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The Effect of Prednisolone® upon the Metabolism and Action of 25-Hydroxy- and 1,25-Dihydroxyvitamin D_3

(vitamin D/intestinal calcium absorption/adrenal steroids)

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ABSTRACT **Treatment of vitamin D-deficient rats with Prednisolone® does not alter the rate of conversion of [^{3}H]25-hydroxyvitamin D_3 to [^{3}H]1,25-dihydroxyvitamin D_3, but the further conversion of [^{3}H]1,25-dihydroxyvitamin D_3 to a more polar metabolite is more rapid in the Prednisolone®-treated animals. This more polar metabolite is biologically inactive, periodate-insensitive, and persists in the intestine as long as 1,25-dihydroxyvitamin D_3. Also, the time course of action of 1,25-dihydroxyvitamin D_3 upon intestinal calcium transport is altered by Prednisolone® treatment. Treatment with Prednisolone® did not change the magnitude of the initial response to 1,25-dihydroxyvitamin D_3 at 7 hr, but did decrease the response at 24 and 48 hr after a single dose of 1,25-dihydroxyvitamin D_3. The present results show that one of the means by which large doses of adrenal corticoids alter intestinal calcium transport is by stimulating the further metabolism of 1,25-dihydroxyvitamin D_3 to a more polar, biologically inactive intestinal metabolite.**

Adrenal glucocorticoids inhibit the intestinal absorption of calcium (1–10). They may act by antagonizing the action of vitamin D (5, 10). However, administration of either vitamin D_3 or 25-hydroxyvitamin D_3 (25-OH D_3) does not reverse the inhibitory effect of the corticoids (5–7). Glucocorticoids might interfere with the metabolism of vitamin D_3 or 25-OH D_3 as proposed by Avioli and coworkers (11), but Kimberg, Favus, and coworkers (2, 6) did not find any significant effect of hydrocortisone therapy upon the metabolism of either vitamin D_3 or 25-hydroxycholecalciferol in the rat, and both Lukert *et al.* (7) and Favus *et al.* (3) have reported finding normal amounts of 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] in the intestinal mucosal cells of the corticoid-treated rats. Our present results indicate that, contrary to the previous evidence, adrenal corticoids do alter the metabolism of 1,25-$(OH)_2D_3$ and this effect may play an important role in the steroid-induced change in intestinal calcium transport.

MATERIALS AND METHODS

Weanling male Wistar rats were grown in individual cages in a dark room on a vitamin D-free diet for 6 weeks (4). The animals weighed between 120 and 140 g. Appropriate groups of animals (3–10 animals/group) were then given Prednisolone® subcutaneously at a dosage of 20 mg/kg per day for 5 days, and control animals were injected with solvent. In some animals nephrectomy was performed immediately before administration of [^{3}H]25-OH D_3 or [^{3}H]1,25-$(OH)_2D_3$.

Abbreviations: 25-OHD_3, 25-hydroxyvitamin D_3; 1,25-$(OH)_2D_3$, 1,2-dihydroxyvitamin D_3.

For the studies of 25-OH D_3 metabolism, each animal received intravenously 9×10^5 dpm of [*26,27*-^{3}H]25-OH D_3 specific radioactivity, 19.7 Ci/mmol (Amersham, England). The animals were killed 7 hr later. The intestinal mucosa, kidneys, bone, and liver were removed and extracted with chloroform–methanol (2:1) by the method of Bligh and Dyer (12). After extraction, the chloroform phase was dried in a rotary evaporator, taken up in a small volume of solvent, and subjected to chromatography on a 1×60-cm column of Sephadex LH20. Stepwise elution with chloroform–hexane (65:35), followed by chloroform–hexane (75:25) was used (13). Fractions (2 ml) were collected, dried, and counted in a Packard Tricarb Scintillation Counter (model 3214) or an Intertechnique SL-40 with an automatic external standardization system. The standard scintillation fluid contained 2 g of 2,5-diphenyloxazole (PPO) and 100 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)](POPOP)-benzene per liter of toluene.

