Effect of Renal Insufficiency on the Active Transport of Calcium by the Small Intestine

RICHARD D. BAERG, DANIEL V. KIMBERG, and ELAINE GERSHON

From the Department of Medicine, Columbia University College of Physicians and Surgeons, New York 10032 and the Department of Medicine, Harvard Medical School, and the Gastrointestinal Unit of the Department of Medicine, Beth Israel Hospital, Boston, Massachusetts 02215

ABSTRACT The intestinal absorption of calcium is often depressed in patients with chronic renal insufficiency. Furthermore, the malabsorption of calcium and the osteodystrophy which occur in association with chronic renal disease are often "resistant" to vitamin D; the basis for this resistance remains uncertain however. Recent studies by others have emphasized the role of an abnormality in the metabolism of vitamin D in accounting for the alterations in the calcium absorption and the apparent vitamin D–resistance which accompany the uremic syndrome.

The present studies with an experimentally uremic animal model demonstrate a defect in the active transport of calcium by duodenal gut sacs in vitro. This abnormality is not due to the semistarvation associated with renal insufficiency and cannot be corrected by the administration of physiologic amounts of vitamin D_3; it is reversed by massive doses of the vitamin.

Neither the metabolism of vitamin D_3 nor the levels of calcium binding protein activity in the duodenal mucosa are affected by renal insufficiency under the conditions employed in the present studies. The results of the present studies strongly suggest that in addition to the recently proposed mechanism involving an interference with the metabolism of vitamin D renal insufficiency also affects the cellular mechanisms for calcium transport in a manner which, while opposite in direction to that of vitamin D, is independent of a direct interaction with the vitamin or its metabolites.

INTRODUCTION

The abnormalities of calcium metabolism that occur with chronic renal insufficiency and which are associated with bone disease have become well recognized during the past 25 yr. Calcium balance studies in uremic patients with osteodystrophy have revealed that the negative balance which accompanies renal failure is due to excessive fecal loss of calcium (1–6). Furthermore, the results of metabolic studies performed in such patients have led to the conclusion that the malabsorption of calcium and the osteomalacia of chronic renal insufficiency may be reversed by treatment with vitamin D, but large amounts are required (2, 3, 6). It seems clear that in uremia there is a relative insensitivity to the biological effects of vitamin D, but the basis for this apparent "resistance" to the vitamin remains uncertain.

The relative resistance of the intestinal calcium absorptive mechanism to vitamin D in uremic subjects has been studied by Stanbury and Lumb (7, 8). They demonstrated that the administration of large amounts of vitamin D to uremic patients, doses capable of producing plasma levels of vitamin D activity 5–10 times normal (as measured by the rachitic rat bioassay), was ineffective in altering the malabsorption of calcium in these subjects. Larger amounts of vitamin D invariably increased the absorption of calcium in these individuals. Kessner and Epstein (9), in a study of calcium transport in everted duodenal gut sacs from chronically uremic rats, were unable to statistically differentiate the depression in transport induced by renal insufficiency from that produced by the semistarvation of paired feeding in their control animals. With the large doses of vitamin D which they employed, they were further unable to demonstrate resistance to vitamin D in their experimental model.

While the etiology of the relative insensitivity to vitamin D in azotemic subjects remains obscure, there are several possible mechanisms which deserve consideration. (*a*) Some effect of uremia on cellular biochemical reactions such that the action of vitamin D cannot be expressed, (*b*) the presence in uremia of a

Received for publication 16 December 1969 and in revised form 20 February 1970.

direct vitamin D antagonist, or (*c*) an abnormality in the metabolism of vitamin D which interferes with the delivery of the active agent(s) to the intestinal mucosal cell. The last possibility has recently been studied by Avioli, Birge, Lee, and Slatopolsky (10), who demonstrated that while the intestinal absorption of vitamin D_3-^3H is normal in uremic subjects, its subsequent fate is indeed abnormal. In addition to a twofold increase in the fractional turnover rate of orally administered vitamin D_3-^3H, there appears to be an abnormal accumulation in the plasma of biologically inactive lipid-soluble metabolites, as well as a urinary loss of both vitamin D_3-^3H and inactive metabolites in the uremic patients. Their experiments support the concept that the resistance to therapeutic doses of vitamin D which often accompanies chronic renal failure may result from an acquired defect in the metabolism and excretion of the vitamin.

Experiments described in this report demonstrate defective transport of calcium and certain other divalent cations by everted gut sacs from rats with experimental renal insufficiency. The abnormality in calcium transport is not due to semistarvation and cannot be reversed by physiologic amounts of vitamin D_3; the administration of massive amounts of vitamin D_3 does, however, correct the defect. The results of experiments concerned with the metabolism of tritiated vitamin D_3 and the levels of the so-called "calcium-binding protein" (CaBP) suggest that in renal insufficiency there is an apparent antagonism to the effects of vitamin D, which under the conditions used in these studies, is unrelated to the presence of an alteration in the metabolism of the vitamin. Furthermore, the studies concerned with the activity of the vitamin D–dependent calcium-binding protein fail to support the hypothesis that there may be a direct vitamin D antagonist. It would appear then as though in renal insufficiency at least part of the observed vitamin D resistance is due to an anti–vitamin D–like effect on the intestinal mucosa which is independent of a direct interaction with the vitamin.

METHODS

Animal preparation. Albino, male rats of the Sherman strain, obtained at a weight of 125–150 g, were used in all of the studies (Camm Research Laboratories, Wayne, N. J.). After 1 wk on the appropriate diet, the animals were subjected to either a five-sixths nephrectomy in a two-stage procedure or to two sham operations under ether anesthesia. The partial nephrectomy was accomplished by a modification of the method of McCance and Morrison (11), and involved the removal of two-thirds of the left kidney followed by a total right nephrectomy 7 days later. Experiments were conducted 7–9 days after the second sham operation or the second stage of the incomplete nephrectomy procedure.

In some of the experiments, the animals were permitted the random consumption of a standard rat chow, adequate in vitamin D (Hemlock Hollow Farm, N. J.), and were housed in groups of five rats per cage. In experiments involving paired feeding, the animals were housed individually and were given a purified "vitamin D–free" diet containing 20% ground gluten, 76% ground maize, 3% $CaCO_3$, and 1% NaCl (Rachitogenic Diet No. 2, U.S.P., Nutritional Biochemicals Corp., Cleveland, Ohio). Sham-operated rats were offered an amount of food equal to the mean consumption of the nephrectomized group during the preceding 24 hr. In addition, in certain experiments, some of the sham-operated rats were permitted random consumption of the "vitamin D–free" diet. All animals had free access to tap water and were fasted overnight before an experiment. When administered, vitamin D_3 (Vi-De 3 Hydrosol, Wander, Berne, Switzerland) was injected subcutaneously in 50% ethanol (v/v), while control animals received 50% ethanol alone.

