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1,24,25-Trihydroxyvitamin D₃
A METABOLITE OF VITAMIN D₃ EFFECTIVE ON INTESTINE*

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MICHAEL F. HOLICK, ANKE KLEINER-BOSSLER, HEINRICH K. SCHNOES, PATRICIA M. KASTEN, IAIN T. BOYLE, AND HECTOR F. DELUCA‡

From the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

SUMMARY

A polar metabolite of 24,25-dihydroxyvitamin D₃ has been generated both in vivo in rats from 25-hydroxyvitamin D₃ and in vitro from 24,25-dihydroxyvitamin D₃ with chicken kidney homogenates. This metabolite has been isolated in pure form and identified as 1,24,25-trihydroxyvitamin D₃ by means of ultraviolet absorption spectrophotometry, mass spectrometry and its reactivity to periodate treatment.

1,24,25-Trihydroxyvitamin D₃ is 60% as active as vitamin D₃ in curing rickets. It is less active on a weight basis than 1,25-dihydroxyvitamin D₃ in stimulating and sustaining intestinal calcium transport and bone calcium mobilization but appears to have preferential action on the intestine.

During the past 6 years the metabolism of vitamin D₃ has been a productive area of investigation. The net result of this work has firmly established that vitamin D₃ is first hydroxylated in the liver on carbon 25 (1, 2) before it travels to the kidney to be hydroxylated either on carbon 1 (3-6) or carbon 24 (7, 8), depending on the physiological state of the animal. Under hypocalcemic or hypophosphatemic conditions the kidney produces 1,25-dihydroxyvitamin D₃ (9, 10), which then travels to the intestine and bone and apparently without further metabolic modification (11, 12) induces intestinal calcium transport and bone calcium mobilization (13-15). On the other hand, normal calcemia, hypercalcemia, and hyperphosphatemia signal the kidney to limit the production of 1,25-(OH)₂D₃ and synthesize instead 24,25-dihydroxyvitamin D₃ (24,25-(OH)₂D₃) (9, 10). The latter metabolite was originally believed to be 21,25-dihydroxyvitamin D₃ (21,25-(OH)₂D₃), but upon more thorough examination it proved to be 24,25-(OH)₂D₃.

Recently, Boyle et al. (9, 16) demonstrated that under normal or hypercalcemic conditions the major circulating metabolite of 25-hydroxyvitamin D₃ is 24,25-(OH)₂D₃. This metabolite is capable of supporting growth, elevating serum calcium, and calcifying bones of rats on a normal calcium, normal phosphorus diet similar to 1,25-(OH)₂D₃ and 25-OH-D₃. Their data clearly demonstrated that 24,25-(OH)₂D₃ is capable of inducing intestinal calcium transport at dose levels similar to 1,25-(OH)₂D₃, but has little ability to mobilize calcium from bone. Furthermore, they showed that 24,25-(OH)₂D₃ is metabolized to a more polar metabolite in the kidney and that this metabolism appears necessary for it to bring about these biological responses.

This work suggested that there might be an alternate pathway for vitamin D₃ metabolism and that the metabolite of 24,25-(OH)₂D₃ might be a tissue-specific hormone which would stimulate only intestinal calcium transport. These peculiar properties prompted an attempt to isolate the metabolite for structural identification and to study directly its biological activity. This metabolite has now been isolated in pure form from chicken kidney homogenates and has been identified as 1,24,25-trihydroxyvitamin D₃ (1,24,25-(OH)₃D₃).

Methods

General Procedures

Radioactive determinations were carried out with a Packard Tri-Carb model 3375 liquid scintillation counter equipped with an automatic external standardization system. Samples were dried in 14 × 45-mm glass vial inserts with a stream of air and dissolved in 4 ml of toluene counting solution (2 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene per 1 liter of toluene). Ultraviolet absorption spectra were recorded with a Beckman DB-G spectrophotometer, while mass spectrometric determinations were carried out with an AEI MS-9 mass spectrometer using a direct probe inlet at temperatures of 120-150° above ambient. All solvents used were of reagent grade and those utilized for chromatography in the later stages of the isolation of the metabolite were doubly distilled before use.

