A few membranes and amorphous material contaminate this preparation, which consists mostly of fibrillar structures (× 51,000). Inset seen at higher magnification in Fig. 2.