Intestinal calcium transport was assessed by the everted-loop technique of Schachter and Rosen (9). Vitamin D-deficient rats were divided into two groups: control and Prednisolone®-treated. The Prednisolone® was given subcutaneously for 5 days at a dose of 20 mg/kg per day. On the last day, 7 hr before they were killed, appropriate groups of control or Prednisolone®-treated animals received a single intravenous dose of 25-OH D_3 or 1,25-$(OH)_2D_3$, or the polar metabolite. 25-OHD_3 was obtained from Dr. Mathieu de Fossey (Roussel Laboratory, Paris). The animals were decapitated; a standard 5-cm segment of duodenum and upper jejunum was removed, washed, everted, and made into a sac, which was incubated in 10 ml of a modified Krebs–Ringer phosphate buffer, pH 7.4, containing 0.4 mM $CaCl_2$ and 0.02 μCi of $^{45}CaCl_2$. Incubations were carried out for 90 min at 37° after the flasks had been oxygenated with 95% O_2. At the end of this time the sac was removed, blotted, and opened. Samples of the inside and outside solutions were counted by the liquid scintillation method, with either Instagel (Packard) or Bray's solution (14). The ratio of inside to outside of radioactivity was recorded as a measure of transport capacity.

The 1,25-$(OH)_2D_3$ used in these studies was produced biosynthetically in renal homogenates obtained from vitamin D-deficient chicks that had been maintained on a vitamin D-deficient diet for 3 weeks (prepared by Mongin and Saaveur, INRA, Nouzilly). They were killed by decapitation; a homogenate of their kidneys was prepared (15) in a Tris-acetate buffer, pH 7.4. An aliquot of 6–8 ml of this homogenate was incubated at 37° for 1 hr after addition of 3 μg of [^{3}H]25-

TABLE 1. *A comparison of the distribution of metabolites of [^{3}H]25-OHD$_3$ in various organs of vitamin D-deficient rats (−D) either untreated or treated with Prednisolone® for 5 days**

Metabolite	Intestine	Bone	Kidney	Liver
	Tissue radioactivity† (dpm/g of tissue)			
−D	76,100	1,780	67,500	25,900
−D + steroid	82,500	1,890	63,000	24,700
	% Of total counts in each chromatographic peak			
Ester				
−D	3.3	2.0	2.3	5.8
−D + steroid	4.6	4.2	2.0	6.0
25OH D$_3$				
−D	32	65	60	70
−D + steroid	41	68	54	65
24,25(OH)$_2$D$_3$				
−D	1.8	0	0	0
−D + steroid	2.9	0	0	0
1,25(OH)$_2$D$_3$				
−D	56	25	28	17
−D + steroid	0	17	22	16
Polar metabolite				
−D	7	8‡	9‡	7‡
−D + steroid	52	9‡	22‡	13‡

* The rats were injected intravenously with [*26,27*-^{3}H]25-OHD$_3$ 7 hr before they were killed.

† Each value represents the mean from three separate experiments.

‡ Values reported as more polar metabolites in these organs may represent different metabolites from those found in the intestine. The metabolite noted in these other organs has not been characterized.