Preparation of 1,24,25-(OH)₃D₃ from Chicken Kidney Homogenates

One-day-old white leghorn cockeral chicks obtained from Northern Hatcheries (Beaver Dam, Wis.) were kept in cages at...
38° and fed ad libitum for 4 weeks on a corn-soy protein radiotrophic diet (17). The animals were killed, their kidneys removed and placed in a buffer solution (pH 7.4) containing 14 mm Tris acetate, 0.19 m sucrose, 1.87 mm magnesium acetate, and 5 mm succinate at 4° (18). The kidneys were teased apart to remove any extraneous connective tissue as well as testes. The kidney tissue (1 part) was homogenized with 9 parts of the buffer solution in three Potter-Elvehjem homogenizers. A homogenate (1.5 ml) was placed in a 25-ml Erlenmeyer flask and then filtered with 1 min with 100% oxygen. 24, 25-(OH)2-[26, 27-3H]D3 (0.5 μg) (specific activity 120,000 dpm per μg) was then added to the homogenate in 10 μl of 95% ethanol. The vessel was placed in a water bath shaker at 37° for 30 min. At the end of the 30 min, 3 ml of methanol (2 parts) were added to each of the flask to stop the reaction. Flask contents were combined and chloroform was added to phase separation as previously described (8, 18). (A total of 50 μg of 24, 25-(OH)2D3 was incubated in 100 separate flasks.)

After extraction the resulting yellow residue (2 g) was dissolved in 1.5 ml of 75:25:2 chloroform-Skellysolve B-methanol and applied to a glass column (2 × 30 cm) containing 15 g of Sephadex LH-20 (a hydroxypropyl ether derivative of Sephadex G-25 from Pharmacia Corp., Piscataway, N.J.) as previously described (12, 19). Sixty 3.4-ml fractions were collected and 1 μl of each fraction was used for tritium determination (Fig. 1). The peak VIb region (tubes 35 to 45) was combined and dried under nitrogen to yield 20 μg of the metabolite in 500 mg of a clear oily residue. This residue was dissolved in 100 μl of methanol and applied to a glass column (1 × 100 cm) packed to a height of 96 cm with Sephadex LH-20 in methanol (5, 8). Sixty 1.3-ml fractions were collected and 1 μl of each fraction was used for tritium determination to reveal the elution position of the metabolite. The contents of the peak tubes (39 to 45) were combined, and dried under nitrogen to give 18 μg of the metabolite in less than 1 mg of a clear lipid. The sample was dissolved in methanol and used for ultraviolet absorption spectrophotometry. The ultraviolet absorption spectrum of the sample demonstrated approximately 100 absorbance units with a λmax at 275 and a λmin at 265 nm. This sample was dissolved in 0.1 ml of 75:23:2 chloroform-Skellysolve B-methanol and applied to a glass column (1 × 150 cm) packed with Bio-Beads S-X8 (a polystyrene resin produced by Bio-Rad Corp., Richmond, Calif.), to a height of 148 cm in the same solvent as previously described (6, 8). Sixty 1.3-ml fractions were collected and 1 μl of each fraction was used for tritium determination. The peak fractions (43 to 46) were dried under nitrogen and then redissolved in methanol and used for ultraviolet absorption spectrophotometry. Based on the ultraviolet absorption spectrum it became clear that the major contaminant migrated one tube later than the metabolite. Tube 46, which contained a major amount of the contaminant, was removed from the sample and the rest of the peak tubes were combined and reapplied to the same column. This procedure was repeated three more times and the final product (13 μg) contained about 3 absorbance units with a λmax at 275 and a λmin at 265 nm. Because of the limited amount of material available it was necessary to develop two new chromatographic systems to separate the metabolite from the remaining contaminants. The sample was dissolved in 100 μl of 75:25 chloroform-Skellysolve B and applied to a column (1 × 60 cm) containing 12 g of Sephadex LH-20, slurried and developed in the same solvent. The metabolite which eluted between 215 to 275 ml was dried under nitrogen and redissolved in methanol, and its ultraviolet absorption spectrum was taken. The ultraviolet absorption spectrum still demonstrated a λmax at 275 and a λmin at 265 nm, but about 50% of the ultraviolet absorbance had been removed with this chromatographic technique. The sample was then dissolved in 65:35 chloroform-Skellysolve B and applied to a glass column (1 × 60 cm) containing 15 g of a phenyl hydroxyethyl derivative of Sephadex LH-20, slurried and developed in the same solvent. The metabolite which eluted between 195 to 225 ml was used for mass spectrometry, ultraviolet absorption spectrophotometry, and biological activity measurements.