Transport studies. Studies of active transport and of the rates of transfer of substances across the intestine were performed by the everted gut sac technique of Wilson and Wiseman (12) as modified by Schachter and Rosen (13). The animals were sacrificed by a blow to the head followed by exaguination. The upper small intestine was removed from its mesenteric attachment, everted over a chilled glass rod, and rinsed in an iced solution containing 0.146 M NaCl and 0.004 M KCl. Gut sacs from the proximal 4–5 cm of duodenum were filled with 0.5 ml of medium and placed in 25-ml Erlenmyer flasks containing 4.0 ml of an identical medium. The standard medium for calcium transport studies had the following composition: 0.151 M NaCl; 0.004 M sodium phosphate of pH 7.4; 0.02 M fructose; 0.0008 M $CaCl_2$; and 0.033 μCi/ml of $^{45}CaCl_2$. Variations in the technique and in the composition of the medium are noted in the text and in the legends to the tables and figures. The gut sacs and incubating medium were equilibrated with 100% O_2 while on ice and the flasks were then sealed with rubber stoppers containing hanging center wells. The wells contained strips of fluted filter paper saturated with 0.2 ml of 10 N KOH in order to remove metabolic CO_2 and thus maintain pH. The flasks were incubated at 37°C for 2 hr in a metabolic water bath oscillating at a rate of 110/min. The sacs were removed from the flasks, blotted, and drained into graduated tubes. The recovery was usually between 90 and 110% of the initial volume. Aliquots of the medium (inside and outside) were counted in Bray's solution (14) with a Packard Tri-Carb liquid scintillation spectrometer or were prepared for calcium determination by atomic absorption spectrometry. The active transport of calcium has been expressed as the ratio of the final concentrations of ^{45}Ca (counts per minute per milliliter) or of total calcium (micromoles per milliliter) in the serosal (inside) medium/mucosal (outside) medium, i.e. the concentration ratio I/O.

In certain experiments, $CaCl_2$ and ^{45}Ca or other test substances were initially present in the mucosal (outside) medium only. In these experiments the suface area (square centimeter) of the gut sacs was determined as previously described (15), and the results expressed either as counts per minute or micromoles transported per square centimeter of gut sac. The oxygen-dependent accumulation of ^{45}Ca by full-thickness slices of duodenum was determined by the method of Schachter, Dowdle, and Schenker (16). All of the isotopes used in this study were procured from the New England Nuclear Corp., Boston, Mass.

Calcium-binding protein. The vitamin D–dependent duodenal mucosal calcium-binding protein was measured by the technique described by Wasserman and Taylor (17). This method depends on the competition between the cation exchange resin, Chelex-100 (Biorad Resins, Calbiochem Corp., Los Angeles, Calif.), and a heat-treated soluble su-

pernatant of a duodenal mucosal homogenate for added ^{45}Ca. Trypsin-treated control samples were routinely employed.

Other determinations. Serum creatinine was determined by a micromodification of the method of Roscoe (18), and serum inorganic phosphate was estimated on protein-free, 5% trichloroacetic acid filtrates (1/10) by the method of Fiske and SubbaRow (19). Calcium was determined in the serum and in the medium by atomic absorption spectrometry. D-Glucose and lactic acid concentrations were estimated by enzymatic methods employing glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold, N. J.) and lactic dehydrogenase (C. F. Boehringer, Mannheim, Germany) respectively (20–22).

Vitamin D metabolites. Vitamin $D_3$1, 2-^3H with a specific activity of 35.4 mCi/mM was purchased from the New England Nuclear Corp. and was purified by thin-layer chromatography on silica gel in a benzene: ethyl acetate (5:1) solvent system. The vitamin D_3-^3H was administered intraperitoneally (55 IU) in 0.25 ml of 0.10% Tween 20 (polyoxyethylene sorbitan monolaurate; Fisher Scientific Co. Pittsburgh, Pa.) and 0.9% NaCl in water to 20 nephrectomized and 18 sham-operated, pair-fed control animals. The animals were sacrificed 20 hr later with a blow to the head followed by exanguination.

Blood was collected in chilled centrifuge tubes and centrifuged 1–2 hr later. The serum was removed, frozen, and stored under N_2 for later analysis. The livers were removed, rinsed in ice-cold 0.25 M sucrose, blotted, frozen over a dry ice-acetone bath, and stored under N_2 for subsequent analysis. The entire small intestine was removed along its mesenteric border, everted over chilled glass rods, rinsed in an ice-cold solution of 0.146 M NaCl and 0.004 M KCl, and blotted on filter paper. The mucosa was removed by scraping the everted gut with a glass microscope slide after placing it on a chilled glass plate. The legs were removed from the animals immediately after the livers and intestines and packed in ice. A short time later, the muscles were removed from the femur, the bones were split open, and the marrow removed. The bone was then stored frozen under N_2 for later analysis.

The serum and homogenates of liver and intestinal mucosa were analyzed for total tritium content after combustion. The tritiated water was counted in a solution which consisted of 40 mg of dimethyl-POPOP (1,4-bis[2,5-phenyloxazolyl])benzene, 4.0 g of PPO (2,5-diphenyloxazole), 200 ml of absolute ethanol, and 800 ml of toluene. The radioactivity present in the liver, bone, serum, and intestinal mucosa was extracted by the method of Bligh and Dyer (23) as modified by Lund and DeLuca (24). The chloroform extracts were concentrated by evaporation with a stream of N_2 and dissolved in *n*-hexane for chromatographic analysis.

Columns, 58 × 1.5 cm, were prepared with activated silicic acid, 100–200 mesh (Unisil, Clarkson Chemical Co., Williamsport, Pa.) in *n*-hexane. The chromatogram was developed by means of a hyperbolic gradient elution as previously described by Ponchon, Kennan, and DeLuca (25). The flow rate was approximately 1.5 ml/min and 10-ml fractions were collected for radioactivity measurements with a Packard Tri-Carb liquid scintillation counter. The samples containing only organic solvents were evaporated in counting vials and dissolved in a counting solution which consisted of 100 mg of dimethyl-POPOP (1,4-bis[5-phenyl-2-oxazolyl]-benzene) and 3.0 g of PPO per liter of toluene (26). Tritium present in samples of the aqueous extract was measured with the counting solution of Bray (14). Internal standards of toluene-^3H were added to all samples and the total distintegrations per minute were calculated.

TABLE I

Effect of Subtotal Nephrectomy on Serum Chemistries

Serum chemistry	No.	Mean	SE	SD	*P*
Serum creatinine, *mg/100 ml*					
Sham-operated	199	1.14	0.02	0.27	<0.001
Nephrectomized	207	2.62	0.08	1.12	
Serum calcium, *mg/100 ml*					
Sham-operated	29	9.61	0.11	0.60	>0.1
Nephrectomized	32	9.43	0.11	0.62	
Serum phosphorus, *mg/100 ml*					
Sham-operated	21	12.37	0.29	1.35	<0.005
Nephrectomized	21	13.99	0.46	2.13	

The chemical determinations were performed by the methods described in the text.

RESULTS

General considerations. Renal insufficiency in rats, in addition to being associated with the derangements described below, causes inappetence and subsequent weight loss. Incompletely nephrectomized rats eating the standard diet sustained an average weight loss of 8 g during the preparatory period while the randomly fed, sham-operated control animals gained an average of 18 g on this diet during the same period. When eating the powdered rachitogenic diet and receiving 2 or 10 IU of vitamin D_3 3 times weekly, nephrectomized animals consumed between 5.9 and 7.3 g/rat per day and lost an average of 23 g during the period, while the pair-fed control rats lost an average of 22 g. Sham-operated animals allowed this diet *ad libitum* gained an average of only 1 g, but they ate less (10.9 g/rat per day) than would be expected had they been offered the regular rat chow (9).

