Effect of Cortisone Treatment on the Active Transport of Calcium by the Small Intestine

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ABSTRACT It is generally recognized that glucocorticoid administration may diminish calcium absorption in vivo as well as the active transport of calcium by the intestine in vitro. Recent studies by others have emphasized the possibility of an alteration in the metabolism of vitamin D to 25-hydroxycholecalciferol in accounting for the steroid effects on calcium absorption. The results obtained in the present studies fail to support this hypothesis.

The present studies confirm that the administration of cortisone or other glucocorticoids to the rat interferes with the active transport of calcium by duodenal gut sacs in vitro. This abnormality is not due to an alteration in the permeability of the intestine to calcium, and it cannot be corrected by the administration of either massive doses of vitamin D_3 or modest doses of 25-hydroxycholecalciferol. Experiments concerned with the effects of cortisone on the level of the vitamin D-dependent duodenal calcium-binding protein, the amount of bioassayable vitamin D activity in the mucosa, and the distribution and metabolism of ^{3}H-vitamin D_3, did not provide evidence in favor of a hormone-related defect in either the localization of vitamin D or its metabolism to 25-hydroxycholecalciferol. Alterations in the transport of iron and D-galactose, not dependent on vitamin D, suggest that cortisone treatment may be responsible for more than a simple antagonism to the effects of vitamin D.

The results of the present studies indicate that cortisone administration affects the cellular mechanisms mediating calcium transport in a manner that is opposite to the effects of vitamin D, but seems to be independent of any direct interaction with the parent vitamin or its metabolites. If a disorder in vitamin D metabolism is at all involved, it is at a step subsequent to 25-hydroxylation.

INTRODUCTION

The effects of vitamin D and certain steroid hormones on calcium metabolism have been recognized for many years, but their mechanisms of action remain unclear. Clinically, there is a well known apparent antagonism between the effects of glucocorticoids and vitamin D, and it is generally recognized that the administration of these steroid hormones may diminish calcium absorption in vivo. In animal studies it has been shown, furthermore, that glucocorticoid administration decreases the intestinal transport and absorption of calcium (1–5), whereas bilateral adrenalectomy increases calcium transport (6).

In certain patients with sarcoidosis, there may be a defect in calcium metabolism due to excesive intestinal absorption of the cation (7–9). This abnormality, correctable by glucocorticoid administration (7–9), may result from increased amounts of circulating vitamin D (8) or an increased sensitivity to its effects (7, 9). Glucocorticoids may also lower the serum calcium in patients with vitamin D intoxication (10, 11), at least in part, by reducing the intestinal absorption of calcium. In patients with idiopathic hypercalcemia of infancy (12, 13), a condition resembling hypervitaminosis D and thought to result from excessive intake or defective metabolism of vitamin D, cortisone has been effective in lowering the serum calcium level. Finally, in patients with hypoparathyroidism, the administration of cortisone may prevent effective replacement therapy with vitamin D (14–16).

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While the effects of glucocorticoids appear to be antagonistic to those of vitamin D, the basis for this apparent antagonism remains uncertain. Possible mechanisms that deserve consideration include: (*a*) steroid hormone-related interference with the metabolism of vitamin D; (*b*) defective localization of vitamin D or its metabolites in the end organ (or the presence of a competitive inhibitor or vitamin D antagonist); and (*c*) glucocorticoid-related alterations in the cellular biochemical reactions, necessary for calcium transport, resulting in an apparent anti-vitamin D effect in the absence of a direct interaction between the hormone and the vitamin or its metabolites.

Recent studies in man by Avioli, Birge, and Lee (17) demonstrated that the administration of prednisone resulted in a rapid turnover of tritiated vitamin D_3 in the plasma with a decrease in the accumulation in the plasma of a biologically active metabolite (peak IV). The authors suggested that the apparent antagonism between glucocorticoids and vitamin D may be related to a decrease in the production of this biologically active vitamin D metabolite, subsequently identified as 25-hydroxycholecalciferol (18), resulting in a decrease in the intestinal absorption of calcium.

The purpose of this report is to present further evidence that in the rat the administration of glucocorticoids leads to a decrease in the active transport of calcium in the intestine in vitro, an effect which appears to be antagonistic to that of vitamin D. The present studies, furthermore, provide evidence that effects of steroid hormones on calcium transport, while opposite to those of vitamin D, may be independent of a direct interaction with the parent vitamin or its metabolites.

