Effect of age on the conversion of 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3 by kidney of rat.

H J Armbrecht, … , T V Zenser, B B Davis

J Clin Invest. 1980;66(5):1118-1123. https://doi.org/10.1172/JCI109941.

Research Article

The decreased absorption of calcium by the small intestine of the adult may reflect changes in vitamin D metabolism with age. The purpose of this study was to compare the capacity of young (1.5 mo of age) and adult (12 mo of age) vitamin D-deficient rats to convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, the physiologically active form of vitamin D. Young rats responded to an oral dose of 25-hydroxyvitamin D3 with significantly increased intestinal absorption of calcium and a three-fold increase in the intestinal content of vitamin D-stimulated calcium-binding protein. Adult rats showed no significant increase in these parameters. The conversion of 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3 was measured in the whole animal by administering a dose of tritiated 25-hydroxyvitamin D3 and determining the appearance of tritiated metabolites in plasma and small intestine. In the adult rat, only 2.1 +/- 0.6% of the plasma radioactivity was in the form of 1,25-dihydroxyvitamin D3 after 24 h compared with 20.8 +/- 3.0% in the young. The conversion of tritiated 25-hydroxyvitamin D3 to its products was also measured directly in isolated slices of renal cortex. 1,25-Dihydroxyvitamin D3 production by adult renal slices was found to be less than one-tenth that of slices from the young. These results indicate that there is a marked decrease in the capacity of the vitamin D-deficient adult rat […]

Effect of Age on the Conversion of 25-Hydroxyvitamin D_3 to 1,25-Dihydroxyvitamin D_3 by Kidney of Rat

H. J. ARMBRECHT, T. V. ZENSER, and B. B. DAVIS, *Geriatric Research, Education and Clinical Center, Veterans Administration Medical Center, St. Louis, Missouri 63125; Departments of Medicine and Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63106*

ABSTRACT The decreased absorption of calcium by the small intestine of the adult may reflect changes in vitamin D metabolism with age. The purpose of this study was to compare the capacity of young (1.5 mo of age) and adult (12 mo of age) vitamin D-deficient rats to convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, the physiologically active form of vitamin D. Young rats responded to an oral dose of 25-hydroxyvitamin D_3 with significantly increased intestinal absorption of calcium and a three-fold increase in the intestinal content of vitamin D-stimulated calcium-binding protein. Adult rats showed no significant increase in these parameters. The conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 was measured in the whole animal by administering a dose of tritiated 25-hydroxyvitamin D_3 and determining the appearance of tritiated metabolites in plasma and small intestine. In the adult rat, only 2.1±0.6% of the plasma radioactivity was in the form of 1,25-dihydroxyvitamin D_3 after 24 h compared with 20.8±3.0% in the young. The conversion of tritiated 25-hydroxyvitamin D_3 to its products was also measured directly in isolated slices of renal cortex. 1,25-Dihydroxyvitamin D_3 production by adult renal slices was found to be less than one-tenth that of slices from the young. These results indicate that there is a marked decrease in the capacity of the vitamin D-deficient adult rat to convert 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3. This is probably due to the decreased capacity of the adult kidney to 1-hydroxylate 25-hydroxyvitamin D_3. These studies also demonstrate the usefulness of renal slices in measuring changes in the renal conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 in the mammal.

This study was presented in part at the Annual Meeting of the American Federation for Clinical Research, Washington D. C., May 1980, and was published in abstract form in 1980. (*Clin. Res.* **28**: 384A.)

Received for publication 23 April 1980 and in revised form 7 July 1980.

INTRODUCTION

Several studies have shown that there is a decrease in the intestinal absorption of calcium with age in humans (1–3). The intestinal absorption of calcium is mediated by vitamin D and its metabolites in the young (4). Therefore, changes in calcium absorption with age may reflect changes in vitamin D metabolism with age. To stimulate calcium absorption by the intestine, vitamin D must first be hydroxylated by the liver to form 25-hydroxyvitamin D (25-OH-D).[1] 25-OH-D is further metabolized to either 1,25-dihydroxyvitamin D (1,25-$[OH]_2$-D) or 24,25-dihydroxyvitamin D (24,25-$[OH]_2$-D) by the kidney. The 1,25-$(OH)_2$-D shows the greatest potency in stimulating intestinal absorption of calcium, and it is considered the major hormonal form of the vitamin (5). In young animals, the conversion of 25-OH-D to 1,25-$(OH)_2$-D by the kidney is enhanced by feeding a low calcium diet or a vitamin D-deficient diet (6).