Serum creatinine, calcium, and inorganic phosphorus values are shown in Table I. Rats were considered to have renal insufficiency only if their serum creatinine concentration was greater than 2 SD above the mean value in the sham-operated controls. Serum creatinine levels were not affected by variations in the dietary regimen. The serum calcium values were normal in both groups of animals and there was no statistically significant difference. The level of serum calcium was not influenced by changes in diet or by the administration of 2 or 10 IU of vitamin D_3 3 times weekly. The duration of vitamin D deprivation during the feeding of the "vitamin D–free" diet was inadequate to cause a lowering of serum calcium. The serum inorganic phosphorus elevation noted in the nephrectomized animals is significant.

Active calcium transport. The active transport of calcium in everted duodenal gut sacs prepared from par-

TABLE II

Effects of Subtotal Nephrectomy, Diet, and Vitamin D_3 on the Active Transport of Calcium in the Duodenum

Experimental conditions	No.	Mean ^{45}Ca concentration ratio inside/outside	SE	SD	P
Experiment 1—random feeding					
Sham-operated	34	6.5	0.6	1.3	<0.005
Nephrectomized	38	4.3	0.4	1.0	
Experiment 2—paired feeding					
Sham-operated	6	5.9	0.3	1.8	<0.005
Nephrectomized	8	4.0	0.2	1.4	
Experiment 3—random feeding					
Sham-operated + 10,000 IU D_3	18	7.5	0.6	2.5	>0.10
Nephrectomized + 10,000 IU D_3 paired feeding	16	6.6	0.4	1.4	
Sham-operated + 6,000 IU D_3	6	5.9	0.7	1.5	>0.10
Nephrectomized + 6,000 IU D_3	9	5.9	0.6	1.6	

In the random feeding experiments the animals consumed the standard rat chow ad lib., whereas in the paired-feeding experiments the "vitamin D–free" diet was employed. In experiment 3 the randomly fed animals received 10,000 IU of vitamin D_3 48 hr before sacrifice and the pair-fed animals received 6000 IU 24 hr before sacrifice. In all experiments the everted gut sacs were filled with 0.5 ml of the standard medium containing 8.0×10^{-4} M $CaCl_2$ and ^{45}Ca and were bathed in flasks containing 4.0 ml of an identical medium as described in Methods.

tially nephrectomized rats is decreased when compared to results obtained with sham-operated control animals (Table II, experiment 1). In this experiment, however, both groups of animals ate the standard chow ad libitum. Since semistarvation is known to decrease the active transport of calcium in the rat duodenum and since this effect may be difficult to distinguish from that caused by renal insufficiency (9), similar studies were performed with the paired feeding technique. The persistence of the defect in active calcium transport in the nephrectomized group under these conditions (Table II, experiment 2) suggests that dietary restriction alone cannot account for this abnormality. Of interest too, is the fact that the defective calcium transport in gut sacs from partially nephrectomized animals may be restored by the prior administration of large amounts of vitamin D_3 (Table II, experiment 3).

In vivo perfusion studies have demonstrated that the entire small intestine may transport calcium against a concentration gradient, although the rate of transport in the distal intestine is lower than that in the duodenum (27). In vitro, only the proximal one-fifth of the small intestine of young rats on a diet adequate in calcium (1.68%) and vitamin D has been observed to participate in the transfer of calcium against a chemical concentration gradient (28). Under conditions of dietary calcium deprivation, however, there is an adaptive response on the part of the active transport mechanism such that concentration ratios (I/O) in excess of 1.0 may be obtained with gut sacs from almost the entire small intestine (15). Under these conditions in vitro, the ability of the gut to transfer calcium is most marked in the duodenum, less in the distal ileum, and least in the mid-small intestine. The relative effect of vitamin D on the rates of calcium transfer across different levels of the intestine parallels this distribution of the active transport mechanism (29). Furthermore, there is evidence to suggest that the mechanisms involved in calcium transport are similar throughout the small intestine (15, 29).

Experiments were undertaken to determine the effects of renal insufficiency on the ability of different segments of the small intestine to transfer calcium (Table III, experiment 1). The sacs were filled (serosal surface) with 0.5 ml of medium minus both $CaCl_2$ and ^{45}Ca, and they were incubated in an identical medium plus 0.003 M $CaCl_2$ and ^{45}Ca. The transfer of calcium from the mucosal to the serosal medium, expressed as micromoles of calcium transferred per square centimeter of surface area, is decreased in renal insufficiency. This effect, most marked in the duodenum, less in the distal ileum, and insignificant in the jejunum, parallels in distribution the pattern of the vitamin D–dependent active transport mechanism for calcium.

Differences in the endogenous intestinal calcium content could conceivably account for the previously mentioned effects of nephrectomy on the transfer of ^{45}Ca. Experiments were performed in which the inside (serosal) medium used to fill duodenal gut sacs from pair-fed animals was deficient in $CaCl_2$ and ^{45}Ca, both of which were added to an otherwise identical outside (mucosal) medium. The initial calcium concentration in the outside medium was 0.003 M as determined by atomic absorption spectrometry, and the specific activity was 23,630 cpm/μmole. After a 2 hr incubation, the specific activity of ^{45}Ca in the inside and outside medium with sacs from sham-operated and nephrectomized animals varied between 21,782 and 22,437 cpm/μmole, a decrease of between 5 and 8%. There were no significant differences in the specific activities of calcium in the media when the two groups of animals were compared. The rates of transfer of calcium from the mucosal to the serosal medium were, however, strikingly different, with gut sacs from the sham-operated animals transferring 0.44 μmoles/cm² per 2 hr and those from the nephrectomized animals transferring 0.34 μmoles/cm² per 2 hr. It seems

TABLE III

Effects of Subtotal Nephrectomy on Calcium Transfer at Different Levels of the Small Intestine from Rats Repleted with Varying Amounts of Vitamin D_3

Experimental conditions	No.	Vitamin D_3 supplement	Gut segment	Calcium transfer	SE $\times 10^{-3}$	SD $\times 10^{-3}$	*P*
				μmoles $\times 10^{-3}$/ *cm² per 2 hr*			
Experiment 1—paired feeding							
Sham-operated	19	None	Duodenum	282.3	7.7	33.7	<0.005
Nephrectomized	17	None	Duodenum	163.3	9.3	38.3	
Sham-operated	18	None	Jejunum	111.5	5.5	23.3	>0.10
Nephrectomized	16	None	Jejunum	99.4	9.2	36.7	
Sham-operated	19	None	Ileum	76.0	4.5	19.8	<0.01
Nephrectomized	18	None	Ileum	62.8	3.1	13.0	
Experiment 2—paired feeding							
Sham-operated	8	2 IU 3 x/wk	Duodenum	303.4	21.6	61.0	<0.005
Nephrectomized	12	2 IU 3 x/wk	Duodenum	192.2	13.7	47.4	
Sham-operated	9	2 IU 3 x/wk	Jejunum	112.8	14.6	43.7	>0.10
Nephrectomized	11	2 IU 3 x/wk	Jejunum	113.6	11.5	38.2	
Sham-operated	11	2 IU 3 x/wk	Ileum	111.1	10.7	35.4	>0.10
Nephrectomized	11	2 IU 3 x/wk	Ileum	108.8	9.9	32.8	
Experiment 3—paired feeding							
Sham-operated	12	10 IU 3 x/wk	Duodenum	317.1	36.6	126.7	<0.005
Nephrectomized	20	10 IU 3 x/wk	Duodenum	228.0	15.0	67.1	
Sham-operated	13	10 IU 3 x/wk	Jejunum	131.7	9.0	32.5	>0.10
Nephrectomized	20	10 IU 3 x/wk	Jejunum	110.0	9.1	40.5	
Sham-operated	13	10 IU 3 x/wk	Ileum	138.2	10.4	37.4	>0.10
Nephrectomized	18	10 IU 3 x/wk	Ileum	146.6	13.6	57.9	
Experiment 4—paired feeding							
Sham-operated	8	None	Duodenum	457.4	28.4	80.2	<0.005
Nephrectomized	12	None	Duodenum	307.6	23.6	81.8	
Sham-operated	5	10,000 IU	Duodenum	506.1	57.8	129.3	>0.10
Nephrectomized	7	24 hr before sacrifice	Duodenum	468.2	32.5	86.0	

In these experiments the paired feeding technique and the "vitamin D–free" diet were employed. In experiments 2 and 3 the animals received either 2 or 10 IU of vitamin D_3 3 times weekly during the 2 wk before sacrifice. In experiment 4, the animals repleted with vitamin D received one dose 24 hr before sacrifice.