METHODS

Animal preparation. Albino, male rats of the Sherman strain, obtained at a weight of 120–140 g (Camm Research Institute, Inc., Wayne, N. J.), were maintained on a stock diet adequate in vitamin D (Hemlock Hollow Diet, Camm Research Institute, Inc.). The animals were injected subcutaneously for 7 days with 5 mg of cortisone acetate (Cortone acetate in saline suspension, Merck, Sharp & Dohme, West Point, Pa.), while control animals received injections of isotonic sodium chloride. In certain experiments the animals received 3 mg of hydrocortisone acetate (Hydrocortone acetate in saline suspension, Merck, Sharpe & Dohme), or 1.25 mg of prednisolone acetate (Meticortelone acetate in aqueous suspension, Schering Corp., Bloomfield, N. J.) daily for 7 days. In certain experiments vitamin D_3 (Vi-De 3 Hydrosol, Dr. A. Wander, Berne, Switzerland) was injected subcutaneously in 50% ethanol (v/v), while control animals received 50% ethanol alone. Bovine growth hormone (NIH-GH-B13, 0.95 USP units per mg) was generously provided by the Endocrinology Study Section of the National Institute of Arthritis and Metabolic Diseases.

Transport studies. Studies of transport were performed by the everted gut sac technique of Wilson and Wiseman (19) as modified by Schachter and Rosen (20), and Baerg, Kimberg, and Gershon (21). Gut sacs from the proximal 4–5 cm of duodenum were filled with 0.5 ml of medium and placed in 25-ml Erlenmeyer 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 per 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 Fig. 1. The flasks were incubated at 37°C for 2–2½ hr as indicated in a metabolic water bath oscillating at a rate of 100 per min. After incubation 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. Portions of the medium (inside and outside) were counted in Bray's solution (22) with a Packard Tri-Carb liquid scintillation spectrometer or were prepared for calcium determination by a titrimetric procedure with EDTA (23). The 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. Measurement of concentration ratios at times varying between 30 min and 3 hr indicated that a steady state had been achieved between 2 and 3 hr.

In certain experiments, $CaCl_2$ and ^{45}Ca or other test substances, as indicated in the text and legends, were initially present in the mucosal (outside) medium only. In these experiments the surface area (square centimeter) of the gut sacs was determined as previously described (6), 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 (24).

Calcium flux studies were conducted in vitro using a modification of the apparatus of Ussing and Zerahn (25), according to the technique described by Walling and Rothman (26). Rats weighing 120–140 g were injected with either isotonic saline or cortisone acetate for 7 days as described above, and the uppermost portion of the duodenum was mounted in vitro as the partition between two round half-chambers (mounted tissue area = 0.49 cm^2). Each compartment contained 11 ml of the same medium used for the sac transport studies with $^{45}CaCl_2$ (30 μCi) initially present in one of the compartments. The solutions were maintained at 37°C and were gassed with 100% O_2. 1 ml samples were taken at 10 min intervals from the initially nonradioactive compartment and immediately replaced by an equal volume of nonradioactive medium. Samples were counted in Bray's solution (22). The transmural electric potential differences were monitored as described (26). Examination of individual flux measurements beginning 10 min after the addition of radioisotope indicated that a steady state was reached within 60 min. Flux values for individual tissues represent the mean of five to six steady-state flux measurements.

Experiments were performed in which the calcium concentration within the mucosa and underlying intestinal coats (tissue remaining after the mucosa was scraped from the intestinal wall) was determined by a modification of the method described by Schachter, Kowarski, Finkelstein, and Wong Ma (27). Everted gut sacs were prepared and incubated as described above with the same media used to fill the sacs (serosal medium) and to bathe them (mucosal medium). After incubation at 37°C the sacs were drained,

blotted, placed immediately on chilled glass plates, and the mucosa was scraped from the underlying intestinal coats with a dull spatula. The mucosal scrapings from two or more gut sacs were pooled as were the underlying intestinal coats. The material was weighed, homogenized in 1.5 ml of 0.1 M EDTA and a portion of the supernatant after centrifugation was counted as described above. Estimates of tissue water were obtained in preliminary experiments in which the samples were dried to constant weight in a vacuum oven. A mean value of 87.8% was determined for both the mucosa and underlying coat fractions, and there were no differences between the control and cortisone-treated groups. All of the isotopes used in this study were procured from the New England Nuclear Corp., Boston, Mass.