There is circumstantial evidence that there are changes in vitamin D metabolism with age. It has been shown in humans that serum 1,25-$(OH)_2$-D_3 levels decrease with age (7). The decrease in serum 1,25-$(OH)_2$-D_3 levels is paralleled by a decrease in calcium absorption. In the same study, the serum 25-OH-D_3 levels were found to remain constant with age (7). The mechanism responsible for the decrease in the steady-state level of 1,25-$(OH)_2$-D_3 with age is not known. The decrease may be due to decreased 1,25-$(OH)_2$-D_3 production by the kidney or increased 1,25-$(OH)_2D_3$ catabolism with age.

The first purpose of the present study was to compare the response of the young and adult rat to 25-OH-D_3 in terms of intestinal absorption of calcium. The second purpose of the study was to compare the conver-

[1] *Abbreviations used in this paper*: CaBP, calcium binding protein; 1,25-$(OH)_2$-D, 1,25-dihydroxyvitamin D; 24,25-$(OH)_2$-D, 24,25-dihydroxyvitamin D; 25-OH-D, 25-hydroxyvitamin D; HPLC, high-pressure liquid chromatography.

sion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3 in the mammalian kidney of young and adult rats. The rat was used because the intestinal absorption of calcium (8), the plasma levels of 1,25-$(OH)_2$-D_3 (9), and the intestinal levels of the vitamin D-stimulated calcium-binding protein (CaBP) (8, 10) all decrease with age in the rat. The results of these experiments indicated that there was a marked decrease in the intestinal response of the vitamin D-deficient adult rat to 25-OH-D_3. The lack of intestinal response was due in large part to decreased 1,25-$(OH)_2$-D_3 production by the adult rat. This was probably due to the decreased capacity of the adult kidney to convert 25-OH-D_3 to 1,25-$(OH)_2$-D_3.

METHODS

Rats and diets. Male F344 rats aged 4 wk (young) or 12 mo (adult) were purchased from Charles River Breeding Laboratories, Wilmington, Mass. Rats were initially fed Purina rodent laboratory chow (Ralston Purina Co., St. Louis, Mo.). Their plasma 25-OH-D_3 levels on this diet were 15.5 ng/ml for the young (average of 11 rats) and 15.6 ng/ml for the adult (average of 9 rats). Plasma 25-OH-D_3 was measured using the technique of Shepard et al. (11). The lower limit of detectability for this assay was 3 ng/ml, and the estimated standard deviation of the assay was 3.1 ng/ml. Rats were made vitamin D-deficient by feeding a vitamin D-deficient diet (Teklad test diet 79093, Teklad Test Diets, Madison, Wis.) containing 0.02% calcium and 0.30% phosphorus for 6 wk. Rats were housed in the dark and allowed diet and deionized, distilled water ad lib. After 6 wk, no 25-OH-D_3 could be detected in the plasma of young or adult rats. Rats on this diet also exhibited low levels of active transport of calcium by the intestine, and they had only small amounts of vitamin D-stimulated CaBP in the intestine. Other studies have also reported residual amounts of CaBP in vitamin D-deficient rats (12, 13).

Measurement of intestinal calcium absorption and CaBP. To test the effect of 25-OH-D_3, vitamin D-deficient rats received 30 ng/g body weight 25-OH-D_3 in propylene glycol via stomach tube. At various times after dose, rats were killed by decapitation; and the intestinal absorption of calcium and the intestinal CaBP content were measured. Active transport of calcium was measured using the everted gut sac technique as described previously (8). In this method, the distribution of ^{45}Ca on the inside (serosal) and outside (mucosal) of the everted intestinal sac was measured after 1.5 h of incubation. The ratio of the serosal (S) to mucosal (M) radioactivity was calculated and called the S/M ratio. The CaBP content of the intestinal mucosa was assayed as previously described (8). After incubation, the mucosa was scraped from the everted sac and homogenized in 2 ml homogenizing buffer. The homogenate was centrifuged, and the supernate was assayed for CaBP by single radial immunodiffusion, using specific antisera to rat intestinal CaBP (12). A linear relationship was obtained between the area of the circular immunoprecipitate minus the area of the well and the CaBP concentration (5–37 μg/ml). In this assay, the average interassay variability of identical samples was 12.6%, and the lower limit of detection was 100 ng. Plasma calcium was measured by a fluorometric technique (14), and plasma phosphorus was measured by the method of Fiske and SubbaRow (15).