Gut sacs were prepared from the proximal 5 cm of duodenum, the jejunum (15–20 cm from the pylorus), and the terminal 5 cm of the ileum. The sacs were filled with 0.5 ml of medium containing 0.151 M NaCl, 4.0×10^{-3} M Tris-HCl buffer at pH 7.4, and 2.0×10^{-2} M fructose, and were bathed in 4.0 ml of a similar medium which contained, in addition, 3.0×10^{-3} M $CaCl_2$ and ^{45}Ca. After a 2 hr incubation at 37°C the sacs were drained, the volume recorded, aliquots of the inside (serosal) and outside (mucosal) medium counted, and the sac area determined (see Methods).

The results of the four experiments are presented together for clarity, but they were conducted on different days as separate studies. While comparisons between groups within an experiment are valid, direct comparisons between groups in different experiments are probably not justified.

TABLE IV

Effect of Vitamin D_3 on Active Transport of Calcium by Duodenal Gut Sacs from Control and Uremic Rats

Dose of vitamin D_3	Sham-operated animals: Net transport of calcium, Δ Mucosal	Sham-operated animals: Net transport of calcium, Δ Serosal	Sham-operated animals: Ca concentration ratio (I/O)	Nephrectomized animals: Net transport of calcium, Δ Mucosal	Nephrectomized animals: Net transport of calcium, Δ Serosal	Nephrectomized animals: Ca concentration ratio (I/O)
U	*μmole/gut sac*			*μmole/gut sac*		
None	−1.13	+1.03	5.7	−0.93	+0.74	4.1
2	−1.12	+1.03	5.7	−0.92	+0.79	4.3
10	−1.12	+1.03	5.7	−1.00	+0.91	4.9
10,000	−1.12	+1.08	5.9	−1.12	+1.10	5.9

The animals used in this experiment were pair-fed the "vitamin D–free" diet. Groups of four to five rats each received either 50% ethanol, 2 or 10 IU of vitamin D_3 thrice weekly during the 2 wk before sacrifice, or a single dose of 10,000 IU 24 hr before sacrifice. One duodenal gut sac from each animal was incubated in the standard medium as described in Methods, with $CaCl_2$ initially present in both the inside (serosal) and outside (mucosal) medium at a concentration of 7.8×10^{-4} M as determined by atomic absorption spectrometry. After a 2 hr incubation at 37°C, the gut sacs from each group were drained, the serosal and mucosal media were pooled separately, and calcium was determined by atomic absorption spectrometry.

unlikely, therefore, that differences in the endogenous tissue calcium content could contribute to the observed effects of renal insufficiency on the intestinal transfer of ^{45}Ca. Additional short-term experiments were performed in which both the inside (serosal) and the outside (mucosal) medium contained 8.0×10^{-4} M $CaCl_2$, but ^{45}Ca was initially present only in the inside medium. These experiments failed to reveal significant differences in the rates of diffusion of calcium (serosal towards mucosal) in sacs prepared from sham-operated (2.61×10^{-4} μmoles/cm² per min [range = $2.17–3.00 \times 10^{-4}$]) and nephrectomized (2.47×10^{-4} μmoles/cm² per min [range = $2.42–2.58 \times 10^{-4}$]) animals.

Response to vitamin D. The effects of renal insufficiency on the transfer of calcium are most marked in the duodenum, the site where the vitamin D–dependent active transport mechanism is maximal. In order to evaluate the responsiveness of the defect in calcium transport to vitamin D, a series of separate experiments were performed in which the rate of transfer of calcium from the mucosa to the serosa was measured in gut sacs from different levels of the small intestine of pair-fed animals repleted with varying amounts of vitamin D (Table III, experiments 2–4).

In animals given 2 or 10 IU of vitamin D_3 3 times weekly, a more than adequate maintenance dose for normal rats, the defect in calcium transport associated with renal insufficiency is seen to persist in the duodenum, but is no longer apparent in the ileum. This defect in the vitamin D–dependent calcium transport mechanism in uremic animals is corrected by the administration of a massive (10,000 IU/rat) dose of the vitamin (Table III, experiment 4).

The response to varying amounts of vitamin D was carefully explored in a single experiment with duodenal gut sacs from groups of 5 or 6 sham-operated, pair-fed, and nephrectomized animals given varying doses of vitamin D (Table IV). Calcium concentration ratios (I/O) in the sham-operated animals varied between 5.7 and 5.8 and were not influenced by the administration of vitamin D. In gut sacs from the nephrectomized animals deprived of vitamin D, the ratio was 4.1 and rose to a level of 5.7 (comparable to the sham group) in only those animals given massive doses of vitamin D (10,000 IU). Furthermore, while neither the disappearance of calcium from the mucosal medium nor its transfer to the serosal medium were influenced by vitamin D administration in the sham-operated animals, both of these parameters were affected by subtotal nephrectomy as expected and were restored to normal only with massive doses of vitamin D.

Bilateral total nephrectomy. The effect of the duration of uremia on the ability of the duodenum to actively transport calcium was tested in experiments in which animals were subjected to either sham procedures, unilateral nephrectomy, or bilateral total nephrectomy. 8 days before sacrifice the animals underwent either a unilateral nephrectomy procedure or a sham operation. 24 hr before sacrifice the previously nephrectomized animals were subjected to either a contralateral nephrec-

TABLE V

Effect of Bilateral Total Nephrectomy on the Active Transport of Calcium by Duodenal Gut Sacs

Operations	No.	^{45}Calcium concentration ratios	Serum creatinine	Serum phosphorus
		(I/O)	*mg/100 ml*	*mg/100 ml*
Sham-sham	11	6.7 ±1.6	0.86 ±0.29	13.04 ±2.80
Neph.-sham	11	6.0 ±1.1	0.92 ±0.19	13.23 ±2.09
Neph.-neph.	15	3.9 ±0.6	3.73 ±1.23	26.34 ±2.33

The animals were prepared as described in the text and were maintained on the standard rat chow ad lib. until the time of the second surgical procedure (20 hr before sacrifice). Duodenal gut sacs were incubated for 2 hr at 37°C in the standard medium as described in Methods, with $CaCl_2$ and ^{45}Ca initially present in both the inside (serosal) and outside (mucosal) bathing solutions.