Vitamin D–deficient animals. Calcium transport in vitro was studied in duodenal gut sacs prepared from groups of vitamin D–deficient control and cortisone-treated animals, half of each group having been repleted with vitamin D before the experiment. For these experiments, albino male rats of the Sherman strain were obtained as weanlings (30–50 g) from mothers maintained on a vitamin D-deficient diet (Camm Research Institute, Inc., Wayne, N. J.), and they were housed in cages shielded from light, and fed a vitamin D–deficient diet (Rachitogenic Diet 2, Nutritional Biochemicals Corps., Cleveland, Ohio). The animals were adequately depleted of the vitamin after 4–5 wk on this regimen. During each of the 7 days before sacrifice, the animals were divided into two groups and were given subcutaneous injections of either 3.0 mg of cortisone acetate or isotonic sodium chloride.

In certain studies, groups of eight vitamin D–deficient control and cortisone-treated animals were prepared as described above, and were then given by means of gastric tube, either: (*a*) 0.25 ml of cottonseed oil: ethanol, 40: 1 (v/v) 24 hr before sacrifice; (*b*) 50 IU of vitamin D_3 in 0.25 ml of the cottonseed oil: ethanol mixture 24 hr before sacrifice; or (*c*) 50 IU of 25-hydroxycholecalciferol in 0.25 ml of the cottonseed oil: ethanol mixture 20 hr before sacrifice. After an overnight fast the animals were sacrificed and the transport of calcium in everted duodenal gut sacs was studied as described above.

In studies concerned with the bioassay of vitamin D activity in the intestinal mucosa, the vitamin D–depleted control and cortisone-treated groups were further subdivided 48 hr before sacrifice, one-half receiving 4000 IU of vitamin D_3 subcutaneously and the remainder receiving an injection of 50% ethanol (v/v). After an overnight fast, the animals were sacrificed, duodenal gut sacs were prepared from the proximal 5 cm of the small intestine, and the transport of calcium was studied as described above. The next distal 15 cm of small intestine was everted, placed on a chilled glass plate and the mucosa was scraped with a glass microscope slide. An aqueous homogenate of mucosa containing between 33.6 and 50.4 mg of protein per ml was prepared, and this material was administered as a test substance for the bioassay of vitamin D–activity as described below.

Bioassay of vitamin D in mucosa. The bioassay of vitamin D, performed by the method of Schachter, Kimberg and Schenker (28), is dependent upon the ability of small doses of vitamin D to restore the transport of calcium in the intestine of rats depleted of the vitamin. The procedure is outlined as follows: (*a*) weanling rats were depleted of vitamin D as described above; (*b*) each of 5 or 6 depleted rats was given 0.25–0.30 ml of the test material (mucosal homogenate) prepared from control and cortisone-treated, vitamin D-free and repleted animals as described above. This material was administered by gastric tube. (*c*) Reference standards of 0.25, 0.50, 1.0 and 2.0 IU of vitamin D_3 in ethanol: propylene glycol, 1: 5 (v/v) were similarly administered to groups of five or six animals. One group of animals received the vehicle alone. (*d*) 48 hr later, duodenal slices were prepared and the oxygen-dependent accumulation of calcium was determined as previously described (24). The amount of vitaminD–activity in the test material (mucosa) could be determined by referring to the log-dose response curve obtained with the reference standards, and the results were expressed as IU of vitamin D–activity per g of mucosal protein.

Calcium-binding protein. The vitamin D–dependent duodenal mucosal calcium-binding protein was measured by the technique described by Wasserman and Taylor (29), using mucosal scrapings from the proximal 5–7 cm of duodenum. This method depends on the competition between the cation-exchange resin, Chelex-100 (Biorad Resins, Calbiochem Corp., Los Angeles, California), and a heat-treated soluble supernatant of a duodenal mucosal homogenate for added ^{45}Ca. Trypsin-treated control samples were routinely employed. These experiments were performed with pooled material from groups of four or more control and cortisone-treated animals maintained on a stock diet adequate in vitamin D. In addition, experiments were performed with duodenal mucosa from groups of vitamin D–deficient control and cortisone-treated animals, half of each group having received 20,000 IU of vitamin D_3 subcutaneously 48 and 24 hr before sacrifice.