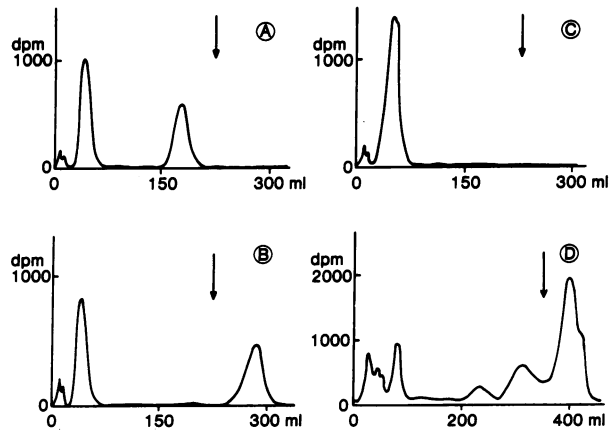

FIG. 1. Chromatographic profiles of radioactive metabolites observed in intestinal mucosal extracts obtained from vitamin D-deficient control and Prednisolone®-treated rats 7 hr after the intravenous administration of [^{3}H]25-OH D$_3$ or [^{3}H]1,25-(OH)$_2$D$_3$. Profiles are from the mucosal extracts of vitamin D-deficient animals: (*A*) given [^{3}H]25-OH D$_3$; (*B*) treated with Prednisolone® and given [^{3}H]25-OH D$_3$; (*C*) treated with Prednisolone®, nephrectomized, and given [^{3}H]25-OH D$_3$; (*D*) treated with Prednisolone®; nephrectomized, and given [^{3}H]1,25-(OH)$_2$D$_3$. Separations in *A*, *B*, and *C* were done on 1 × 60-cm columns of Sephadex LH20 with stepwise elution with chloroform–hexane 65:35, followed by chloroform–hexane 75:25 at *arrow*. The first major peak, appearing at about 50 ml of effluent, was identified as 25-OH D$_3$; the second major peak (*A*), appearing at 170 ml of effluent, was 1,25-(OH)$_2$D$_3$; and the third major peak (*B*), appearing at 290 ml of effluent, was identified as a more polar metabolite of 1,25-(OH)$_2$D$_3$. The separation in experiment *D* was done on a 2 × 30-cm column of Sephadex LH 20, and the major peak seen at the 400-ml elution volume is the more polar metabolite.

OH D$_3$ (about 8,000 dpm/μg). The homogenate was extracted first with chloroform–methanol (2:1), then with petroleum ether (30°–60°), and then again with chloroform. The extract was dried in a rotary evaporator; the residue was taken up in a small amount of solvent and subjected to chromatography on a 2 × 80-cm column of Sephadex LH20 with chloroform–hexane (65:35) as eluting solvent. The tubes containing the peak corresponding to [^{3}H]1,25-(OH)$_2$D$_3$ were collected, evaporated, and again subjected to LH20 chromatography, with methanol as eluting solvent. The material isolated by this method was tested for biological activity by the standard *in vivo* assay (9).

The sensitivity of 25-OH D$_3$ metabolites to periodate was examined by the technique of Holick *et al.* (16). Half milliliter of a solution containing the eluted metabolite was dried, dissolved in 1 ml of MeOH, and then treated with 0.1 ml of 0.5% aqueous $NaIO_4$. After 16 hr at 22°, 1 ml of chloroform was added with 0.5 ml of H_2O. The recovered chloroform phase was applied to a 2 × 30-cm column of Sephadex LH20 and eluted in the standard fashion.

RESULTS

Chromatographic profiles of lipid extracts of intestinal mucosa of vitamin D-deficient control and Prednisolone®-treated rats 7 hr after administration of [^{3}H]25-OH D$_3$ are shown in Fig. 1 *A* and *B*. Total radioactivity recovered and the percentage remaining as [^{3}H]25-OH D$_3$ in the two extracts was approximately the same. However, there was a striking difference in the location of the second major chromatographic peak. In the profile from the vitamin D-deficient control animals (Fig. 1*A*) the major radioactive peak, other than [^{3}H]25-OH D$_2$, eluted between 160 and 215 ml and before the switch in eluting solvent (*arrow*, Fig. 1*A* and *B*). This is the chromatographic position of 1,25-(OH)$_2$D$_3$. In contrast, the major radioactive peak, other than [^{3}H]25-OH D$_3$, seen in the profile (Fig. 1*B*) of the extracts from vitamin D-deficient, Prednisolone®-treated animals eluted between 270 and 305 ml and after the switch in eluting solvent. Based upon this difference, a more complete study of the distribution of metabolites of [^{3}H]25-OH D$_3$ in the two types of animals was undertaken. As shown in Table 1, there was a difference in the metabolism of [^{3}H]25-OH D$_3$ in control and Prednisolone®-treated rats when the distribution of radioactivity was determined 7 hr after [^{3}H]25-OH D$_3$ was injected. The total radioactivity recovered in the various organs in the two types of experimental animals was essentially the same, indicating that Prednisolone® therapy did not alter the gross organ distribution of 25-OH D$_3$ or its metabolites. Also, the relative percentage of 25-OH D$_3$ that remained unchanged in the organs of the two types of animals was essentially the same, indicating that the overall rate of conversion of 25-OH D$_3$ was not significantly different in the two types of animals. The most striking difference was found in the intestinal extracts. About 56% of the total radioactivity

TABLE 2. *The effect of the administration of 25-OH D_3, 1,25-$(OH)_2D_3$, or polar metabolite upon intestinal calcium transport in vitamin D-deficient control and Prednisolone®-treated rats*