Results are expressed as the mean ±SD. There are no significant differences ($P > 0.1$) between the sham-sham and the neph.-sham groups with respect to ^{45}Ca concentration ratios, serum creatinine, or serum phosphorus. The effect of bilateral nephrectomy on all three parameters is significant when compared to either the sham-sham or the neph.-sham group ($P < 0.005$).

tomy or a sham procedure, whereas the previously sham-operated animals underwent a second sham operation on the opposite side. The animals were then fasted overnight before the experiment. As seen in Table V, there were no significant differences in the values for serum creatinine or inorganic phosphorus when the unilaterally nephrectomized animals were compared to the sham-operated controls. Furthermore, as might be anticipated, the ^{45}Ca concentration ratio (I/O) in the bilaterally nephrectomized group was 3.9, a value which was quite significantly different from the values of 6.7 and 6.0 obtained with gut sacs from the sham and unilateral nephrectomy groups respectively.

Transfer of other divalent cations. In order to determine whether or not the defective intestinal transport of calcium in the uremic animals is unique for this cation, the transfer of other divalent cations was also studied. In the experiments shown in Table VI, the divalent cation and its isotope were initially present only in the outside (mucosal) medium and the results express the rate of transfer of cation to the inside (serosal) medium per square centimeter of gut sac area.

Renal insufficiency did not have a significant effect on the intestinal transfer of barium, however there was a small but probably significant effect on the transfer of strontium. The transfer of iron was strikingly reduced, representing an effect of uremia on a non–vitamin D–dependent transport mechanism which nonetheless may have features in common with the calcium transport system (29, 30). This effect will be discussed later.

Transport of hexoses and L*-leucine.* Neither the active transport of D-glucose nor the production of L-lactate by duodenal or jejunal gut sacs in vitro was affected by the nephrectomy procedure. This lack of a nephrectomy effect was noted in experiments using sham-operated animals which were either randomly fed or pair-fed. In a sense, this confirms the report of Boucot, Nurser, and Merrill (31) who demonstrated normal absorption of glucose in the intact uremic rat.

D-Galactose is also actively transported, but in contrast to D-glucose, it is only poorly metabolized by the intestinal mucosa. As shown in Table VII, the rate of transfer of D-galactose across the jejunum was enhanced by semistarvation, but was not influenced by the nephrectomy procedure per se. These results as well as those of others suggest that the active transport of hexoses may facultatively adapt to conditions of dietary hexose deprivation (32, 33).

The jejunal transport of L-leucine was unaffected by either semistarvation or the subtotal nephrectomy procedure.

Calcium-binding protein. The administration of vitamin D_3 to rachitic rats or chicks results in the appearance in the intestinal mucosa of a calcium-binding protein (Ca-BP) (17, 34–36). The amount of Ca-BP in the mucosa correlates well with the capacity of the intestine to absorb calcium and with the physiological need of the animal for calcium (17, 34–36). Furthermore, recent studies in the rachitic chick demonstrated a correlation between the amount of the vitamin D–induced Ca-BP and the dose of administered vitamin D (36). The role of this protein in calcium transport, if any, is still unknown.

In view of the possibility that the vitamin D resistance in uremia may be related to interference with the formation of active metabolites of vitamin D (10) or their localization in the intestinal mucosa, it was of interest to estimate the levels of the Ca-BP in homogenates of duodenal mucosa from sham-operated and partially nephrectomized rats (Table VIII). Despite the readily demonstrable defect in the active transport of calcium in the duodenum of nephrectomized rats deprived of vitamin D or maintained on modest doses, there were no significant differences in the levels of the Ca-BP when compared to sham-operated animals subjected to the semistarvation of paired feeding. The effects of experimental renal insufficiency in decreasing the active transport of calcium appear then to be at least in part, independent of the levels of the vitamin D-related Ca-BP. As noted in Table VIII, sham-operated rats permitted the random consumption of the powdered "D-free" diet, had higher levels of Ca-BP than did either the sham-pair-fed or nephrectomized animals. These results suggest that the effect of starvation per se on calcium transport, an ef-

TABLE VI

Effect of Subtotal Nephrectomy on the Intestinal Transfer of Barium, Strontium, and Iron

Divalent cation	No.	Gut segment	Cation transfer	SE	SD	*P*
			cpm/cm² per 2 hr			
Experiment 1—barium (^{133}Ba)						
Sham-operated	21	Duodenum	3959	200	948	<0.10
Nephrectomized	18	Duodenum	3476	282	1228	>0.05
Sham-operated	21	Ileum	2524	141	670	>0.10
Nephrectomized	18	Ileum	2545	223	989	
Experiment 2—strontium (^{85}Sr)						
Sham-operated	15	Duodenum	4488	200	806	<0.05
Nephrectomized	15	Duodenum	3734	387	1509	>0.025
Sham-operated	15	Ileum	2315	264	1024	>0.10
Nephrectomized	16	Ileum	2143	223	911	
Experiment 3—iron (^{55}Fe)						
Sham-operated	18	Duodenum	2954	346	1516	<0.01
Nephrectomized	18	Duodenum	1643	244	1095	

The animals were randomly fed the standard rat diet and surgery was performed as described in Methods. Gut sacs were prepared from the proximal 5 cm of duodenum and the terminal 5 cm of ileum. In experiments 1 and 2 everted sacs were filled with 0.5 ml of a medium containing 0.151 M NaCl, 4.0×10^{-3} M sodium phosphate buffer of pH 7.4, and 2.0×10^{-2} M fructose. In experiment 1, the sacs were bathed in 4.0 ml of a similar medium which contained, in addition, 3.0×10^{-3} M $BaCl_2$ and ^{133}Ba (9500 cpm/0.1 ml of medium). In experiment 2, the sacs were bathed in 4.0 ml of a similar medium which contained, in addition, 3.0×10^{-3} M $SrCl_2$ and ^{85}Sr (10,000 cpm/0.1 ml of medium). In experiment 3, the everted sacs were filled with 0.5 ml of a medium containing 0.145 M NaCl, 4.0×10^{-2} M D-mannose, 4.0×10^{-3} M Tris-HCl buffer at pH 7.4, and 8.0×10^{-4} M sodium ascorbate. In this experiment the sacs were bathed in 4.0 ml of a similar medium which contained, in addition, 5.0×10^{-5} M $FeSO_4$ and ^{55}Fe (6200 cpm/0.1 ml of medium). In all of the experiments the everted gut sacs were incubated at 37°C for 2 hr and the results are expressed as the rate of cation transfer to the inside (serosal) medium (cpm/cm² per 2 hr) as described in Methods.

fect previously described (9) and also noted in the present studies, may be related to alterations in the metabolism or localization of vitamin D in the semi-starved state.

Of interest is the fact that repeated experiments failed to demonstrate the presence of a consistent difference in the oxygen-dependent uptake of ^{45}Ca by duodenal tissue slices from sham-operated as opposed to nephrectomized rats when the slices were incubated in medium containing calcium in concentrations between 1.0×10^{-4} M and 1.5×10^{-3} M.

Vitamin D metabolites. It has recently become apparent that after the administration of tritiated vitamin D, radioactive metabolites possessing biologic activity appear in the plasma of man and in the plasma, bone, intestine, kidney, and liver of the rat (see references 37 and 38 for recent reviews). Furthermore, it is likely that the liver is the major site for the metabolic transformation of vitamin D (25, 38). While certain of the metabolites of vitamin D are water soluble and biologically inactive, silicic acid column chromatography of the chloroform-soluble metabolites has led to the separation and identification of numerous peaks, especially those including biologically potent material in peak III (unaltered vitamin D) and peak IV (25-hydroxycholecalciferol). Much recent evidence strongly suggests that 25-hydroxycholecalciferol represents the major and perhaps the sole metabolically active form of vitamin D in the various target tissues (38–42).