Chemical Modifications of Metabolite

Periodate Oxidation—The metabolite (1 μg) was dissolved in 30 μl of methanol and treated with 20 μl of a 5% aqueous solution of sodium metaperiodate. After 4 hours at 22°, 50 μl of methanol were added to the reaction mixture and the sample was applied to a glass column (0.8 × 30 cm) containing 5 g of Sephadex LH-20 in methanol. One-milliliter fractions were collected and the product was found in Fraction 8 as previously described (8).

Trimethylsilylation of 1, 24, 25-(OH)2D3. The metabolite (1 μg) was dissolved in 15 μl of pyridine and reacted with 10 μl of TBT (a special combination of trimethylsilylimidazole, bistri- methylsilylamine, and trimethylchlorosilane, Pierce Chemical Co., Rockford, Ill.) at 22° for 15 min. The reaction mixture was purified on a Sephadex LH-20 column (0.8 × 30 cm) developed in methanol as described above.

Preparation of Phenyl Hydroxyethyl Sephadex LH-20

The phenyl hydroxyethyl derivative of Sephadex LH-20 (PHE Sephadex LH-20) was prepared as previously described (20). Forty grams of Sephadex LH-20 were soaked in 400 ml of methylene chloride in a 2000-ml Erlenmeyer flask. Boron trifluoride etherate (48% BF3) (10 ml) was added and thoroughly mixed by shaking. After 20 min, 20-ml portions of 20% v/v solution of phenylethylene oxide and methylene chloride were added every 10 min with shaking and then cooling the reaction flask in an ice bath. After 300 ml of the phenylethylene oxide solution had been added the reaction was allowed to proceed for another 30 min. The product was filtered on a Buchner funnel and washed with successive portions of chloroform-methanol (96% w/w) and chloroform-methanol (1:1 v/v). The product was then refluxed with stirring in chloroform-methanol (1:1) and washed with chloroform-methanol (1:1). The product was carefully washed with benzene and refluxed for a 4-hour period in benzene. After a final washing with benzene the product (64 g) was dried at room temperature.

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**Fig. 1.** Sephadex LH-20 column (2 × 30 cm packed in 75:23:2 chloroform-Skellysolve B-methanol) profile of lipid extract from kidney homogenates incubated with 24, 25-(OH)2-[26, 27-3H]D3.

![Fig. 1](http://www.jbc.org/DownloadedFromOctober14,2017)
Metabolism of 25-OH[26,27-3H]D₃

Weanling male rats (Holtzman Co., Madison, Wis.) were fed an adequate calcium and phosphorus, vitamin D-deficient diet for 3 weeks (9) and were supplemented with 0.05 µg of vitamin D₃ orally during the last week. Three rats received 312 pmoles of 25-OH[26,27-3H]D₃ intrajugularly in 0.05 ml of 95% ethanol. Forty-eight hours later the animals were killed and the blood collected and extracted as previously described (21). The lipid extract was dissolved in 75:25:2 chloroform:Skellosolve B-methanol and applied to a glass column (1 × 60 cm) containing 12 g of Sephadex LH-20 slurred and developed in the same solvent.