Experiment	Vitamin D-deficient (I/O)	Vitamin D-deficient rat given Prednisolone® (I/O)
1. Control	0.9 ± 0.1 (8)	—
25-OH D_3, 0.125 μg	2.2 ± 0.1 (5)	—
1,25-$(OH)_2$ D_3, 0.1 μg	2.7 ± 0.1 (6)	—
Polar metabolite, 0.1 μg	0.9 ± 0.1 (4)	—
Polar metabolite, 1.00 μg	1.1 ± 0.2 (5)	—
2. Control	1.0 ± 0.1 (16)	0.9 ± 0.1 (14)
25-OH D_3, 0.25 μg i.v.	2.3 ± 0.2 (5)	1.1 ± 0.1 (5)
25-OH D_3, 125 μg i.v.	2.2 ± 0.1 (7)	1.2 ± 0.1 (8)
25-OH D_3, 0.05 μg/day for 5 days orally	1.8 ± 0.2 (4)	1.4 ± 0.2 (5)
25-OH D_3, 25 μg/day for 5 days orally	2.1 ± 0.2 (6)	1.3 ± 0.2 (6)
1,25-$(OH)_2D_3$, 0.125 μg i.v.	2.7 ± 0.1 (9)	2.5 ± 0.1 (8)
1,25-$(OH)_2D_3$, 0.125 μg/day for 5 days orally	1.5 ± 0.1 (3)	1.2 ± 0.2 (5)

Calcium transport was measured by a standard method (9). The results are reported in terms of the ratio (*I/O*) of counts per min of ^{45}Ca inside (serosal medium) and outside (mucosal medium) an everted sac of small intestine. When single doses of the agents to be tested were used, the animals were given the agent intravenously (*i.v.*), and 7 hr later the upper small intestine was removed. A sac of standard length was prepared. The inverted sac was incubated with ^{45}Ca in a modified Krebs–Ringer phosphate buffer for 1 hr at 37°. Initially, the ^{40}Ca and ^{45}Ca concentrations were equal on the inside and outside of the sac. The ratio of these concentrations after 90 min of incubation was taken as a measure of calcium transport. A ratio of less than 1 (seen when rats were treated with Prednisolone®) indicates that the flux of calcium from serosal to mucosal fluids was greater in these animals than the reverse. When prolonged oral therapy was used, intestinal sacs were obtained 7 hr after the last dose of sterol.

Numbers in *parentheses* represent the number of animals in each group. Values are means ± SEM. —, not done.

found in the control intestinal extracts was 1,25-$(OH)_2D_3$, but none of the radioactivity found in the extracts of the intestine from Prednisolone®-treated animals appeared as this metabolite (Table 1). However, in these extracts a large peak of a more polar metabolite was identified. It accounted for nearly 52% of the total radioactivity. In contrast, this peak accounted for only 7% of the radioactivity found in the extracts from control animals.

In contrast to the intestinal extracts, renal and hepatic extracts from the two types of animals contained nearly equivalent amounts of 25-OH D_3 and 1,25-$(OH)_2D_3$. Extracts of bone from the Prednisolone®-treated animals contained about two-thirds of the 1,25-$(OH)_2D_3$ found in extracts from control animals, but there was no increase in the percentage of more polar metabolite(s) in extracts of bone from Prednisolone®-treated animals. The only other organ, besides the intestine, in which Prednisolone® treatment led to an increase in the percentage of more polar metabolite(s) was the kidney. In this organ the percentage of more polar metabolite(s) doubled after Prednisolone® treatment.

Characterization of Intestinal Polar Metabolite. Three aspects of this newly discovered intestinal metabolite were examined: (*i*) its biological activity; (*ii*) its sensitivity to periodate; and (*iii*) whether it was derived directly from 25-OH D_3 or from 1,25-$(OH)_2D_3$.

As shown in Table 2 (Expt. 1), administration of either 25-OH D_3 or 1,25-$(OH)_2D_3$ to vitamin D-deficient rats led to a definite increase in the calcium transport ratio (I/O) observed in subsequently isolated intestinal loops, but the polar-metabolite had no effect at a comparable or larger dose.

The more polar metabolite was treated with periodate and then again subjected to chromatography on LH20. After periodate treatment 85% of the initial radioactivity was recovered in the peak that chromatographed with untreated polar metabolite.