In view of the recent report by Avioli et al. (10) demonstrating an abnormality in the metabolism and turnover of plasma D_3-^{3}H in subjects with chronic renal failure, it was of interest to study the effects of experimental uremia on the metabolic transformation of tritiated vitamin D_3. 20 hr after the intraperitoneal administration of 55 IU of vitamin D_3-^{3}H to groups of sham-operated, pair-fed, and nephrectomized animals, the liver, intestinal mucosa, bone, and serum were extracted and the chloroform-soluble metabolites chromatographed as indicated in the Methods section. The tissue distribution of radioactivity (disintegrations per minute per milligram of tissue protein or per milliliter of serum), the distribution between the aqueous and chloroform phases, and the proportion of the chromatographed radioactivity in the biologically active peaks III and IV are not altered by subtotal nephrectomy (Table IX). It

seems unlikely, therefore, that alterations in the metabolism or localization of vitamin D (or its metabolites) can account for the defect in calcium transport and the apparent vitamin D resistance noted in the present studies.

DISCUSSION

While our understanding of the pathogenesis of renal osteodystrophy is far from complete (8), it is generally held that chronic renal failure may be accompanied by a relative resistance to the effects of vitamin D on the intestine and bone resulting in skeletal abnormalities and parathyroid hyperplasia (1–3, 6–8). Buffering of excessive acid by calcium salts may contribute to skeletal demineralization (43), but it is unlikely that this factor plays a major role (8). Experimentally induced acidosis in the rat in the absence of uremia does not influence the active transport of calcium in the intestine (9). While the presence of a state of relative vitamin D resistance with impaired intestinal calcium absorption seems likely, the basis for this defect remains uncertain.

The recent studies by Avioli et al. (10, 44) have provided evidence which suggests that, at least in part, the relative resistance to vitamin D in uremia may be related to alterations in the metabolic transformation of the vitamin to its biologically active form. The authors suggested that in patients with chronic renal disease there may be an abnormal nonultrafiltrable substance or substances which may alter the hepatic or intestinal enzymes responsible for the transformation of vitamin D_3 to its active metabolite(s). In studies concerned with experimentally induced chronic uremia in rats and dogs, Avioli, Lee, Birge, Slatopolsky, and DeLuca (44) confirmed the presence of a derangement in vitamin D metabolism by demonstrating decreased plasma and intestinal mucosal levels of the biologically active 25-hydroxycholecalciferol (chromatographic peak IV). In their studies the intestine from uremic rats showed a decrease in the active transport of calcium and a diminution in the levels of the mucosal calcium-binding protein (44, 45). Whereas treatment of the uremic animals with modest amounts of vitamin D_3 did not significantly affect either the level of calcium-binding protein or the active transport of calcium, both of the defects did respond substantially to the administration of 25-hydroxycholecalciferol, the metabolically active form of vitamin D_3. Of especial interest, however, is the fact that the active transport mechanism remained quite defective despite the administration of supraphysiologic amounts (500 IU) of metabolite which the authors considered lacking (45). Larger, massive doses were not used in these studies.

Since starvation per se may depress the active transport of calcium in the intestine (9), an observation confirmed by the present studies, experiments employing the paired feeding technique were conducted in a relatively short-term uremic animal model. These studies clearly document the presence of a defect in the active transport of calcium in the duodenum of partially nephrectomized uremic animals when compared to sham-operated, pair-fed controls. This defect resists repair with "physiological" doses of vitamin D, and only with large, pharmacological doses of the vitamin can the active transport of calcium in the duodenum be restored to normal.

TABLE VII

Effects of Subtotal Nephrectomy and Diet on Galactose Transfer in Jejunal Gut Sacs

Experimental conditions	No.	Galactose-^{14}C transfer	SE	SD	P
		cpm/cm² per 2 hr			
Experiment 1—random feeding					
Sham-operated	25	7,271	640	3204	<0.01
Nephrectomized	33	11,000	768	4418	
Experiment 2—paired feeding					
Sham-operated	16	10,841	556	2231	>0.10
Nephrectomized	25	9,516	632	3162	

In experiment 1 the animals were randomly fed the standard diet, whereas in experiment 2, the "vitamin D–free" diet and the paired feeding technique were employed. Gut sacs were prepared from 5-cm segments of intestine 15–20 cm and 20–25 cm from the pylorus in each animal, and the mean value of the two sacs from each animal was employed. The sacs were filled with 0.5 ml of a medium containing 0.151 M NaCl, 4.0×10^{-3} M sodium phosphate buffer at pH 7.4, and 2.0×10^{-2} M fructose. The sacs were incubated for 2 hr at 37°C in 4.0 ml of an otherwise identical medium which, in addition, contained 4.0×10^{-4} D-galactose and galactose-^{14}C (8000 cpm/0.1 ml of medium). The results are expressed as the rate of galactose-^{14}C transfer to the inside (serosal) medium (cpm/cm² per 2 hr) as described in Methods.

The effects of renal insufficiency on the rates of calcium transfer noted in these studies are more marked in the duodenum than in either the jejunum or ileum. Furthermore, the defect in the more distal segments requires only modest doses of vitamin D for repair. While previous studies have made it clear that the magnitude of the vitamin D effect is greatest in the duodenum, there is a substantial body of evidence to suggest that the vitamin does affect transport throughout the entire small intestine (29). There is also good reason to believe that the same general mechanisms for calcium transport are operative in both proximal and distal segments of the small intestine (29). It is not at all surprising, therefore, to find that in the present experiments as well as in other studies dealing with factors regulating calcium transport, the most striking effects have been noted in the duodenum (15, 29, 46). Since much of the dietary calcium normally absorbed in intact animals probably traverses the distal small intestine, one may question the physiologic significance of the in vitro findings in the present study. The fact that renal insufficiency of such mild degree has even a small effect on transport in those areas responsible for much of the calcium absorption in vivo suggests that these findings may indeed be of great importance in the intact uremic subject.

The results of the present studies strongly suggest that in addition to the recently proposed mechanism involving an interference with the metabolism of vitamin D (10, 44, 45) renal insufficiency also affects the cellular mechanisms for calcium transport in a manner which, while opposite in direction to that of vitamin D, is independent of a direct interaction with the vitamin or its metabolites. Of note is the fact that in the present studies the decrease in the active transport of calcium in the intestine of the uremic rats is independent of any alteration in the level of the duodenal mucosal calcium-binding protein. This lack of correlation between the transport of calcium and the level of the Ca-BP in the present studies, in and of itself, suggests that the observed effect of renal insufficiency may be unrelated either to alterations in the metabolism of vitamin D or to a direct interaction with the vitamin or its metabolites. Further evidence in support of this view may be derived from the results of the studies concerned with the fate of tritiated vitamin D_3 in the sham-operated, pair-fed controls and the nephrectomized animals. Since the available preparation of D_3-^3H had a relatively low specific activity, a moderately large dose per rat was required. As a result, the per cent of the dose recovered as 25-hydroxycholecalciferol was relatively low in comparison to that noted in certain studies employing smaller doses and longer periods of observation (24, 37, 38). Of importance, however, is the fact that there were no demonstrable differences in either the tissue distribution or metabolism of vitamin D_3 in the sham-operated as opposed to the nephrectomized animals. Furthermore, total bilateral nephrectomy is associated with a depression in the active transport of calcium by the intestine within 20 hr, an effect which is not likely due to depletion of vitamin D stores or an alteration in its metabolism. Additional evidence of a defect in the cellular mechanisms for calcium transport which is somewhat independent of a direct interaction with vitamin D may actually be derived from the studies of Avioli et al. (44, 45). In their experiments, despite improvement with the direct administration of 25-hydroxycholecalciferol to uremic animals, a substantial impairment in calcium transport did persist.