Biological Assays

Weanling male rats (Holtzman Co., Madison, Wis.) were housed in overhanging wire cages and fed the adequate calcium and phosphorus, vitamin D-deficient diet for 2 weeks and for an additional week the low calcium (0.02%), vitamin D-deficient diet (22). At the end of the third week the rats weighed approximately 100 to 110 g and had an average serum calcium concentration of 4.5 mg/100 ml.

Intestinal Calcium Transport Assay

Groups of six rats received either 62.5 pmoles of 1,25-(OH)₂D₃ or 1,24,25-(OH)₃D₃ intraocularly in 0.05 ml of 95% ethanol while the controls received only EtOH. At the desired time after administration, the animals were decapitated and the blood and duodena were collected. The duodena were prepared according to the procedure of Martin and DeLuca (23) for measuring intestinal calcium transport activity by the everted gut sac technique. Samples from both the inside and outside (100 µl) of the intestinal sac were spotted on filter paper discs, dried, and placed in 20-ml counting vials containing 10 ml of scintillation counting solution.

Bone Calcium Mobilization

The blood from the rats was centrifuged and 0.1 ml of serum was mixed with 1.9 ml of 0.1% lanthanum chloride solution. Serum calcium concentration was determined with a Perkin-Elmer atomic absorption spectrometer, model 403.

Antirachitic Activity Assay

Rachitic rats (24) were given 6.5 pmoles and 62.5 pmoles of 1,24,25-(OH)₃D₃ and 1α,25-(OH)₂D₃, intrajeugularly in 0.05 ml of 95% EtOH or just 0.05 ml of 95% EtOH for 5 days. Two days after the last dose the animals were killed, and their radii and ulnae were removed and stained with 15% silver nitrate and ulnae were removed and stained with 15% silver nitrate.

RESULTS

The results of Boyle et al. (16) demonstrating that 24,25-(OH)₂D₃ could be metabolized by the kidney to a more polar metabolite prompted an investigation to see whether this more polar metabolite could be generated from 25-OH-D₃ under circumstances where both the 25-OH-D₃-24-hydroxylase and 25-OH-D₃-1-hydroxylase were active. Animals fed a normal calcium and phosphorus diet supplemented with vitamin D were dosed with tritiated 25 OH-D₃, and 48 hours later the blood profile (Fig. 2) demonstrated that the 25-OH-D₃ was metabolized to 24,25-(OH)₂D₃ and 1,25-(OH)₂D₃ as well as two more polar metabolites. The metabolite designated as Peak VIb was found to co-chromatograph on a 75:25:2 CHCl₃-Skellosolve B-MeOH Sephadex LH-20 column with the more polar metabolite of 24,25-(OH)₂D₃ described by Boyle et al. (16). It was interesting to note, however, that very small amounts of this metabolite (less than 0.1 ng per ml of blood) were observed. It was clear from these results that this would not be a very good source of the metabolite for isolation and identification.

Since chicken kidney homogenates have been successfully used in preparing 1,25-(OH)₂D₃ from 25-OH-D₃, it was conceivable that the 25-OH-D₃-1-hydroxylase enzyme could also hydroxylate 24,25-(OH)₂D₃. Chicken kidney homogenates were prepared and incubated with 24,25-(OH)₂D₃. The results clearly demonstrated that 24,25-(OH)₂D₃ is metabolized to a more polar metabolite which migrates with Peak VIb from recovered rat plasma of animals that had received either 25-OH-D₃ or 24,25-(OH)₂D₃. Furthermore, it appeared that the maximum amount of this more polar metabolite was generated after 30 min of incubation and that this metabolite could also be generated with chicken kidney mitochondria prepared as previously described (18).