When [^3H]25-OH D_3 was administered to nephrectomized, vitamin D-deficient animals, there was no [^3H]1,25-$(OH)_2D_3$ found in subsequently isolated intestinal extracts. Also, when [^3H]25-OH D_3 was given to nephrectomized, Prednisolone®-treated, vitamin D-deficient animals (Fig. 1*C*), there was no polar metabolite peak found. On the other hand, when [^3H]1,-25-$(OH)_2D_3$ was given to vitamin D-deficient, Prednisolone®-treated, nephrectomized rats, both unchanged [^3H]1,25-$(OH)_2D_3$ and the more polar metabolite were found in the extracts prepared 7 hr after [^3H]1,25-$(OH)_2D_3$ administration (Fig. 1*D*).

When [^3H]1,25-$(OH)_2D_3$ was administered to vitamin D-deficient control animals and intestinal mucosal extracts were examined 7 hr later (Fig. 2*A*), nearly all the radioactivity (>90%) appeared as a single peak of unchanged [^3H]1,25-$(OH)_2D_3$. In contrast, in similar extracts prepared from Prednisolone®-treated, vitamin D-deficient animals (Fig. 2*B*) there were three distinct peaks: one at the origin, one in the position of [^3H]1,25-$(OH)_2D_3$ (about 60% of the radioactivity), and the third in the position of the more polar metabolite, (about 25% of the radioactivity). Thus, the more polar metabolite was shown to be derived from 1,25-$(OH)_2D_3$ in both control and nephrectomized, vitamin D-deficient, Prednisolone®-treated animals. Similar experiments were performed on intestinal extracts obtained from Prednisolone®-treated, vitamin D-deficient animals given [^3H]1,25-$(OH)_2D_3$ either 24 or 48 hr previously. When extracts were obtained from vitamin D-deficient, Prednisolone®-treated animals 24 hr after [^3H]1,25-$(OH)_2D_3$ injection, nearly 80% of the recoverable radioactivity was found as the more polar peak and only 10% as 1,25-$(OH)_2$-D_3 (Fig. 2*C*). When extracts were obtained from vitamin D-deficient, Prednisolone®-treated animals 48 hr after [^3H]1,25-$(OH)_2D_3$ injection, very little radioactivity remained in the intestine, but that which did was nearly all in the form of the more polar metabolite. Total radioactivity recovered in these experiments with [^3H]1,25-$(OH)_2D_3$ was 6944 dpm/g of fresh

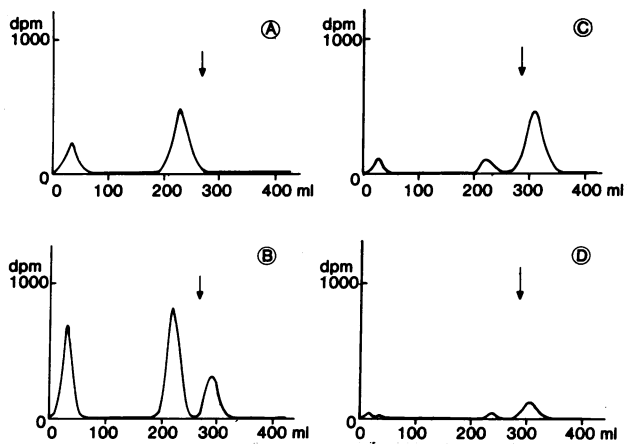

FIG. 2. Chromatographic profiles of radioactive metabolites from intestinal mucosal extracts of vitamin D-deficient rats given a single dose of [^{3}H]1,25-(OH)$_2$D$_3$ 7, 24, and 48 hr before they were killed. Separation was done on 2 × 30-cm columns of Sephadex LH20 by stepwise elution with chloroform–hexane mixtures, 250 ml of 65:35, then 150 ml of 75:25, as indicated by *arrow*. Profiles are from the extracts obtained from vitamin D-deficient animals: (*A*) given [^{3}H]1,25-(OH)$_2$D$_3$ 7 hr before they were killed; (*B*) treated with Prednisolone® and given [^{3}H]1,25-(OH)$_2$D$_3$ 7 hr before they were killed; (*C*) treated with Prednisolone® and given [^{3}H]1,25-(OH)$_2$D$_3$ 24 hr before they were killed; (*D*) treated with Prednisolone® and given [^{3}H]1,25-(OH)$_2$D$_3$ 48 hr before they were killed.