Measurement of 25-OH-D_3 metabolism in the intact rat. Vitamin D-deficient rats were injected intraperitoneally with tritium-labeled 25-OH-D_3 (25-OH-[26,27-^3H]-D_3) purchased from Amersham Corp. (Arlington Heights, Ill.) or New England Nuclear (Boston, Mass.). Purity of radioactive material was monitored by high-pressure liquid chromatography (HPLC) and found to be 98% or greater. Rats were injected with 0.30 μCi/100 g body wt in 0.1 ml ethanol. The specific activity of the tritiated 25-OH-D_3 was adjusted to 9 Ci/mmol using unlabeled 25-OH-D_3 obtained from Dr. J. C. Babcock (Upjohn Co., Kalamazoo, Mich.).

24 h after dosing, blood was drawn from the rats by heart puncture. Preliminary experiments showed that the conversion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3 was maximal at this time in young rats. The 5 cm of the small intestine distal to the pylorus was also removed. The small intestine was everted, the mucosa scraped from the muscle layer and homogenized in 2 ml of saline. Plasma samples and the intestinal homogenate were extracted twice using 3.75 vol of methanol/methylene chloride (2:1, vol/vol) followed by 1.25 vol of methylene chloride to produce a phase separation (11). The combined methylene chloride extracts were evaporated under nitrogen, and the residues were solubilized in 1 ml hexane/chloroform/methanol (9:1:1).

The tritium-labeled vitamin D metabolites were isolated according to the general procedures of Shepard et al. (11). The lipid extracts were chromatographed on a column (0.6 × 12 cm) of Sephadex LH-20 in hexane/chloroform/methanol (9:1:1). The first 10 ml contained tritiated 25-OH-D_3, and the second 14 ml contained the tritiated 24,25-$(OH)_2$-D_3 and 1,25-$(OH)_2$-D_3. This second 14-ml fraction was evaporated, redissolved in 0.05 ml of 2-propanol/hexane (1:9), and rechromatographed using HPLC to separate the 24,25-$(OH)_2$-D_3 from the 1,25-$(OH)_2$-D_3. The rechromatography took place on a Zorbax-SIL column (E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.) equilibrated with 2-propanol/hexane (1:9) at a flow rate of 2.6 ml/min. 1-ml fractions were collected and evaporated, and the amount of radioactivity was determined by scintillation counting. Results were expressed as the percentage of radioactivity found in each metabolite compared with the total amount of radioactivity extracted from the sample.

Efficiency of extraction of the tritiated metabolites was measured in separate experiments and was found to be similar for each age group. The recovery of tritiated 25-OH-D_3 from the plasma was 78.4±1.0% in the young and 74.4±0.8% in the adult, and the recovery of tritiated 1,25-$(OH)_2$-D_3 was 76.5±0.6% and 72.7±1.9% in young and adult rats, respectively. Intestinal recovery of 25-OH-D_3 was 81.6±1.4% in young and 77.4±1.7% in adult, and the recovery of 1,25-$(OH)_2$-D_3 was 68.5±0.9% and 68.4±1.0%, respectively.

Production of vitamin D metabolites by renal slices. Kidneys were removed from rats anesthetized with diethyl ether, and the kidneys were placed in ice-cold saline. The renal capsules were removed, and slices of cortex were prepared with a Stadie-Riggs microtome. Slices were stored in 30 ml of cold saline before incubation. They were then weighed and incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1 mg/ml of glucose, 2.5 μM 25-OH-D_3, and tritiated 25-OH-D_3 (0.04–0.08 Ci/mmol). In a typical experiment, 150–250 mg of slices were incubated in 1 ml of buffer with 95% O_2, 5% CO_2 as the gas phase. After 1 h of incubation in a shaking water bath at 37°C, the slices were removed from the buffer and homogenized in 1 ml of deionized, distilled water. The whole homogenate was extracted and analyzed for tritiated vitamin D metabolites using HPLC. Preliminary experiments indicated that the vitamin D metabolites produced by the slices remained in them and were not released into the incubation media. Production of vitamin D metabolites by renal slices was expressed per milligram slice weight per hour. The production of 1,25-$(OH)_2$-D_3 by renal cortical slices was

found to be linear with tissue weight between 40–250 mg and with incubation time from 30 to 90 min.

