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Research Article

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Effects of 1,25-Dihydroxycholecalciferol on Intestinal Calcium Transport in Cortisone-Treated Rats

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ABSTRACT The administration of glucocorticoids may decrease intestinal calcium absorption in vivo and the active transport of calcium in rat duodenum in vitro. It has been suggested that this apparent "anti-vitamin D-like" effect of steroid hormones may be related to alterations in vitamin D metabolism. In order to test this hypothesis, vitamin D-deficient control and cortisone-treated rats were given an intraperitoneal injection of 5.5 IU of 1,25-dihydroxycholecalciferol (1,25-DHCC), the probable end-organ active vitamin D metabolite in the intestine, and 16 h later studies of duodenal calcium transport were performed in modified Ussing chambers. In the vitamin D-deficient state, cortisone administration was associated with a diminution in J_{MS}, J_{Net}, and the flux ratio (J_{MS}/J_{SM}). While the magnitude of the increases in J_{MS} and J_{Net} that resulted from 1,25-DHCC treatment were approximately the same in control and cortisone-treated animals, 1,25-DHCC failed to restore these parameters to "normal levels" in the steroid-treated rats. Furthermore, contrary to the results obtained in the saline-treated controls, 1,25-DHCC failed to reduce J_{SM} in the duodenum from cortisone-treated rats. The cortisone-related defect in calcium transport was due to alterations in both unidirectional calcium fluxes (decrease in J_{MS} and increase in J_{SM}), such that the J_{Net} and the flux ratio (J_{MS}/J_{SM}) were only approximately 50% of the levels achieved in vitamin D-deficient control animals repleted with the same dose of 1,25-DHCC.

The administration of 1,25-DHCC was accompanied by a marked increase in the serum calcium levels of control rats, but there was no such response in the cortisone-treated group.

The results support the concept that under the conditions of these experiments in the rat the apparent antagonism between glucocorticoids and vitamin D may be due to steroid hormone-related alterations in end organ function that are independent of any direct interaction between the hormone and the vitamin and that cannot be reversed by the vitamin.

INTRODUCTION

The administration of glucocorticoids may decrease the intestinal absorption of dietary calcium in normal humans (1), as well as in those with sarcoidosis and vitamin D intoxication (2, 3). Furthermore, these hormones can decrease the active transport of calcium in vitro with preparations of rat small intestine (4–6). It has been shown that cortisone administration in the rat does not affect the hepatic conversion of cholecalciferol, the parent vitamin D_3, to its circulating active form, 25-hydroxycholecalciferol (25-HCC) [1] (6, 7). Moreover, the impairment in calcium transport in the intestine of cortisone-treated animals cannot be corrected by the administration of pharmacologic doses of either cholecalciferol or 25-HCC (6). These observations have led to the concept that the apparent antagonism between vitamin D and glucocorticoid administration may be due to either: (*a*) a steroid hormone-related defect in the formation and/or localization of the more polar dihydroxyl tissue active forms of the vitamin; or (*b*) an influence of glucocorticoids on the cellular

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[1] *Abbreviations used in this paper:* 25-HCC, 25-hydroxycholecalciferol; 1,25-DHCC, 1,25-dihydroxycholecalciferol; J_{MS}, unidirectional flux mucosal to serosal; J_{SM}, unidirectional flux serosal to mucosal; J_{Net}, net flux defined as $J_{MS} - J_{SM}$; PD, transmural electric potential difference; SCC, short-circuit current.

mechanisms mediating calcium transport in a manner that is independent of any direct interaction with the parent vitamin or its metabolites (6–8).