Vitamin D metabolites. Vitamin D_3, 1,2-^3H with a specific activity of 154 mCi/mmole 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. Weanling rats maintained on the vitamin D–free diet for 5 wk were given subcutaneous injections of either 0.2 ml of isotonic saline or 0.2 ml of cortisone acetate suspension (3.0 mg) daily for 7 days. On the 6th day of treatment 25 control and 26 cortisone-treated animals were given an intraperitoneal injection of 15 IU of ^3H vitamin D_3 in 0.25 ml of 0.10% Tween 20 (polyoxyethylene sorbitan monolaurate; Fisher Scientific Co., Pittsburgh, Pa.) and 0.9% NaCl in water. The animals were sacrificed 45 hr later with a blow to the head followed by exsanguination. In a similar experiment, 20 control and 24 cortisone-treated animals were given 15 IU of ^3H vitamin D_3 on the 7th day of treatment and sacrified 20 hr later.

The serum and homogenates of liver and intestinal mucosa were prepared and analyzed for total tritium content after combustion as previously described (21). The radioactivity present in the liver, serum, and intestinal mucosa was extracted by the method of Bligh and Dyer (30) as modified by Lund and DeLuca (31). The chloroform extracts were concentrated by evaporation with a stream of N_2 and dissolved in *n*-hexane for chromatographic analysis on columns of activated silicic acid using the gradient elution scheme described by Ponchon, Kennan, and DeLuca (32). Tritium present in the 10-ml fractions obtained from the columns as well as that present in samples of the aqueous extracts was measured by previously described methods (21). Internal standards of toluene-^3H were added to all samples and the total disintegrations per minute were calculated.

TABLE I

Effect of Various Glucocorticoids on the Transport of Calcium by Duodenal Gut Sacs

Experiment	Treatment	Number	Mean ^{45}Ca concentration ratio inside/outside	SE	P
1	Control	44	8.1	0.3	<0.005
	Cortisone	59	4.0	0.1	
2	Control	19	8.6	0.3	<0.005
	Hydrocortisone	23	3.7	0.2	
3	Control	13	8.3	0.5	<0.005
	Prednisolone	15	3.4	0.2	

The everted gut sacs were filled with 0.5 ml of the standard medium and were bathed in flasks containing 4.0 ml of an identical medium for 2.5 hr as described in Methods.

RESULTS

Calcium transport. The transport of calcium in everted duodenal gut sacs prepared from cortisone-treated rats maintained on a stock diet adequate in vitamin D was decreased when compared to results obtained with saline-treated control animals (Table I). Similar results were obtained after treatment with either hydrocortisone or prednisolone. The steroid effect was not demonstrable 24 hr after a single injection of 25 mg of cortisone acetate, but was present after 3 days of treatment with 5 mg of cortisone or with doses as low as 2 mg administered daily for 5–7 days. The glucocorticoid effect on calcium transport persisted for at least 5 days after the cessation of a 7 day course of treatment with 5 mg of cortisone daily. The daily subcutaneous administration of 0.5 mg (0.48 USP units) of bovine growth hormone along with cortisone, did not influence the steroid effect on calcium transport in the intestine.

TABLE II

Effect of Supplementary Vitamin D_3 on the Transport of Calcium in Cortisone-Treated Rats

Treatment	Number	Mean ^{45}Ca concentration ratio inside/outside	SE	P
Control, no extra D_3	17	6.6	0.4	<0.005
Cortisone, no extra D_3	22	3.8	0.2	
Control, plus extra D_3	17	7.0	0.6	<0.005
Cortisone, plus extra D_3	24	4.1	0.3	

In these experiments, the animals consumed the standard diet which was adequate in vitamin D. When administered, supplementary vitamin D_3 was given subcutaneously in doses of 12,000 IU, 7, 5, 3, and 1 day before sacrifice. The remaining conditions are as described in the legend to Table I and in Methods.

The administration of multiple, large doses of vitamin D failed to have a significant effect on transport in either the control or steroid-treated groups, and the difference in the concentration ratios (I/O) persisted (Table II). These results at least suggest that the hormonal effect may not be due to a subtle alteration in the rate of turnover or metabolism of vitamin D as had been suggested (17).