The homogenates of renal slices were extracted twice using methanol/methylene chloride (11), and the extracts were prepared for HPLC chromatography by passing them through an LH-20 column (0.6 × 3 cm) equilibrated with hexane/chloroform/methanol (9:1:1). The eluent was evaporated and redissolved in 0.1 ml of the HPLC solvent system of hexane/methanol/methylene chloride (8:1:1). Extracts (0.05 ml) were then chromatographed on a Zorbax-SIL column (0.6 × 25 cm). The flow rate was 1.1 ml/min; and 1-min fractions were collected, evaporated, and assayed for radioactivity using scintillation counting. These extraction and chromatographic procedures resulted in the recovery of 79–84% of the tritiated 25-OH-D_3 and 70–74% of the tritiated 1,25-$(OH)_2$-D_3 present in the original homogenate.

Statistics. The data from these experiments are reported as the mean±SEM. Statistical analyses were performed using Student's two-tailed *t* test, and a confidence level of 95% or greater was considered significant (16).

RESULTS

The capacity of young and adult vitamin D-deficient rats to increase the active transport of calcium by the intestine and the intestinal content of CaBP in response to an oral dose of 25-OH-D_3 was compared. In the young rat, active transport was significantly elevated over the 0 h control at 12, 24, and 48 h after dosing (Fig. 1). The adult rats showed no significant increase in calcium transport at the same times after an equivalent dose (per gram body weight) of 25-OH-D_3. The intestinal CaBP content of the young rats was increased >threefold by 25-OH-D_3 at 48 h after dose (Fig. 2). However, 25-OH-D_3 produced only a slight increase in CaBP content in the adult after 48 h. In the same experiments, the capacity of 25-OH-D_3 to increase plasma calcium and phosphorus levels was measured. After 12 h, 25-OH-D_3 significantly increased plasma calcium by 0.8 mg/100 ml and plasma phosphorus by 1.8 mg/100 ml in young rats. However, no significant increase in plasma calcium and phosphorus was observed in adult rats.

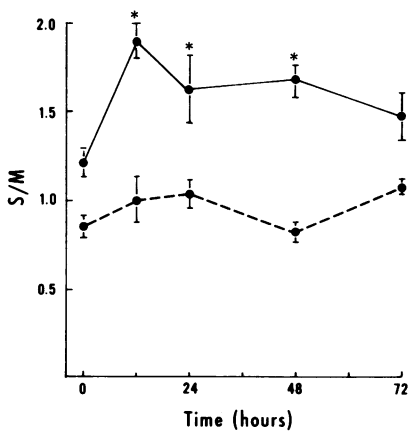

FIGURE 1 Changes in active transport of calcium serosal to mucosal ratio (S/M) with time after oral administration of 25-OH-D_3 (30 ng/g body wt) to young (● —— ●) and adult (● - - - ●) vitamin D-deficient rats. Data are expressed as the mean±SEM of four to eight rats. An asterisk indicates that transport was significantly increased above zero hour control (propylene glycol only).