There is now a great deal of evidence to indicate that 25-HCC, formed from the parent vitamin in the liver (9), is subsequently converted in the kidney (10–12) to a more polar metabolite that has been shown to be 1,25-dihydroxycholecalciferol (1,25-DHCC) (13–15). This latter dihydroxyl metabolite is the predominant form of the vitamin in the intestinal mucosa (16, 17), and it is probably the end-organ active form of the vitamin in the gut (18, 19). Recent animal studies in this laboratory demonstrated that cortisone administration influences neither the metabolic conversion of 25-HCC to 1,25-DHCC nor the localization of this metabolite in end organs including intestinal mucosa (8). The results of these studies strongly suggested that defective calcium transport following cortisone administration in the rat is not due to an alteration in the production or localization of 1,25-DHCC.

The present study was undertaken to determine directly whether or not the administration of 1,25-DHCC, the probable end-organ active metabolite, could overcome the steroid hormone-related defect in the active transport of calcium in the intestine. The results furnish additional support for the concept that glucocorticoids may alter calcium transport quite independently of any direct interaction with vitamin D metabolites.

METHODS

Animal preparation. Albino male rats of the Sherman strain were obtained as weanlings weighing 30–50 g (Camm Research Institute, Inc., Wayne, N. J.), and were raised in the dark on a vitamin D-deficient diet containing 0.80% calcium, 0.35% phosphorous, and 0.24% magnesium (General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio). During the 5th wk on the diet the animals were randomized into four treatment groups that were matched for weight (mean = 218 g). Two groups received 10 mg of cortisone acetate (cortone acetate in saline suspension, Merck, Sharp & Dohme, West Point, Pa.) subcutaneously each day for the 7 days prior to sacrifice. Two control groups received daily subcutaneous injections of 0.4 ml of isotonic saline during the 7 days prior to sacrifice. 16 h before sacrifice, 5.5 IU of 1,25-dihydroxycholecalciferol (1,25-DHCC) was administered intraperitoneally in 0.25 ml ethanol: propanediol, 50:50 (vol/vol) to each of the animals in one control and one steroid-treated group. The remaining animals received an intraperitoneal injection of the vehicle. All animals were then fasted until time of sacrifice. The 1,25-DHCC was generously provided by Dr. Anthony W. Norman, Riverside, Calif.

Transport studies. 16 h following the administration of either 1,25-DHCC or the vehicle, the fasting animals were stunned by concussion and exsanguinated. Blood was collected for subsequent determinations of serum calcium and magnesium by atomic absorption spectrometry and of serum inorganic phosphorous by the method of Fiske and Subbarow (20). Unidirectional transmural fluxes of calcium across the proximal duodenum were studied in vitro using a modification of the apparatus of Ussing and Zerahn (21) as described by Walling and Rothman (22) except that each compartment contained 10 ml of a bicarbonate-buffered Krebs-Ringer solution (pH 7.4), and the exposed tissue area was 0.45 cm^2. The bicarbonate-buffered Krebs-Ringer solution had the following ionic composition in millimoles per liter: Na, 144; K, 4.7; Ca, 0.5; Mg, 1.2; Cl, 127; HCO_3, 25; SO_4, 1.2; D-glucose, 11; and no inorganic phosphorous. Calcium fluxes were measured with $^{45}CaCl_2$ as previously described (22). Net flux was measured on paired tissues from the same animal and was calculated by the equation $J_{Net} = J_{MS} - J_{SM}$, where J_{Net} is net flux and J_{MS} and J_{SM} are the unidirectional transmural fluxes, mucosal to serosal and serosal to mucosal, respectively. Fluxes were calculated by the method of Schultz and Zalusky (23), and only mean steady-state values are reported.

Electrical measurements on the mounted tissues were made in a manner similar to that described by Field, Fromm, and McColl (24). The transmural electric potential difference (PD) across the mounted duodenum was monitored at the beginning and end of the experiment and was otherwise nulled by passing a short-circuit current (SCC). Tissue resistance was measured at 60 min, after the preparation was permitted to reach steady state under open-circuited conditions.