Based on the results of the present studies as well as others (9, 10, 44, 45), it is obvious that the effects of uremia on intestinal transport mechanisms are quite

TABLE VIII

Effects of Subtotal Nephrectomy, Diet, and Vitamin D_3 on Calcium-Binding Protein Activity

Experimental conditions	Calcium-binding protein activity
	cpm bound/ mg of protein
Experiment 1—no additional vitamin D_3	
Sham-operated, random feeding	4,945
Sham-operated, paired feeding	3,588
Nephrectomized, paired feeding	4,225
Experiment 2—vitamin D_3, 2 IU 3 x/wk	
Sham-operated, random feeding	6,547
Sham-operated, paired feeding	4,450
Nephrectomized, paired feeding	4,977
Experiment 3—vitamin D_3, 10 IU 3 x/wk	
Sham-operated, random feeding	Not done
Sham-operated, paired feeding	7,302
Nephrectomized, paired feeding	7,929
Experiment 4—vitamin D_3, 10,000 IU	
Sham-operated, random feeding	13,457
Sham-operated, paired feeding	14,276
Nephrectomized, paired feeding	13,487

The animals were operated upon as described in the text and were maintained on the "vitamin D–free" diet with either the paired or random feeding technique as indicated. Vitamin D supplementation was administered as described in Methods and in the legend to Table IV. The activity of the duodenal mucosal calcium-binding protein was measured as described (17), employing pooled material from three to five animals in each group.

While the results of the four experiments are presented together for clarity, they were conducted on separate days with separate calcium-binding protein assays. Comparisons between groups within an experiment are valid, but direct comparisons between groups in different experiments are probably not justified.

TABLE IX

Serum and Tissue Chromatographic Fractions 20 hr after Injection of 55 IU Vitamin D_3-^3H

Sample	I	II_a	II	III	IV_a	IV	V_{a+b}	VI_{a+b}	VII_{a+b}
Serum									
Sham	0.2	0.6	0.8	66.2	4.6	15.9	3.7	2.5	4.0
Neph.	0.3	0.2	0.5	72.1	1.9	16.1	2.1	1.7	1.8
Small intestine									
Sham	2.7	2.2	6.1	71.7	3.1	4.1	3.2	3.7	3.0
Neph.	2.0	2.3	5.0	78.0	3.4	3.6	1.8	1.5	2.4
Liver									
Sham	1.7	3.0	3.2	72.9	2.9	6.5	2.8	3.1	3.1
Neph.	0.6	3.0	3.2	74.0	3.0	7.2	2.3	3.5	2.4
Bone									
Sham	3.4	0.8	1.7	60.4	5.2	11.1	3.4	7.1	3.3
Neph.	1.3	0.3	1.6	65.5	2.4	12.0	4.9	7.1	2.5

The data are expressed as the per cent of the chromatographed radioactivity and represent values from pooled samples of 18 sham-operated, pair-fed controls and 20 nephrectomized animals (see Methods).

complex. The starvation which accompanies uremia may in and of itself affect not only the intestinal transport of calcium (9), but may also affect the levels of the Ca-BP and the transport of hexoses as noted in the present study. Whether or not starvation may, at least in part, account for the previously described alterations in vitamin D metabolism (10, 44), a process which occurs largely in the liver, remains uncertain. The present studies also demonstrate an impairment in iron transport in the intestine of uremic rats. While the transport of iron is not influenced by vitamin D (29), there is evidence which suggests that the active transfer mechanisms for Ca and Fe do compete for one or more rate-limiting cellular constituents or reactions and that the major site for this competition is not in the mucosal uptake per se, but in the transfer to or towards the serosal surface (30). The alterations in intestinal transport mechanisms associated with uremia may go beyond those processes which are sensitive to vitamin D, suggesting the presence of a more basic defect in intestinal mucosal function. The defect has some specificity, however, for the transport of hexoses and L-leucine are not affected.

The active transport of calcium in the intestine is a two-step process involving uptake at the mucosal surface and a rate-limiting process of transfer to or towards the serosal surface (29). Both of these steps are affected by vitamin D. In experiments measuring the oxygen-dependent uptake of calcium by duodenal slices, a process which is largely a reflection of the first step of transport or uptake at the mucosal surface (29), there were no consistent differences observed between the results with tissue slices from the sham as opposed to the nephrectomized animals. These results, as well as the finding of normal levels of Ca-BP (a factor which probably best correlates with the first step in transport) and the data obtained in experiments dealing with Fe transfer, all suggest that the major defect in calcium transport demonstrable in these studies is in the second or rate-limiting step of transfer to or towards the serosal surface.

The results of the present studies probably complement those of Avioli et al. (10, 44, 45). In their studies dietary intake may well have differed from the conditions employed in these experiments, and the duration of the uremic process was considerably longer. It seems quite probable then that the basis for the so-called vitamin D resistance in uremia is complex and that it is due not only to a derangement in the metabolism of vitamin D as noted previously (10, 44), but also to a vitamin D–independent effect of uremia on the biochemical processes mediating calcium transport in the intestine. It is not surprising to find an effect of uremia on the cellular mechanisms responsible for calcium transport in the gut, for there have been reports concerned with the effects of uremia or of uremic serum on a host of biochemical reactions in a variety of tissues (47–49). Elucidation of the basis for this defect in uremia and the effects of massive doses of vitamin D awaits a more complete understanding of the processes mediating transcellular calcium transport in the intestine.

ACKNOWLEDGMENTS

This work was supported by Grants AM-05234, AM-07625, and AM-13696 from the National Institutes of Health of the United States Public Health Service.