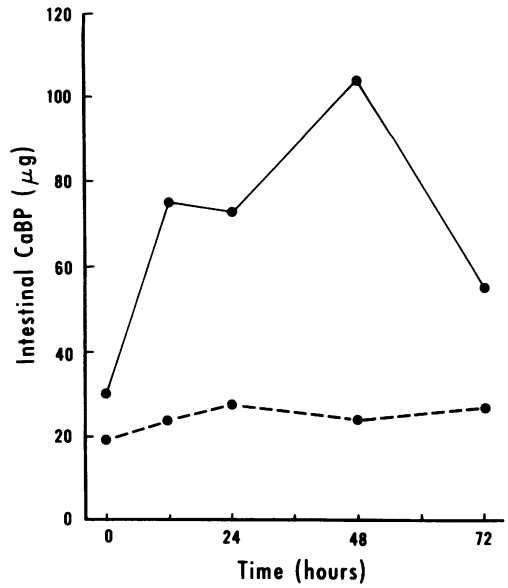

FIGURE 2 Changes in intestinal CaBP content with time after 25-OH-D_3 administration in young (● —— ●) and adult (● - - - ●) vitamin D-deficient rats. Total CaBP content of the first 5 cm of duodenum was quantified using radial immunodiffusion. CaBP content was measured in pooled intestinal supernates from the same segments used to measure active calcium transport (Fig. 1). The wet weights of the duodenal segments were 540±13 (n = 24) mg/5 cm for the young and 746 ±10 (n = 19) mg/5 cm for the adult.

Since the oral administration of 25-OH-D_3 had little effect in the adult compared with the young rat, a study was made of the metabolism of 25-OH-D_3 in young and adult rats. Vitamin D-deficient rats were injected intraperitoneally with tritiated 25-OH-D_3. 24 h after injection, tritiated vitamin D metabolites were isolated from plasma and intestine using LH-20 chromatography and HPLC. In the young rat, 20.8 ±3.0% of the plasma radioactivity was recovered as 1,25-$(OH)_2$-D_3 as compared with 2.1±0.6% in the adult (Table I). There was no significant difference between young and adult in the percentage of radioactivity in the 24,25-$(OH)_2$-D_3 fraction. The intestine of the young rat contained 58.4%±4.1% of the radioactivity as 1,25-$(OH)_2$-D_3 compared with only 2.2±1.6% in the adult. In terms of radioactivity, the young rat intestine contained 816±227 cpm as 1,25-$(OH)_2$-D_3 per gram wet weight mucosa, but the adult intestine contained only 58.5±29.9 cpm per gram mucosa as 1,25-$(OH)_2$-D_3.

The lack of appearance of tritiated 1,25-$(OH)_2$-D_3 in the plasma and intestine of the adult was consistent with a decreased conversion of 25-OH-D_3 to 1,25-

TABLE I
*Effect of Age on Percentage of Radioactivity in Vitamin D Metabolites after [^3H]25-OH-D_3 Injection**

	25-OH-D_3	24,25-$(OH)_2$-D_3	1,25-$(OH)_2$-D_3	Other
Plasma				
Young	76.1±3.3	0.7±0.2	20.8±3.0	2.4±1.2
Adult	94.3±1.3‡	2.1±0.6	2.1±0.6‡	1.3±0.6
Intestine				
Young	37.5±5.1	0	58.4±4.1	4.6±1.9
Adult	86.6±10.1‡	7.8±7.8	2.2±1.6‡	3.6±1.5

* Numbers are percentage of radioactivity found in each metabolite 24 h after injection with [^3H]25-OH-D_3. Percentage is based on total amount of radioactivity extracted from samples. Numbers are mean±SEM of four animals. Young (1.5 mo of age) and adult (12 mo of age) rats were placed on a vitamin D-deficient, low calcium diet for 6 wk before experiment.
‡ Significantly different from corresponding young value ($P < 0.05$).

$(OH)_2$-D_3 by the adult kidney. Therefore, the capacity of isolated renal slices to convert 25-OH-D_3 to 1,25-$(OH)_2$-D_3 was measured in slices from young and adult animals. Renal cortical slices were incubated with tritiated 25-OH-D_3 for 1 h, and the tritiated metabolites produced by the slices were isolated using HPLC. After a 1-h incubation, slices from young rats contained significant amounts of tritiated 1,25-$(OH)_2$-D_3 in addition to the 25-OH-D_3 (Fig. 3, top). Slices from adult rats produced almost no detectable 1,25-$(OH)_2$-D_3 when incubated under the same conditions (Fig. 3, bottom). Little 24,25-$(OH)_2$-D_3 was seen in slices from either age group under these conditions. Because production of 1,25-$(OH)_2$-D_3 was found to be linear with time and tissue weight, 1,25-$(OH)_2$-D_3 production was quantitated on a per milligram wet weight per hour basis (Table II). The rate of 1,25-$(OH)_2$-D_3 production was ~10-fold greater in vitamin D-deficient young rats compared with vitamin D-deficient adult rats. 1,25-$(OH)_2$-D_3 production was markedly reduced when young rats were fed a vitamin D-replete, high Ca diet (3 IU vitamin D/g diet, 0.6% Ca) or when slices were heat-treated before incubation.