Identification of Metabolite as 1,24,25-(OH)₃D₃

The ultraviolet absorption spectrum of the metabolite λ max 265 nm was similar to those previously reported for the 5,6-cis triene system of vitamin D and its metabolites (1, 4, 5, 8). The mass spectrum of the metabolite and its trimethylsilyl ether derivative (Fig. 3, A and B) showed molecular ion peaks at m/e 432 and 720, demonstrating that an additional hydroxyl function had been incorporated into 24,25-(OH)₃D₃. The fragments of m/e 432 (287, 269 (287.H₂0), 24,25-(OH)₃D₃ intrajugularly 48 hours earlier. The column was stripped with 1:1 CHCl₃-MeOH.

FIG. 2. Sephadex LH-20 column (1 × 60 cm packed in 75:25:2 CHCl₃-Skellosolve B-methanol) profile of a lipid extract from blood of rats that received 312 pmoles 25-OH[26,27-3H]D₃ intrajugularly 48 hours earlier. The column was stripped with 1:1 CHCl₃-MeOH.

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tional oxygen function in ring A. The compelling evidence for the 5,6-cis triene system (λ_max 265 for the metabolite) eliminated carbon 6 and carbon 7 as possible sites for hydroxyl substitution.

Treatment of the metabolite with periodate provided essential information. The mass spectrum of the periodate product showed a molecular ion peak at m/e 372 corresponding to a C-24 aldehyde which results from the C-24,C-25 periodate cleavage (Fig. 3C). This demonstrated that the 24,25-hydroxyl functions are present in the metabolite. Furthermore peaks at m/e 152 and 134 firmly established that the additional hydroxyl function in ring A must be on carbon 1 since an additional hydroxyl function on either C 4 or C 2 would be vicinal to the hydroxyl on carbon 3, and thus be sensitive to periodate oxidation (4, 5).

These data firmly establish that the additional oxygen function is on carbon 1 and therefore the structure of this metabolite is 1,24,25-(OH)_3D<sub>3</sub>.

Fig. 4 shows that 62.5 pmoles of 1,24,25-(OH)_3D<sub>3</sub> are capable of eliciting an intestinal calcium transport response 6 hours after administration and a maximum response at 18 hours, while 62.5 pmoles of 1,25-(OH)_2D<sub>3</sub> show a maximum response at 6 hours. Furthermore, 62.5 pmoles of 1,24,25-(OH)_3D<sub>3</sub> were less active on a weight basis than 1,25-(OH)_2D<sub>3</sub> during the entire time observed, and the maximum response of this metabolite began to decline at 72 hours, and by 96 hours was near control levels. On the other hand, the response induced by 1,25-(OH)_2D<sub>3</sub> in the intestine was sustained at 96 hours and only showed signs of declining at 144 hours after administration.

Fig. 5 illustrates the ability of 1,24,25-(OH)_3D<sub>3</sub> to mobilize calcium from the bone. There is a small but significant rise in serum calcium (increase of 0.5 mg%) presumably due to bone calcium mobilization at 24, 48, and 72 hours and at 96 hours was down to control values. In comparison, 1,25-(OH)_2D<sub>3</sub> showed a rapid response with a marked rise in serum calcium at 6 hours (an increase of 2 mg%) with a sustained 1 mg% increase for 72 hours after which time the serum calcium dropped to control values. The biopotency of this polar metabolite in comparison to 1,25-(OH)_2D<sub>3</sub> was also reflected in the calcification results (Table I). The trihydroxy metabolite is about 60% as active as vitamin D<sub>3</sub> and 20 times less active than 1,25-(OH)_2D<sub>3</sub> in its ability to heal rachitic lesions.
therefore, making the polar metabolites elute in less volume than
the basis of molecular weight, was useful in removing greater
the phenyl hydroxyethyl groups decreased the polarity of the gel,
metabolite, probably because of the high degree of polarity that
ally ineffective. Even when the methanol was decreased lo-fold
75: 23 : 2 chloroform-Skellysolve B-methanol Sephadex LH-20
chromatographic system was not useful in the purification of this
ently had a molecular weight similar to the metabolite. The
cation. A major contaminant in the preparation had an ultra-
violet absorption spectrum (X,,, 275 and Xmin 265) and appar-
response by the line test method (United States Pharmacopoeia,
957, EtOH intraperitoneally every 24 hours for 5 days. After
pmoles of either 1,24,25-(OH),D, or 1,25-(OH),Da in 0.05 ml of
Vitamin D3 ............................
1,24,25-(OH)3D3 ................................
1,25-(OH),Da ............................