With gut sacs prepared from young rats maintained on a diet adequate in calcium and vitamin D, only the proximal one-fifth of the small intestine has been shown to participate in the in vitro transfer of calcium against chemical concentration gradients (33). After dietary calcium deprivation, however, there is an apparent adaptation of the transport mechanism such that concentration ratios (I/O) in excess of 1.0 may be observed with everted sacs from almost all levels of the small intestine (6). There is strong evidence to suggest that the mechanisms involved in the transport of calcium are similar throughout the small intestine and that vitamin D affects transport at all levels (6, 28). The effects of cortisone treatment on the transfer of calcium by different segments of the small intestine were observed (Table III). 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. While the rate of transfer of calcium across the duodenum was diminished with gut sacs obtained from cortisone-treated animals, the more distal segment (ileum) transferred calcium more readily after glucocorticoid administration. These results, which will be discussed later, suggest that the effects of cortisone administration on calcium transport are not simply those related to an anti-vitamin D–like action.

TABLE III

Effect of Cortisone Treatment on Calcium Transfer at Different Levels of the Small Intestine

Treatment	Number	Gut segment	Calcium transfer	SE $\times 10^{-3}$	P
			μmoles $\times 10^{-3}$/cm² per 2 hr		
Control	19	Duodenum	673.3	27.7	<0.005
Cortisone	17	Duodenum	479.1	22.3	
Control	19	Ileum	105.8	11.4	<0.01
Cortisone	19	Ileum	142.3	13.5	

The animals were maintained on the standard diet, adequate in vitamin D. Gut sacs were prepared from the proximal 5 cm of duodenum 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, portions of the inside (serosal) and outside (mucosal) medium counted, and the sac area determined (see Methods).

Effects of varying calcium concentrations in vitro. The results described thus far suggest that the administration of glucocorticoids may affect the active transport mechanism. It has been suggested however, that these hormones may influence active transport indirectly, affecting primarily the rate of simple diffusion of calcium at the luminal surface of the cell, thus limiting the amount of cation available to the active transport mechanism (2). If steroid hormones only affected the rates of simple diffusion of calcium, then hormonal effects on the transfer of calcium would be minimized in the presence of higher concentrations of calcium in the medium; under these conditions, the maximal rates of transport observed with intestine from the two groups of animals should approach equality.

Table IV summarizes the results of experiments in which pools of duodenal tissue slices from control and cortisone-treated animals maintained on a diet adequate in vitamin D were incubated with varying concentrations of $CaCl_2$. The oxygen-dependent accumulation of calcium was greater with slices from the control animals at all concentrations of calcium employed, even at higher concentrations. The rates of accumulation of calcium were maximal with calcium concentrations in excess of 0.6 μmole/ml and the maximal values for the control and treated groups were 0.92 and 0.67 μmole/g slice per hr, respectively.

Similar results were observed in experiments in which duodenal gut sacs were incubated in media containing 0.20–2.40 μmole/ml of calcium (Table V). Net absorption of calcium from the mucosal medium was observed at all concentrations tested, and increased directly with initial calcium concentration. Transfer to the serosal medium remained constant at concentrations in the medium of 0.8 μmole/ml and greater, and as shown previously (28, 33), the transfer to the serosal medium is rate limited. Cortisone treatment decreased calcium transport at all concentrations of calcium, and the effect was not diminished in the presence of higher concentrations. With sacs from control animals, the maximal rate of transfer to the serosal medium was 1.5 μmole/gut sac, whereas with sacs from cortisone-treated animals, the maximal rate was 0.9 μmole/gut sac. Cortisone administration, therefore, may indeed cause a defect in the active transport mechanism rather than a decrease in the simple diffusion of calcium into the cell.

In order to demonstrate an effect of cortisone administration on the active transport mechanism for calcium, flux studies were conducted as described in Methods, and the results appear in Table VI. Rat small intestine is electrically polarized such that the serosal surface is positively charged relative to the mucosal. No significant differences were noted in the transmural electric

TABLE IV

Effect of Cortisone Treatment on Accumulation of Calcium by Slices of Duodenum

Initial Ca^{++} concentration	O_2-dependent accumulation of Ca^{++}		
	Control	Cortisone	*P*
M	*μmoles/g slices per 1 hr*		
1.0×10^{-4}	0.27 ±0.02	0.21 ±0.02	<0.01
2.0×10^{-4}	0.42 ±0.05	0.35 ±0.05	<0.025
4.0×10^{-4}	0.75 ±0.06	0.57 ±0.06	<0.005
6.0×10^{-4}	0.88 ±0.05	0.66 ±0.04	<0.005
8.0×10^{-4}	0.90 ±0.09	0.67 ±0.08	<0.01
1.5×10^{-3}	0.92 ±0.02	0.67 ±0.01	<0.001

The results represent the means ±1 SEM of values obtained in six separate experiments.