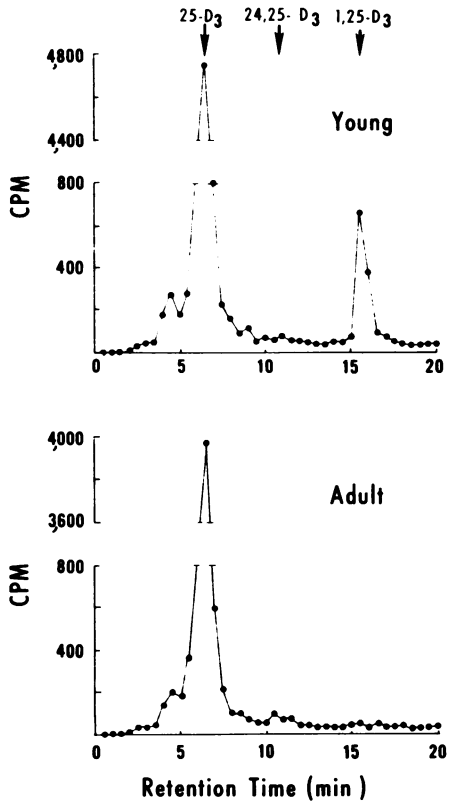

FIGURE 3 Distribution of tritiated vitamin D metabolites in renal cortical slices from young (A) and adult (B) vitamin D-deficient rats. Slices were incubated with tritiated 25-OH-D_3 for 1 h and extracted. Extracts, containing about 10,000 cpm each, were chromatographed on HPLC using a Zorbax-SIL column (0.6 × 25 cm) equilibrated with hexane/methanol/methylene chloride (8:1:1) at a flow rate of 1.1 ml/min. 0.5-min fractions were collected and analyzed for radioactivity. The position of the absorbance maxima (measured at 254 nm) of standard vitamin D compounds chromatographed under identical conditions is indicated at the top of the figure.

DISCUSSION

The present study demonstrated that there was a marked decrease in the capacity of the adult vitamin D-deficient rat to convert 25-OH-D_3 to 1,25-$(OH)_2$-D_3. It is possible that the decreased amount of 1,25-$(OH)_2$-D_3 in the plasma of the adult could be attributed to more rapid catabolism of 1,25-$(OH)_2$-D_3 by the adult. However, the measurement of 1,25-$(OH)_2$-D_3 production by isolated renal slices demonstrated that there was a very low rate of conversion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3 by the adult kidney. This indicated that the decreased amount of plasma 1,25-$(OH)_2$-D_3 in the adult was due,

TABLE II
*Effect of Age and Diet on 1,25-$(OH)_2$-D_3 Production by Renal Slices**

Diet	Age	1,25-$(OH)_2$-D_3 Production
		pg/mg tissue/h
D-deficient, low Ca	Young	244±12 (4)
D-deficient, low Ca	Adult	23.4±1.2(4)
D-replete, high Ca	Young	34.2±7.8(3)
D-deficient, low Ca (heated)‡	Young	9.1±0.7(3)

* Values are mean±SEM of number of animals indicated in parentheses. 150–250-mg wet wt slices were incubated 1 h.
‡ Slices were heated for 5 min at 100°C before incubation.

at least in part, to the reduced production of 1,25-$(OH)_2$-D_3 by the adult kidney. It may be that the factors that regulate the renal conversion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3 (5) change with age, or it may be that the response of the kidney to these regulators decreases with age.

The present study demonstrated the usefulness of using isolated renal slices to study the conversion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3 and its regulation in the mammal. Although it has been possible to measure this conversion in the chick kidney using tissue homogenates (6) or isolated mitochondria (17), these preparations had not been successfully applied to the mammalian kidney in the past. This has been attributed to the presence of the plasma binding protein for 25-OH-D_3 in these preparations (18). Therefore, the renal slice technique was developed to study this key enzyme system in the metabolic pathway of vitamin D in the mammal. Apparently, the 25-OH-D_3 plasma binding protein was washed away during the slice preparation, allowing the measurement of the conversion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3. Recently, a technique for measuring this conversion in mammalian renal mitochondria has been reported. This technique uses high substrate concentrations and an incubation temperature of 25°C (19). However, the slice preparation does not involve the isolation of subcellular organelles, and it may be useful in studying the cytoplasmic components involved in the regulation of the conversion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3 by the mitochondria.