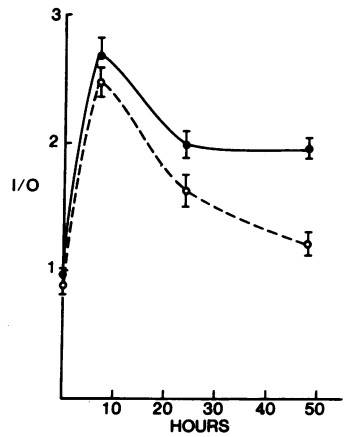

FIG. 3. A comparison of the effects of 1,25-(OH)$_2$D$_3$ treatment upon calcium transport in intestinal sacs obtained from vitamin D-deficient (*solid line*) and vitamin D-deficient, Prednisolone®-treated (*dashed line*) rats. The animals were given the vitamin D metabolites 7, 24, and 48 hr before they were killed. After decapitation, a 5-cm segment of duodenum was removed and transport ability was assessed by measuring the ratio of ^{45}Ca in serosal and mucosal fluids (*I/O*) after 90 min of incubation under standard conditions in which the initial ratio, I/O, was 1. Note that 1,25-(OH)$_2$D$_3$ was effective in increasing the ratio in both control and Prednisolone®-treated animals at 7 hr, but the action decreased more rapidly in the Prednisolone®-treated animals. Each value is a mean ± SEM.

mucosa 7 hr after injection; 4324 dpm/g at 24 hr; and 1779 dpm/g at 48 hr.

Effect of Metabolites and Analogs on Intestinal Calcium Transport. Prednisolone® treatment caused a small but significant decrease in inside/outside in vitamin D-deficient animals so that inside/outside was less than 1. When 25-OH D$_3$ was given intravenously as a single injection of either 0.25 or 125 μg per rat and intestinal sacs were prepared 7 hr later, there was a 2.2-fold increase in inside/outside in the sac from vitamin D-deficient animals, but no significant increase in inside/outside in Prednisolone®-treated, vitamin D-deficient animals receiving the smaller dose, and only a 1.3-fold increase in animals given the larger dose. Groups of vitamin D-deficient animals, either controls or Prednisolone®-treated, were also treated with daily oral doses of 25-OH D$_3$, either 0.05 μg or 25 μg per day, for 5 days (the same total dose as the single intravenous injection), and sacs were prepared at the end of 5 days. In this case 25-OH D$_3$ treatment caused an approximately 2-fold increase in inside/outside in the vitamin D-deficient group and a 1.5-fold increase in the Prednisolone®-treated animals. In contrast, when 0.125 μg of 1,25-(OH)$_2$D$_3$ were given, either as a single intravenous dose or orally for 5 days, the relative increases in inside/outside were nearly the same (a 2.7-fold increase after the intravenous and a 1.5-fold increase after the oral doses) in the vitamin D-deficient control and Prednisolone®-treated animals (Table 2).

The time course of the change in inside/outside after a single intravenous injection of 1,25-(OH)$_2$D$_3$ to vitamin D-deficient control and Prednisolone®-treated rats was also examined. The peak effect of 1,25-(OH)$_2$D$_3$ upon intestinal calcium transport was approximately the same in the two groups of animals, but the effect decreased more rapidly in the Prednisolone®-treated animals (Fig. 3).