Statistical analyses. Paired *t* tests were employed to determine the significance of differences between J_{MS} and J_{SM} within groups. Since J_{Net} is a dependent variable of J_{MS} and J_{SM}, two-tailed *t* tests were used to evaluate differences in J_{Net} between the various treatment groups. One-way analyses of variance were employed to examine differences between J_{MS}, J_{SM}, and serum chemistries in the various groups, and a table of "least significant difference" was constructed to evaluate confidence intervals (25).

RESULTS

The effects of 1,25-DHCC administration on in vitro duodenal calcium transport and on serum levels of calcium, magnesium, and phosphorous in control and cortisone-treated, vitamin D-deficient rats are summarized in Tables I and II. Net fluxes, J_{Net} in the absorptive direction and ratios of J_{MS}/J_{SM} in excess of 1.0, findings consistent with active calcium transport in the duodenum, were observed in all but the vitamin D-deficient, cortisone-treated group where J_{SM} actually exceeded a markedly reduced J_{MS}, resulting in net calcium secretion (Table I). While the magnitude of the increases in J_{MS} and in J_{Net} which resulted from 1,25-DHCC treatment were approximately the same in both control and cortisone-treated animals, 1,25-DHCC failed to restore these parameters to "normal levels" in the steroid-treated group. Furthermore, contrary to the results obtained in the saline-treated controls, 1,25-DHCC did not reduce J_{SM} in the duodenum from cortisone-treated animals. The failure of 1,25-DHCC to completely overcome the cortisone-related defect in J_{MS}, coupled with the lack of responsiveness of J_{SM} to 1,25-DHCC in the steroid-treated group, accounted for the persistent decrease of approximately 50% in both the net flux of calcium (J_{Net}) and in the flux ratio (J_{MS}/J_{SM}) in the glucocorticoid-treated animals.

TABLE I

Effects of Cortisone and 1,25-Dihydroxycholecalciferol (1,25-DHCC) Administration on Duodenal Calcium Transport

Experimental conditions	Number	Calcium flux			J_{MS}/J_{SM}	1 h PD	1 h SCC	R_I
		J_{MS}	J_{SM}	J_{Net}*				
		$nmol \cdot cm^{-2} \cdot h^{-1} \pm SEM$				mV	$\mu A \cdot cm^{-2}$	$\Omega \cdot cm^{-2}$
Vitamin D-deficient, saline	6	19.1±2.3[1]	10.6±0.4	8.5±2.6[6,10]	1.8	2.4±0.4	38.7±4.6	37.7±2.2[18]
Vitamin D-deficient, cortisone	6	9.0±0.5	11.0±0.7	−2.0±0.7[7]	0.8	2.3±0.2	41.2±5.6	40.9±3.1[18]
1,25-DHCC, saline	6	30.6±2.7[2,3]	6.4±0.6[5]	24.2±2.5[8,11,12]	4.8	3.1±0.2	41.5±5.1	48.0±5.5
1,25-DHCC, cortisone	7	23.3±2.9[4]	10.7±0.9	12.6±2.2[9,13]	2.2	5.2±0.3[14,15]	80.6±9.7[16,17]	38.3±5.4

The preparation of animals and the measurement of transmural fluxes and electrical changes are described in Methods. PD, transmural potential difference; SCC, short-circuit current; R_I, intestinal resistance.

* $J_{Net} = J_{MS} - J_{SM}$. Negative values are secretion; positive values are absorption.

[1] Vitamin D-deficient, saline J_{MS} > vitamin D-deficient, cortisone J_{MS}; $P < 0.01$.

[2] 1,25-DHCC, saline J_{MS} > vitamin D-deficient, saline J_{MS}; $P < 0.005$.

[3] 1,25-DHCC, saline J_{MS} > 1,25-DHCC, cortisone J_{MS}; $P < 0.05$.

[4] 1,25-DHCC, cortisone J_{MS} > vitamin D-deficient, cortisone J_{MS}; $P < 0.001$.

[5] Vitamin D-deficient, saline J_{SM} > 1,25-DHCC, saline J_{SM}; $P < 0.001$.