Table I
Antirachitic activity of 1,24,25-(OH),Da, 1a,25-(OH),Da, and vitamin D3

Rats were fed a high calcium (1.2%) low phosphorus (0.3%) diet for 3 weeks prior to the experiment. They were divided into
four groups of eight rats. Each rat in the control group received
0.05 ml of 95% EtOH, each rat in another group received 4 i.u.
of vitamin D3, and each animal in the other groups received 0.5
pmoles of either 1,24,25-(OH),Da or 1,25-(OH),Da in 0.05 ml of
95% EtOH intraperitoneally every 24 hours for 3 days. After
an additional 2 days the rats were killed and used for antirachitic assay by the line test method (United States Pharmacopoeia,
1935).

\[
\begin{array}{|c|c|}
\hline
\text{Compound} & \text{Antirachitic activity} \\
\hline
\text{EtOH} & 0 \\
\text{Vitamin D3} & 40 \\
1,25-(OH),Da & 400 \\
1,24,25-(OH),Da & 24 \\
\hline
\end{array}
\]

Discussion

The polar metabolite of 24,25-(OH),Da first described by
Boyle et al. (16) has been synthesized in vitro from chicken kidney
homogenates and identified as 1,24,25-(OH),Da. The incor-
poration of an additional hydroxyl function into the parent
24,25-(OH),Da structure provided special problems in its purifi-
cation. A major contaminant in the preparation had an ultra-
 violet absorption spectrum (Xmax 275 and Xmin 265) and ap-
parently had a molecular weight similar to the metabolite. The
75:23:2 chloroform-Skellysolve B-methanol Sephadex LH-20
chromatographic system was not useful in the purification of this
metabolite, probably because of the high degree of polarity that
methanol imparted making the liquid-gel partition system virtu-
ally ineffective. Even when the methanol was decreased 10-fold
no further purification was observed. Instead, Bio-Beads S-X8
molecular sieve chromatography, which separates compounds on
the basis of molecular weight, was useful in removing greater
than 90% of the contaminants. However, because of the limited
amount of material it was also necessary to develop two new
chromatographic systems. Sephadex LH-20 slurried and de-
veloped in 75:25 chloroform-Skellysolve B proved to be useful in
the further purification of the metabolite. The phenyl hy-
droxyethyl derivative of Sephadex LH-20 was also used in the
purification of this metabolite. It appeared that the addition of
the phenyl hydroxyethyl groups decreased the polarity of the gel,
therefore, making the polar metabolites elute in less volume than
would be expected for Sephadex LH-20. Furthermore, both of
these chromatographic systems are capable of separating the mono-
,di-, and trihydroxy derivatives of vitamin D3. In com-
parison, the 65:35 chloroform-Skellysolve B Sephadex LH-20
chromatographic system is effective in eluting the mono- and
dihydroxy derivatives of vitamin D but retains the trihydroxy
metabolites (19).

That the peak VIb metabolite proved to be 1,24,25-(OH),Da
was expected. Based on the ultraviolet absorption spectrum
(λmax 265) and mass spectrum of the metabolite (M = 432) and
fractograms m/e 257, 269, and 251 and its trimethylsilyl ether
derivative M = 720 and fragment m/e 200, it was clear that the
5,6-cis triene system had remained intact and that an insertion
of a hydroxyl function had occurred in the A ring of the precursor
24,25-(OH),D4. The sensitivity of the side chain to periodate
left little doubt that the original vicinal hydroxyls on C-24 and
C-25 had remained intact. Furthermore the fragments at m/e
152 and 134 in the mass spectrum were unchanged from that
seen for 1,25-(OH),D3 establishing that the additional hydroxyl
function was on carbon 1. The mass spectrum of the metabolite
showed relatively intense fragments at m/e 140 and m/e 105,
and the mass spectrum of the trimethylsilyl ether derivative
showed a fragment at m/e 147. The former peaks were observed
also in the solvent coming from Sephadex LH-20 columns and
are probably due to phthalates and related contaminants. The
peak at m/e 147 ((CH3)3Si—O—Si(CH3)3) is commonly observed
in the spectra of silyl derivatives and can be attributed to the
reagent or intramolecular silyl transfer during fragmentation.