TABLE V

Effects of Calcium Concentration in Medium on Calcium Transport by Duodenal Gut Sacs from Control and Cortisone-Treated Rats

Initial calcium concentration	Control		Cortisone	
	ΔMucosal	ΔSerosal	ΔMucosal	ΔSerosal
mM	*Net calcium transport, μmoles per gut sac*			
0.2	−0.40	+0.88	−0.32	+0.49
0.5	−1.40	+1.02	−1.12	+0.59
0.8	−1.75	+1.41	−1.57	+0.84
1.5	−3.56	+1.45	−2.27	+0.82
2.4	−3.96	+1.49	−2.84	+0.89

Duodenal gut sacs from each of five control and five cortisone-treated animals were tested at each concentration of calcium. At each concentration, $CaCl_2$ was initially present in both the inside (serosal) and outside (mucosal) medium. After a 2.5 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 as indicated in Methods. The results represent the means of values obtained in two separate experiments.

TABLE VI

Effect of Cortisone Treatment on Steady-State Calcium Fluxes

Experimental conditions	Steady-state calcium flux		
	MS flux	SM flux	Net flux
	mμmoles/cm² per hr		
Control	32.6 ±6.4 (6)	8.9 ±1.9 (4)	23.7 ±8.2 ($P < 0.02$)
Cortisone	15.6 ±1.8 (7)	14.6 ±2.2 (5)	0.9 ±2.9 ($P > 0.5$)
ΔControl-cortisone	17.0 ±6.2 ($P < 0.02$)	−5.7 ±3.0 ($0.05 < P < 0.1$)	22.8 ±8.0 ($P < 0.02$)

The number of experiments is given in parentheses. The results are expressed as the means ±1SEM.