The present studies also demonstrated that vitamin D-deficient adult animals do not respond to exogenous 25-OH-D_3 with increased calcium absorption by the intestine (Fig. 1) or increased CaBP content in the intestine (Fig. 2). However, 1,25-$(OH)_2$-D_3 has been shown to significantly increase calcium absorption and CaBP levels in adult rats (20). Therefore, the lack of adult response to 25-OH-D_3 is not due to a lack of intestinal response to the 1,25-$(OH)_2$-D_3 product. Rather, the lack of response is due to the fact that only a very small amount of labeled 1,25-$(OH)_2$-D_3 is found in the blood and, therefore, in the intestine of the adult after dosing with labeled 25-OH-D_3 (Table I). This is consistent with a previous study in vitamin D-replete rats that reported that there was a decrease in the accumulation of 1,25-$(OH)_2$-D_3 by the intestine that correlated with the decrease in calcium absorption with age (21). The fact that 25-OH-D_3 did not increase intestinal absorption of calcium in adult rats, but that 1,25-$(OH)_2$-D_3 did is consistent with the results of human studies. 1α-Hydroxyvitamin D_3 administration has been shown to increase intestinal absorption in older adults (22). Moderate doses of 25-OH-D_3, however, result in no increase in the intestinal absorption of older adults, although plasma 25-OH-D_3 levels are increased (23).

The lack of 1,25-$(OH)_2$-D_3 production in the adult and the subsequent lack of intestinal absorption of calcium may be of nutritional importance. Adult F344 rats fed a low calcium diet for 14 d demonstrate a negative calcium balance due to loss of calcium in the feces (data not shown). It has been reported that patients suffering from osteoporosis have decreased calcium absorption compared with age-matched controls (7). Osteoporotic patients may also exhibit negative calcium balance (5). Both calcium absorption and calcium balance are improved by administration of 1,25-$(OH)_2$-D_3. The nutritional consequences of decreased calcium absorption by the adult may be compounded by the fact that a high percentage of the elderly consume less than two-thirds of the recommended daily allowance of calcium (24).

ACKNOWLEDGMENTS

The antiserum to rat intestinal CaBP used in this study was generously provided by Dr. Elizabeth Bruns (Department of Pathology, University of Virginia Medical Center, Charlottesville, Va.). The authors gratefully acknowledge the excellent technical assistance of Mrs. Cindy Gross, Mr. Mark Palmier, and Mr. Harold Buhle, and the secretarial assistance of Miss Sharon Smith.

REFERENCES

1. Bullamore, J. R., R. Wilkinson, J. C. Gallagher, B. E. C. Nordin, and D. H. Marshall. 1970. Effects of age on calcium absorption. *Lancet.* **II:** 535–537.