[6] Vitamin D-deficient, saline J_{MS} > vitamin D-deficient, saline J_{SM}; $P < 0.025$.

[7] Vitamin D-deficient, cortisone J_{SM} > Vitamin D-deficient, cortisone J_{MS}; $P < 0.05$.

[8] 1,25-DHCC, saline J_{MS} > 1,25-DHCC, saline J_{SM}; $P < 0.001$.

[9] 1,25-DHCC, cortisone J_{MS} > 1,25-DHCC, cortisone J_{SM}; $P < 0.005$.

[10] Vitamin D-deficient, saline J_{Net} > Vitamin D-deficient, cortisone J_{Net}; $P < 0.005$.

[11] 1,25-DHCC, saline J_{Net} > vitamin D-deficient, saline J_{Net}; $P < 0.005$.

[12] 1,25-DHCC, saline J_{Net} > 1,25-DHCC, cortisone J_{Net}; $P < 0.01$.

[13] 1,25-DHCC, cortisone J_{Net} > vitamin D-deficient, cortisone J_{Net}; $P < 0.001$.

[14] 1,25-DHCC, cortisone PD_{60} > 1,25-DHCC, saline PD_{60}; $P < 0.001$.

[15] 1,25-DHCC, cortisone PD_{60} > vitamin D-deficient, cortisone PD_{60}; $P < 0.001$.

[16] 1,25-DHCC, cortisone I_{SCC60} > 1,25-DHCC, saline I_{SCC60}; $P < 0.001$.

[17] 1,25-DHCC, cortisone I_{SCC60} > vitamin D-deficient, cortisone I_{SCC60}; $P < 0.001$.

[18] Tissue resistances for all groups are not different; $F_{3,23} = 1.147$; $P < 0.25$.

Another noteworthy effect of cortisone administration was found in the measurements of PD and SCC in the group given both cortisone and 1,25-DHCC (Table I). These values were nearly double those observed in the other three groups, while the resistance of the intestine was the same for all groups. These changes in PD and SCC are probably related to the enhanced transport of glucose (26) observed in the small intestine of glucocorticoid-treated rats, if the stimulation in SCC is assumed to be the result of sodium and glucose cotransport (23). Of note is the fact that the effect of cortisone administration on the transmural PD was not observed in a previous study from this laboratory (6) in which fructose, a nonactively transported hexose, replaced glucose in the incubation medium.

As noted in Table II, serum calcium levels were initially somewhat higher in the cortisone-treated, vitamin D-deficient animals than in the vitamin D-deficient controls. While the administration of 1,25-DHCC was followed by a striking increase in the serum calcium levels of control rats, there was no such response in the cortisone-treated group. 1,25-DHCC caused a moderate elevation in serum phosphate levels in both the control and cortisone-treated groups, whereas serum magnesium levels were uninfluenced by all of the experimental conditions.

DISCUSSION

The results of the present study show that a 5.5 IU dose of 1,25-DHCC failed to restore duodenal calcium transport to "normal levels" in vitamin D-deficient, cortisone-treated rats. The cortisone-related defect in calcium transport is due to alterations in both unidirectional calcium fluxes (decrease in J_{MS} and increase in J_{SM}), such that the net flux (J_{Net}) and the flux ratio (J_{MS}/J_{SM}) are only approximately 50% of the levels achieved in vitamin D-deficient control animals repleted with the same dose of 1,25-DHCC. The fact that

TABLE II

Effect of Cortisone and 1,25-Dihydroxycholecalciferol (1,25-DHCC) Administration on Serum Calcium, Magnesium and Phosphorous

Experimental conditions	Number	Serum calcium	Serum magnesium	Serum phosphorous
		mM	*mM*	*mM*
Vitamin D-deficient, saline	11	1.60±0.04*	1.13±0.05[5]	3.36±0.24
Vitamin D-deficient, cortisone	12	1.81±0.08[1]	1.11±0.03	3.41±0.24
1,25-DHCC, saline	8	2.35±0.12[2,3]	1.18±0.05	4.10±0.19
1,25-DHCC, cortisone	9	1.95±0.06[4]	1.08±0.05	4.16±0.24[6]

The preparation of animals and the procedures employed for determination of serum calcium, magnesium, and phosphorous are described in Methods.