The mass spectrum (Fig. 3B) of the trimethylsilyl ether deriv-
aive compares well with that of silylated 24,25-(OH),D3 reported
by us earlier (8), although differences in the relative abundance
of certain fragments are apparent. For example, the peaks cor-
responding to M-HOSi(CH3)3 (m/e 630) and M-131 (m/e 599)
as well as M-191-HOSi(CH3)3 (m/e 499) are relatively prominent
in Fig. 3B, whereas the analogous fragments in the spectrum of
24,25-(OH),D3-trimethylsilyl ether gave rise to peaks of rather
low intensity.

The biological activity of 1,24,25-(OH),Da parallels the bio-
logical activity reported for 24,25-(OH),Da (10). It is pref-
entially more active in inducing intestinal calcium transport
than mobilizing calcium from bone and is about 60% as effective
as vitamin D3 in the cure of rachitic lesions. Although on a
weight basis it is less active both in the magnitude of the response
and in its duration than 1,25-(OH),Da, it is probably much more
active than its precursor 24,25-(OH),Da. This has not been
examined in the present report. It is interesting, however, that
1,24,25-(OH),Da is preferentially active on the intestine, and
may therefore be useful in the treatment of kidney diseases in
which an influx of calcium from the intestine is preferred to an
influx of calcium at the expense of bone mobilization.

The long acting intestinal calcium transport response observed
for 62.5 pmoles of 1,25-(OH),Da was quite unexpected. It has
been previously reported that in chickens 1,25-(OH),Da elicits
a maximum response at 9 hours, and by 24 hours the response
diminishes considerably (17, 26, 27). However, careful ex-
amination of the data reveals that the transport in those cases
had not returned to deficient levels. It now appears that in
rats on a low calcium diet, 1,25-(OH),Da given as a single pulse
(62.5 pmoles) is capable of maintaining a response as long as
96 hours after administration and diminishes only slightly after
144 hours. The surprising long lasting effect of 1,25-(OH),Da
has been noted in man (28) and in dogs (29). This interesting
phenomenon certainly merits additional investigation in view of

Fig. 5. Bone calcium mobilization response of vitamin D-
deficient rats (22) to either 62.5 pmoles of 1,25-(OH),Da (A) or
1,24,25-(OH),Da (B). The vertical bars represent the standard
error of the mean for six animals.
the rapidity with which intestinal cells turnover. Although 1, 24, 25-(OH)D₃ is not as effective as 1, 25-(OH)D₂ in its ability to induce intestinal calcium transport or heal rickets, this does not rule out the possibility that 1, 24, 25-(OH)D₃ may have some significant physiological role in other tissues or under circumstances where 1, 25-(OH)D₂ is not readily available.

Fig. 2 demonstrates that 1, 24, 25-(OH)D₃ is produced in animals that have both the 24-hydroxylase and 1-hydroxylase operative in the kidney, although at this time it is not possible to say whether the 24, 25-(OH)D₃ made by the kidney is further hydroxylated by the 1-hydroxylase or that the 1, 25-(OH)D₂ is further hydroxylated by the 24-hydroxylase. In any case, this may be another pathway by which the animal can call on the reserve of 24, 25-(OH)D₃ to make it biologically active.

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1,24,25-Trihydroxyvitamin D$_3$ : A METABOLITE OF VITAMIN D3 EFFECTIVE ON INTESTINE
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