DISCUSSION

The present results indicate that the conversion of 1,25-(OH)$_2$-D$_3$ to a more polar; biologically inactive metabolite in the intestine is one means by which large doses of adrenal corticoids alter intestinal calcium transport. This conclusion is contrary to previous conclusions (2, 3, 7). The present results concerning the effect of Prednisolone® treatment on intestinal calcium transport agree with previous results (1, 5, 8, 10) insofar as our results show that Prednisolone® blocks the increase in intestinal calcium transport induced by a single dose of 25-OH D$_3$ and partially blocks the effect of daily administered 25-OH D$_3$ (Table 2). However, our results are not in accord with those of Lukert *et al.* (7), Kimberg *et al.* (6), or Favus *et al.* (2, 3), who have reported that the amount of 1,25-(OH)$_2$D$_3$ in the intestinal mucosa is normal in Prednisolone®-treated animals and that 1,25-(OH)$_2$D$_3$ is less effective than normal in restoring calcium transport in these animals. The difference between our results and theirs (2, 3, 7) may be explained by differences in methodology. Lukert *et al.* (7) based their identification of 1,25-(OH)$_2$D$_3$ upon its relative position in a silicic acid chromatographic separation and its sensitivity to periodate. They did not verify that their "1,25-(OH)$_2$D$_3$ peak" actually contained a biologically active metabolite. It is clear from our own work that silicic acid chromatography would not distinguish between 1,25-(OH)$_2$D$_3$ and the more polar intestinal metabolite, and that periodate sensitivity cannot be used as a criterion to distinguish between them. In their studies Favus *et al.* (2) used Sephadex LH20 chromatography to separate intestinal metabolites of [^{3}H]25-OH D$_3$. From an analysis of chromatographic profiles of 25-OH D$_3$ metabolites from an intestinal nuclear fraction, they concluded that there was no difference in the recovery of radioactive 1,25-(OH)$_2$D$_3$ in control and corticoid-treated animals. However, they did not establish that the "1,25-

$(OH)_2D_3$" recovered from these extracts was biologically active. It is also necessary to point out that the doses of Prednisolone® used in the present study were quite large.

A noteworthy characteristic of the more polar metabolite, identified in the present study, is its persistence in the intestine of the Prednisolone®-treated animal (Fig. 2). This result suggests that the polar metabolite may bind to the same intestinal receptor as 1,25-$(OH)_2D_3$. Our data indicate that the polar metabolite can be derived directly from 1,25-$(OH)_2D_3$ (Figs. 1 and 2) and not from 25-OH D_3. Thus, the polar metabolite arises as a consequence of the further metabolism of 1,25-$(OH)_2D_3$ in the intestinal mucosal cells of the Prednisolone®-treated rat. This conclusion is supported by the fact that nearly normal amounts of 1,25-$(OH)_2D_3$ appear in liver, kidney, and bone extracts obtained from corticoid-treated, vitamin D-deficient animals (Table 1).

The other major difference between the present results and those of Favus *et al.* (2, 3) concerns the effectiveness of 1,25-$(OH)_2D_3$ in influencing intestinal calcium transport in the vitamin D-deficient, corticoid-treated animals. Favus *et al.* (3) showed that 1,25-$(OH)_2D_3$ did increase intestinal calcium transport in such animals, but that its effect was never as great in these animals as in appropriate controls. Our results (Fig. 3 and Table 2) show that the relative effectiveness of 1,25-$(OH)_2D_3$ in the two types of animals depends upon the time of measurement of response. When calcium transport was measured 7 hr after 1,25-$(OH)_2D_3$ was given, there was a marked increase in inside/outside in both control (vitamin D-deficient) and Prednisolone®-treated animals (Fig. 3), but there was no significant difference in response in the two types of animals. However, when measurements were made at 24 or 48 hr after 1,25-$(OH)_2D_3$ was given, the response was significantly less in the Prednisolone®-treated animals. Favus *et al.* (3) made their measurements at a single point in time, 16 hr after administration of 1,25-$(OH)_2D_3$.

The present results are of general significance to the study of vitamin D metabolism. They indicate that control of the plasma and/or tissue concentrations of 1,25-$(OH)_2D_3$ can be achieved by regulating either its rate of synthesis (15, 17, 18) or "degradation."

The results of our studies may have practical significance in clinical medicine. One of the serious complications of long-term steroid therapy is the development of osteoporosis (19). Recent animal studies have shown that a significant factor in the pathogenesis of this steroid-induced osteoporosis may be the secondary hyperparathyroidism (20) resulting from an inhibition of intestinal calcium transport (21). The present data suggest that it may be possible to prevent the steroid-induced inhibition of intestinal calcium transport by the administration of 1,25-$(OH)_2D_3$ or an appropriate analog, thereby minimizing or preventing the secondary hyperparathyroidism. If this does prove possible, then analogs or metabolites of vitamin D_3 may become an extremely useful adjunct to long-term adrenal steroid therapy in man.

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