2. Avioli, L. V., J. E. McDonald, and S. W. Lee. 1965. The influence of age on the intestinal absorption of ^{47}Ca in women and its relation to ^{47}Ca absorption in postmenopausal osteoporosis. *J. Clin. Invest.* **44:** 1960–1967.
3. Ireland, P., and J. S. Fordtran. 1973. Effect of dietary calcium and age on jejunal calcium absorption in humans studied by intestinal perfusion. *J. Clin. Invest.* **52:** 2672–2681.
4. Boyle, I. T., R. W. Gray, and H. F. DeLuca. 1971. Regulation by calcium of *in vivo* synthesis of 1,25-dihydroxycholecalciferol and 21,25-dihydroxycholecalciferol. *Proc. Natl. Acad. Sci. U. S. A.* **68:** 2131–2134.
5. DeLuca, H. F. 1979. The vitamin D system in the regulation of calcium and phosphorus metabolism. *Nutr. Rev.* **37:** 161–193.
6. Henry, H. L., R. J. Midgett, and A. W. Norman. 1974. Regulation of 25-hydroxyvitamin D_3-1-hydroxylase *in vivo*. *J. Biol. Chem.* **249:** 7584–7592.
7. Gallagher, J. C., B. L. Riggs, J. Eisman, A. Hamstra, S. B. Arnaud, and H. F. DeLuca. 1979. Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients. *J. Clin. Invest.* **64:** 729–736.
8. Armbrecht, H. J., T. V. Zenser, M. E. H. Bruns, and B. B. Davis. 1979. Effect of age on intestinal calcium absorption and adaptation to dietary calcium. *Am. J. Physiol.* **236:** E769–E774.
9. Pike, J. W., S. Toverud, A. Boass, T. McCain, and M. R. Haussler. 1977. Circulating 1,25-$(OH)_2$-D_3 during physiological states of calcium stress. *In* Vitamin D Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism. A. W. Norman, K. Schaefer, J. W. Coburn, H. F. DeLuca, D. Fraser, H. G. Grigoleit, and D. V. Herrath, editors. Walter de Gruyter & Co., Berlin. 187–189.
10. Wasserman, R. H., and R. A. Corradino. 1973. Vitamin D, calcium, and protein synthesis. *Vitam. Horm.* **31:** 43–103.
11. Shepard, R. M., R. L. Horst, A. J. Hamstra, and H. F. DeLuca. 1979. Determination of vitamin D and its metabolites in plasma from normal and anephric man. *Biochem. J.* **182:** 55–69.
12. Bruns, M. E. H., A. Fausto, and L. V. Avioli. 1978. Placental calcium binding protein in rats. *J. Biol. Chem.* **253:** 3186–3190.
13. Thomasset, M., P. Cuisinier-Gleizes, and H. Mathieu. 1979. 1,25-Dihydroxycholecalciferol: dynamics of the stimulation of duodenal calcium-binding protein, calcium transport and bone calcium mobilization in vitamin D and calcium-deficient rats. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **107:** 91–94.
14. Kepner, B. L., and D. M. Hercules. 1963. Fluorometric determination of calcium in blood serum. *Anal. Chem.* **35:** 1238–1240.
15. Fiske, C. H., and Y. SubbaRow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66:** 375–400.
16. Dixon, W. J., and F. J. Massey. 1969. Introduction to Statistical Analysis. McGraw-Hill, Inc., New York. 638.
17. Gray, R. W., J. L. Omdahl, J. G. Ghazarian, and H. F. DeLuca. 1972. 25-Hydroxycholecalciferol-1-hydroxylase. Subcellular localization and properties. *J. Biol. Chem.* **247:** 7528–7532.
18. Ghazarian, J. B., B. Kream, K. M. Botham, M. W. Nickells, and H. F. DeLuca. 1978. Rat plasma 25-hydroxyvitamin D_3 binding protein: An inhibitor of the 25-hydroxyvitamin D_3-1α-hydroxylase. *Arch. Biochem. Biophys.* **189:** 212–220.
19. Vieth, R., and D. Fraser. 1979. Kinetic behavior of 25-hydroxyvitamin-D-1-hydroxylase and -24-hydroxylase in rat kidney mitochondria. *J. Biol. Chem.* **254:** 12455–12460.
20. Armbrecht, H. J., T. V. Zenser, and B. B. Davis. 1980. Effect of vitamin D metabolites on intestinal calcium absorption and calcium-binding protein in young and adult rats. *Endocrinology.* **106:** 469–475.
21. Horst, R. L., H. F. DeLuca, and N. A. Jorgensen. 1978. The effect of age on calcium absorption and accumulation of 1,25-dihydroxyvitamin D_3 in intestinal mucosa of rats. *Metabolic Bone Disease and Related Research.* **1:** 29–33.
22. Peacock, M., J. C. Gallagher, and B. E. C. Nordin. 1974. Action of 1α-hydroxyvitamin D_3 on calcium absorption and bone resorption in man. *Lancet.* **I:** 385–389.
23. Somerville, P. J., J. W. K. Lien, and M. Kaye. 1977. The calcium and vitamin D status in an elderly female population and their response to administered supplemental vitamin D_3. *J. Gerontol.* **32:** 659–663.
24. Department of Health, Education and Welfare. Ten-state nutrition survey 1968–1970. DHEW Publication No. (HSM) 72-8130 to 72-8134.