* SEM.

[1] Vitamin D-deficient, cortisone serum calcium > vitamin D-deficient, saline serum calcium; $P < 0.05$.

[2] 1,25-DHCC, saline serum calcium > vitamin D-deficient, saline serum calcium; $P < 0.001$.

[3] 1,25-DHCC, saline serum calcium > vitamin D-deficient, cortisone serum calcium; $P < 0.001$.

[4] 1,25-DHCC, cortisone serum calcium > vitamin D-deficient, cortisone serum calcium; $P < 0.30$.

[5] Serum magnesium means for all groups are not different; $F_{3,36} = 1.018$; $P > 0.25$.

[6] 1,25-DHCC, saline and 1,25-DHCC, cortisone serum phosphorous > vitamin D-deficient, saline and vitamin D-deficient, cortisone serum phosphorous; $P < 0.005$.

this impairment in calcium absorption persists despite what appears to be a quantitatively normal response of J_{MS} and J_{Net} to 1,25-DHCC, suggests that glucocorticoids do not simply induce a state of relative end-organ resistance to the effects of vitamin in the intestine.

Studies by Avioli, Birge, and Lee (27) provided evidence suggesting that the administration of prednisone to normal human volunteers might interfere with the conversion of vitamin D_3 (cholecalciferol) to 25-HCC. Unfortunately, only the plasma metabolites were studied, and these were examined at only one point in time (24 h), during what may represent the early phase of a biphasic semilogarithmic disappearance curve with the half-life of the second phase measurable in weeks (28). Recently, Kimberg, Baerg, Gershon, and Graudusius (6) and Schaefer, von Herrath, Koch, and Opitz (7) reported that cortisone treatment in the rat does not interfere with the conversion of vitamin D_3 to 25-HCC. It was furthermore shown that the administration of multiple pharmacologic doses of cholecalciferol (12,000 IU) or 25-HCC (50 IU) to glucocorticoid-treated animals failed to completely correct the hormone-related defect in intestinal calcium transport (6). Based on the results of these previous studies (6, 7) it was clear that if indeed cortisone exerted its apparent anti-vitamin D-like effect by interfering with the metabolism of vitamin D, it did so at a step subsequent to 25-hydroxylation in the liver.

At the present time there is good reason to believe that 1,25-DHCC is the end-organ active metabolite of vitamin D in the intestine (13–19). Recent studies in this laboratory (8) have shown that cortisone treatment does not interfere with the metabolic conversion of 25-HCC to 1,25-DHCC in the rat. Furthermore, the target tissue distribution and the subcellular localization of 1,25-DHCC in the intestinal mucosal cell are not altered by cortisone administration (8). This lack of any apparent cortisone-related defect in the formation and localization of 1,25-DHCC, coupled with the present demonstration of a persistent steroid hormone-related impairment in calcium transport despite a substantial response to exogenous 1,25-DHCC, make it appear rather unlikely that glucocorticoids exert their apparent anti-vitamin D-like effect by interfering with the formation or localization of essential vitamin D metabolites.