REFERENCES

1. Liu, S. H., and H. I. Chu. 1943. Studies of calcium and phosphorus metabolism with special reference to pathogenesis and effect of dihydrotachysterol (A.T. 10) and iron. *Medicine.* **22**: 103.
2. Dent, C. E., C. M. Harper, and G. R. Philpot. 1961. The treatment of renal-glomerular osteodystrophy. *Quart. J. Med.* **30**: 1.
3. Stanbury, S. W., and G. A. Lumb. 1962. Metabolic studies of renal osteodystrophy. I. Calcium phosphorus and nitrogen metabolism in rickets, osteomalacia and hyperparathyroidism complicating chronic uremia and in the osteomalacia of the adult Fanconi syndrome. *Medicine.* **41**: 1.
4. Kaye, M., M. Silverman, and T. Thibault. 1965. Calcium metabolism in chronic renal failure. *J. Lab. Clin. Med.* **66**: 535.
5. Ford, F. J. 1931. Calcium and phosphorus metabolism in nephritis. *Arch. Dis. Childhood.* **6**: 209.
6. Fletcher, R. F., J. H. Jones, and D. B. Morgan. 1963. Bone disease in chronic renal failure. *Quart. J. Med.* **32**: 321.
7. Stanbury, S. W., and G. A. Lumb. 1967. The osteomalacia of renal insufficiency. *In* L'Osteomalacie. Massoon et Cie, Paris. 367.
8. Stanbury, S. W. 1968. Bone disease in uremia. *Amer. J. Med.* **44**: 714.
9. Kessner, D. M., and F. H. Epstein. 1965. Effect of renal insufficiency on gastrointestinal transport of calcium. *Amer. J. Physiol.* **209**: 141.
10. Avioli, L. V., S. Birge, S. W. Lee, and E. Slatopolsky. 1968. The metabolic fate of vitamin D_3-^3H in chronic renal failure. *J. Clin. Invest.* **47**: 2239.
11. McCance, R. A., and A. B. Morrison. 1956. The effect of equal and limited rations of water, and 1, 2, and 3 percent solutions of sodium chloride on partially nephrectomized and normal rats. *Quart. J. Exp. Physiol. Cog. Med. Sci.* **41**: 365.
12. Wilson, T. H., and G. Wiseman. 1954. The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. *J. Physiol. (London).* **123**: 116.
13. Schachter, D., and S. M. Rosen. 1959. Active transport of Ca^{45} by the small intestine and its dependence on vitamin D. *Amer. J. Physiol.* **196**: 357.
14. Bray, G. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**: 279.
15. Kimberg, D. V., D. Schachter, and H. Schenker. 1961. Active transport of calcium by intestine: effects of dietary calcium. *Amer. J. Physiol.* **200**: 1256.
16. Schachter, D., E. B. Dowdle, and H. Schenker. 1960. Accumulation of Ca^{45} by slices of the small intestine. *Amer. J. Physiol.* **198**: 275.
17. Wasserman, R. H., and A. N. Taylor. 1966. Vitamin D_3-induced calcium-binding protein in chick intestinal mucosa. *Science (Washington).* **152**: 791.
18. Roscoe, M. H. 1953. The estimation of creatinine in serum. *J. Clin. Pathol. (London).* **6**: 201.
19. Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375.
20. Saifer, A., and S. Gerstenfeld. 1958. The photometric microdetermination of blood glucose with glucose oxidase. *J. Lab. Clin. Med.* **51**: 448.
21. Hohorst, H. J. 1963. L-(+)-Lactate determination with lactic dehydrogenase and DPN. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim/Bergstr. 266–270.
22. Dell, R. B., and R. W. Winters. 1967. Lactate gradients in the kidney of the dog. *Amer. J. Physiol.* **213**: 301.
23. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**: 911.
24. Lund, J., and H. F. DeLuca. 1966. Biologically active metabolite of vitamin D_3 from bone, liver, and blood serum. *J. Lipid Res.* **7**: 739.
25. Ponchon, G., A. L. Kennan, and H. F. DeLuca. 1969. "Activation" of vitamin D by the liver. *J. Clin. Invest.* **48**: 2032.
26. Herberg, R. J. 1960. Determination of carbon-14 and tritium in blood and other tissues. Liquid scintillation counting of tissues. *Anal. Chem.* **32**: 42.
27. Krawitt, E. L., and H. P. Schedl. 1968. In vivo calcium transport by rat small intestine. *Amer. J. Physiol.* **214**: 232.
28. Schachter, D., E. B. Dowdle, and H. Schenker. 1960. Active transport of calcium by the small intestine of the rat. *Amer. J. Physiol.* **198**: 263.
29. Schachter, D., D. V. Kimberg, and H. Schenker. 1961. Active transport of calcium by intestine: action and bio-assay of vitamin D. *Amer. J. Physiol.* **200**: 1263.
30. Manis, J. G., and D. Schachter. 1962. Active transport of iron by intestine: features of the two-step mechanism. *Amer. J. Physiol.* **203**: 73.
31. Boucot, N. G., E. K. Nurser, and J. P. Merrill. 1960. Carbohydrate metabolism in rats with chronic uremia. *Amer. J. Physiol.* **198**: 797.
32. Crane, R. K., and P. Mandelstam. 1960. The active transport of sugars by various preparations of hamster intestine. *Biochim. Biophys. Acta.* **45**: 460.
33. Neale, R. J., and G. Wiseman. 1968. Active transport of L-glucose by isolated small intestine of the dietary-restricted rat. *J. Physiol. (London).* **198**: 601.
34. Taylor, A. N., and R. H. Wasserman. 1967. Vitamin D_3-induced calcium-binding protein: partial purification, electrophoretic visualization, and tissue distribution. *Arch. Biochem. Biophys.* **119**: 536.
35. Wasserman, R. H., and A. N. Taylor. 1968. Vitamin D-dependent calcium-binding protein. Response to some physiological and nutritional variables. *J. Biol. Chem.* **243**: 3987.
36. Ebel, J. G., A. N. Taylor, and R. H. Wasserman. 1969. Vitamin D-induced calcium-binding protein of intestinal mucosa. Relation to vitamin D dose level and lag period. *Amer. J. Clin. Nutr.* **22**: 431.
37. DeLuca, H. F. 1967. Mechanism of action and metabolic fate of vitamin D. *Vitamins Hormones.* **25**: 315.
38. DeLuca, H. F. 1969. Recent advances in the metabolism and function of vitamin D. *Fed. Proc.* **28**: 1678.
39. Blunt, J. W., Y. Tanaka, and H. F. DeLuca. 1968. The biological activity of 25-hydroxycholecalciferol, a metabolite of vitamin D_3. *Proc. Nat. Acad. Sci. U. S. A.* **61**: 717.
40. Morrii, H., J. Lund, P. F. Neville, and H. F. DeLuca. 1967. Biological activity of vitamin D metabolite. *Arch. Biochem. Biophys.* **120**: 508.
41. Trummel, C. L., L. G. Raisz, J. W. Blunt, and H. F. DeLuca. 1969. 25-Hydroxycholecalciferol: stimulation of bone resorption in tissue culture. *Science (Washington).* **163**: 1450.

42. Olson, E. B., and H. F. DeLuca. 1969. 25-Hydroxycholecalciferol: direct effect on calcium transport. *Science (Washington)*. **165**: 405.
43. Relman, A. S. 1968. The acidosis of renal disease. *Amer. J. Med.* **44**: 706.
44. Avioli, L. V., S. W. Lee, S. J. Birge, E. Slatopolsky, and H. F. DeLuca. 1969. The nature of the defect in intestinal calcium absorption in chronic renal disease. *J. Clin. Invest.* **48**: 4a. (Abstr.)
45. Avioli, L. V., S. Scott, S. W. Lee, and H. F. DeLuca. 1969. Intestinal calcium absorption: nature of defect in chronic renal disease. *Science (Washington)*. **166**: 1154.
46. Finkelstein, J. D., and D. Schachter. 1962. Active transport of calcium by intestine: effects of hypophysectomy and growth hormone. *Amer. J. Physiol.* **203**: 873.
47. McCormick, G. J., L. Shear, and K. G. Barry. 1966. Alteration of hepatic protein synthesis in acute uremia. *Proc. Soc. Exp. Biol. Med.* **122**: 99.
48. Clayton, E. M., D. Seligson, and H. Seligson. 1965. Inhibition of protein synthesis by N-methyl-2-pyridone-5-formamidoacetic acid and other compounds isolated from uremic patients. *Yale J. Biol. Med.* **38**: 273.
49. Hicks, J. M., D. S. Young, and I. D. P. Wootton. 1964. The effect of uremic blood constituents on certain cerebral enzymes. *Clin. Chim. Acta.* **9**: 228.