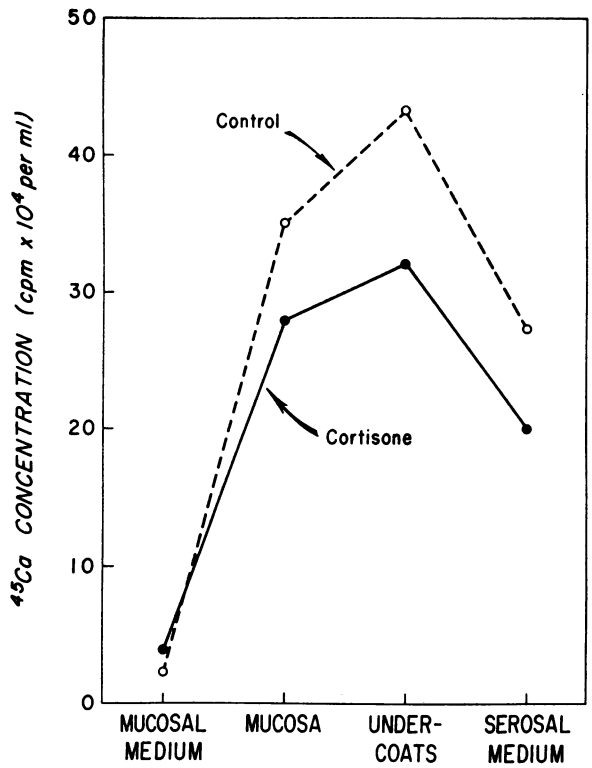

FIGURE 1 Effect of cortisone treatment on ^{45}Ca concentration differences across duodenal gut sacs in vitro. Rats maintained on the standard diet were treated with either isotonic saline or cortisone acetate as described in Methods. One duodenal gut sac was prepared from each of four control and four cortisone-treated animals. The sacs were incubated in the standard medium (see Methods) except that the initial calcium concentration on both the inside and outside was 4.0×10^{-4} M, and the medium contained 90,000 cpm per ml. After a 2 hr incubation the ^{45}Ca tissue concentration differences were determined as described in Methods. The results in the figure represent the mean values obtained in six separate experiments. The mucosal calcium concentrations (mean ±1 SEM) in the control and cortisone-treated sacs were $34.1 \pm 2.2 \times 10^4$ cpm per ml and $28.5 \pm 1.1 \times 10^4$ cpm per ml respectively ($P < 0.005$). The undercoat calcium concentrations were $43.9 \pm 1.8 \times 10^4$ cpm per ml and $32.2 \pm 1.5 \times 10^4$ cpm per ml respectively ($P < 0.001$). The differences at Mucosa-Undercoats were significant at the level $P < 0.001$ in the control and at the level $P < 0.01$ in the cortisone-treated sacs.

potential differences between the control and cortisone-treated groups when the tissues were first mounted in vitro (4.5 mv ±0.4 SEM vs. 4.2 mv ±0.3 SEM, respectively), or at the beginning (1.0 mv ±0.1 SEM vs. 0.9 mv ±0.2 SEM, respectively) and end (0.5 mv ±0.1 SEM vs. 0.4 ±0.1 SEM, respectively) of the steady-state periods. In the cortisone-treated animals the unidirectional flux mucosal to serosal (MS)[1] was diminished to less than one-half of the control flux. Although the mean flux serosal to mucosal (SM) was increased by cortisone treatment, this effect was not significant at the 95 per cent confidence level. Contrary to the results obtained in the controls, the MS and SM fluxes were not different in the cortisone-treated animals. It is clear then, that cortisone administration affects the active calcium transport mechanism.

Tissue concentration differences. Schacter and his co-workers have demonstrated two vitamin D-dependent steps in the movement of calcium across everted duodenal gut sacs in vitro: uptake at the mucosal surface and transfer toward the serosa (28, 34). In other studies (27), estimation of the calcium concentration differences in the tissue did indeed show two oxygen-requiring, vitamin D–dependent uphill gradients corresponding to the net steps in transport: (*a*) mucosal medium to mucosa and (*b*) mucosa to underlying coats. The measured mucosal calcium concentrations probably represent the mean values of low and high concentration compartments within the cell. The concentration in the underlying coats on the other hand, may reflect diffusion from the high concentration compartment in the adjacent mucosa (27).

In an attempt to demonstrate the effects of cortisone on both vitamin D–dependent steps in transport, studies of the tissue concentration differences during the transport of calcium by the intestine were undertaken as described in Methods. The results in Fig. 1, indicate that cortisone administration decreased both uphill differences, mucosal medium-mucosa, and mucosa-underlying coats, in keeping with the apparent "antagonism" to the effects of vitamin D.

Transport of other divalent cations. In order to determine whether the defect in the intestinal transport of calcium after cortisone treatment was unique for this cation, the transfer of other divalent cations was also studied. In the experiment in Table VII the divalent cation and its isotope were presented initially on the outside (mucosal medium) of the gut sac only, and the results express the rate of transfer of cation to the inside (serosal) medium per square centimeter of gut sac area.

As noted earlier, cortisone treatment diminished the rate of transfer of calcium in duodenal gut sacs, but enhanced transfer in the ileal sacs. Similar results were noted with strontium, which, while less well transported than calcium, does seem to share a common vitamin D-dependent transport mechanism (28). Cortisone administration did not have a significant effect on the transfer of barium. The transfer of iron was reduced

[1] *Abbreviations used in this paper:* Ca-BP, calcium-binding protein; MS, unidirectional flux mucosal to serosal; SM, unidirectional flux serosal to mucosal.

TABLE VII

Effect of Cortisone Treatment on the Intestinal Transfer of Barium, Strontium, and Iron

Divalent cation	Number	Gut segment	Cation transfer	SE	*P*
			cpm/cm² per 2 hr		
Experiment 1, barium (^{133}Ba)					
Control	28	Duodenum	9,780	661	>0.10
Cortisone	36	Duodenum	11,039	734	
Control	24	Ileum	4,735	606	<0.10
Cortisone	32	Ileum	5,755	491	
Experiment 2, strontium (^{85}Sr)					
Control	20	Duodenum	10,271	696	<0.025
Cortisone	23	Duodenum	8,352	521	
Control	21	Ileum	2,250	204	<0.01
Cortisone	24	Ileum	3,006	240	
Experiment 3, iron (^{55}Fe)					
Control	15	Duodenum	2,081	270	<0.005
Cortisone	18	Duodenum	898	101	
Control	18	Ileum	75	10	<0.005
Cortisone	18	Ileum	152	27	

The animals were maintained on the standard diet, adequate in vitamin D. 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 at 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 (10,200 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 (4400 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. The values of *P* refer to the effects of cortisone in each segment.

in duodenal gut sacs and enhanced in ileal gut sacs from cortisone-treated animals despite the fact that iron is transported by a mechanism that is not dependent on vitamin D (28).