It now seems probable that in the rat the cortisone-induced defect in the intestinal transport of calcium is due to hormone-related biochemical alterations in intestinal epithelial cell functions which interfere with the calcium transport mechanism, but which do not involve any direct interaction between the steroid and the vitamin. Although not observed in previous studies of calcium transport employing the in vitro gut sac technique (6, 29), cortisone did interfere with calcium transport even in the vitamin D-deficient animals employed in the present experiments. Also of significance in this regard are the observations that cortisone administration, while depressing calcium transport, does not interfere with the formation of the vitamin D-dependent calcium-binding protein in the intestinal mucosa (6, 29) or with the vitamin D-related rise in in-

testinal mucosal Ca^{++}-ATPase activity (29). Moreover, it has been shown that glucocorticoid administration impairs intestinal iron transport (6) and enhances the transport of both glucose (26) and galactose (6). Although these transport functions are presumably not directly dependent upon vitamin D (30), calcium and iron (31), and calcium and actively transported hexoses (30) may compete for one or more constituents or reactions involved in transmural transport. The present observation that the cortisone-induced increases in PD and SCC, presumably due to sodium and glucose cotransport occurred only after 1,25-DHCC administration, suggests that in this regard there may be some type of a synergistic interaction between the steroid hormone and the vitamin.

The significant elevation in serum calcium concentration following 1,25-DHCC administration in control animals and the lack of such a response to the vitamin D metabolite following cortisone treatment, suggests that glucocorticoids may, in addition to their effects on the intestine, alter the bone-mobilizing properties of 1,25-DHCC (32). Further studies will be required to elucidate the nature of the interaction of 1,25-DHCC and cortisone on both bone metabolism and certain intestinal transport mechanisms. Finally, the relevance, if any, of the present observations to those clinical situations in which glucocorticoids may lower serum calcium levels remains to be established (2, 3).

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REFERENCES

1. Wajchenberg, B. L., V. G. Pereira, J. Kieffer, and S. Ursic. 1969. Effect of dexamethasone on calcium metabolism and ^{47}Ca kinetics in normal subjects. *Acta Endocrinol.* **61**: 173.
2. Henneman, P. H., E. F. Dempsey, E. L. Carroll, and F. Albright. 1956. The cause of hypercalcuria in sarcoid and its treatment with cortisone and sodium phytate. *J. Clin. Invest.* **35**: 1229.
3. Verner, J. V., Jr., F. L. Engel, and H. T. McPherson. 1958. Vitamin D intoxication: report of two cases treated with cortisone. *Ann. Intern. Med.* **48**: 765.
4. Harrison, H. E., and H. C. Harrison. 1960. Transfer of Ca^{45} across intestinal wall in vitro in relation to action of vitamin D and cortisol. *Am. J. Physiol.* **199**: 265.
5. Williams, G. H., E. N. Bowser, W. J. Henderson, and V. Uzgiries. 1961. Effects of vitamin D and cortisone on intestinal absorption of calcium in the rat. *Proc. Soc. Exp. Biol. Med.* **106**: 664.
6. Kimberg, D. V., R. D. Baerg, E. Gershon, and R. T. Graudusius. 1971. Effect of cortisone on the active transport of calcium by the small intestine. *J. Clin. Invest.* **50**: 1309.
7. Schaefer, K., D. von Herrath, H.-V. Koch, and A. Opitz. 1971. Effect of cortisone on vitamin D metabolism. *Isr. J. Med. Sci.* **7**: 533.
8. Favus, M. J., D. V. Kimberg, G. N. Millar, and E. Gershon. 1973. Effect of cortisone administration on the metabolism and localization of 25-hydroxycholecalciferol in the rat. *J. Clin. Invest.* In press.
9. Ponchon, G., A. L. Kennan, and H. F. DeLuca. 1969. "Activation" of vitamin D by the liver. *J. Clin. Invest.* **48**: 2032.
10. Fraser, D. R., and E. Kodicek. 1970. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature (Lond.)*. **228**: 764.
11. Norman, A. W., R. J. Midgett, J. F. Myrtle, and H. G. Nowicki. 1971. Studies on calciferol metabolism I. Production of vitamin D metabolite 4B from 25-OH-cholecalciferol by kidney homogenates. *Biochem. Biophys. Res. Commun.* **42**: 1082.
12. Gray, R., I. Boyle, and H. F. DeLuca. 1971. Vitamin D metabolism: the role of kidney tissue. *Science (Wash. D. C.)*. **172**: 1232.
13. Lawson, D. E. M., D. R. Fraser, E. Kodicek, H. R. Morris, and D. H. Williams. 1971. Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. *Nature (Lond.)*. **230**: 228.
14. Holick, M. F., H. K. Schnoes, and H. F. DeLuca. 1971. Identification of 1,25-dihydroxycholecalciferol, a form of vitamin D_3 metabolically active in the intestine. *Proc. Natl. Acad. Sci. U. S. A.* **68**: 803.
15. Norman, A. W., J. F. Myrtle, R. J. Midgett, H. G. Nowicki, V. Williams, and G. Popják. 1971. 1,25-Dihydroxycholecalciferol: identification of the proposed active form of vitamin D_3 in the intestine. *Science (Wash. D. C.)*. **173**: 51.
16. Myrtle, J. F., M. R. Haussler, and A. W. Norman. 1970. Evidence for the biologically active form of cholecalciferol in the intestine. *J. Biol. Chem.* **245**: 1190.
17. Lawson, D. E. M., P. W. Wilson, and E. Kodicek. 1969. Metabolism of vitamin D: a new cholecalciferol metabolite, involving loss of hydrogen at C-1, in chick intestinal nuclei. *Biochem. J.* **115**: 269.
18. Myrtle, J. F., and A. W. Norman. 1971. Vitamin D: a cholecalciferol metabolite highly active in promoting intestinal calcium transport. *Science (Wash. D. C.)*. **171**: 79.
19. Omdahl, J., M. Holick, T. Suda, Y. Tanaka, and H. F. DeLuca. 1971. Biological activity of 1,25-dihydroxycholecalciferol. *Biochemistry*. **10**: 2935.
20. Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorous. *J. Biol. Chem.* **66**: 375.
21. Ussing, H. H., and K. Zerahn. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**: 110.
22. Walling, M. W., and S. S. Rothman. 1969. Phosphate-independent carrier-mediated active transport of calcium by rat intestine. *Am. J. Physiol.* **217**: 1144.
23. Schultz, S. G., and R. Zalusky. 1964. Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes. *J. Gen. Physiol.* **47**: 567.
24. Field, M., D. Fromm, and I. McColl. 1971. Ion trans-

port in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. *Am. J. Physiol.* **220**: 1388.
25. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. The Iowa State University Press, Ames. 6th edition. 272.
26. Banerjee, S., and S. D. Varma. 1966. Effect of diabetogenic hormones on transport of glucose in small intestine in vitro. *Proc. Soc. Exp. Biol. Med.* **123**: 212.
27. Avioli, L. V., S. J. Birge, and S. W. Lee. 1968. Effects of prednisone on vitamin D metabolism in man. *J. Clin. Endocrinol. Metab.* **28**: 1341.
28. Mawer, E. B., G. A. Lumb, and S. W. Stanbury. 1969. Long biological half-life of vitamin D_3 and its polar metabolites in human serum. *Nature (Lond.)*. **222**: 482.
29. Krawitt, E. L. 1972. The role of intestinal transport proteins in cortisone-mediated suppression of Ca^{2+} absorption. *Biochim. Biophys. Acta.* **274**: 179.
30. Schachter, D., D. V. Kimberg, and H. Schenker. 1961. Active transport of calcium by intestine: action and bioassay of vitamin D. *Am. J. Physiol.* **200**: 1263.
31. Manis, J. G., and D. Schachter. 1962. Active transport of iron by intestine: features of the two-step mechanism. *Am. J. Physiol.* **203**: 73.
32. Tanaka, Y., and H. F. DeLuca. 1971. Bone mineral mobilization activity of 1,25-dihydroxycholecalciferol, a metabolite of vitamin D. *Arch. Biochem. Biophys.* **146**: 574.