Transfer of D-*galactose and* L-*leucine.* The ileal transfer of L-leucine was not affected by previous treatment with cortisone, but the rate of transfer of D-galactose in the jejunum was increased (Table VIII). This effect of cortisone on the rate of transfer of D-galactose, an actively transported hexose which is poorly metabolized by the intestinal mucosa, is consistent with the results of previous studies in which effects of glucocorticoids on hexose transport have been noted (35).

Repletion with vitamin D_3 and 25-hydroxycholecalciferol. Recent studies have indicated that vitamin D_3 must be converted in the liver to a biologically "active form," 25-hydroxycholecalciferol (32). Experiments were undertaken in order to examine the effects of vitamin D_3 and 25-hydroxycholecalciferol on the transport of calcium in duodenal gut sacs from control and cortisone-treated animals maintained on a vitamin D–deficient diet.

As noted in Table IX, the concentration ratios (I/O) in duodenal gut sacs from control and cortisone-treated animals given the vehicle alone were low (2.5 vs. 2.2) and there was no significant difference between the two groups. While the intestinal transport mechanism in both the control and steroid-treated animals responded to the administration of either vitamin D_3 or 25-hydroxycholecalciferol, the response on the part of the cortisone group was less marked, and a very striking difference between the two groups was apparent. Furthermore, repletion with modest doses of the biologically active

TABLE VIII

Effect of Cortisone Treatment on the Intestinal Transfer of Leucine and Galactose

Experimental conditions	Number	Rate of transfer	SE	P
		cpm/cm² per 2 hr		
Experiment 1, L-leucine				
Control	25	19,010	580	>0.10
Cortisone	25	19,898	741	
Experiment 2, D-galactose				
Control	25	13,815	536	<0.005
Cortisone	23	18,179	819	

The animals were maintained on the standard diet, adequate in vitamin D. In experiment 1, gut sacs were prepared from 5-cm segments of intestine 15–20 cm and 20–25 cm from the ileocecal valve 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 1.2×10^{-3} M L-leucine and L-leucine-^{14}C (5400 cpm/0.1 ml of medium). In experiment 2, 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 inside (serosal) medium was identical with that used in experiment 1. The outside (mucosal) medium contained 4.0×10^{-4} M D-galactose and D-galactose-^{14}C (7800 cpm/0.1 ml of medium). The results are expressed as the rate of L-leucine-^{14}C or D-galactose-^{14}C transfer to the inside (serosal) medium (cpm/cm² per 2 hr) as described in Methods.

25-hydroxycholecalciferol failed to fully restore the transport mechanism in cortisone-treated animals.

Calcium-binding protein. The administration of vitamin D_3 to rachitic rats or chicks results in the appearance in the small intestinal mucosa of a calcium-binding protein (Ca-BP) (29, 36-38). The role, if any, of this protein in calcium transport, remains unclear. Nonetheless, the levels of Ca-BP in the mucosa usually correlate well with the transport capacity, the physiologic need for calcium, and the dose of administered vitamin D (29, 36-38).

Since the glucocorticoid effect on calcium transport might be related to either an alteration in the metabolism of vitamin D or the localization of active metabolites in the intestinal mucosa, the effects of cortisone treatment on the levels of the vitamin D–dependent Ca-BP were studied (Table X). Despite the clearly demonstrable defect in the transport of calcium in the duodenum of cortisone-treated rats maintained on a stock diet (Table I), the levels of Ca-BP in homogenates of duodenal mucosa from such animals exceeded those of the saline-injected control animals. Furthermore, in experiments with vitamin D-deficient animals, cortisone treatment did not impair the formation of the Ca-BP in response to vitamin D administration. In fact, the level of Ca-BP activity in cortisone-treated, vitamin D-repleted animals exceeded that of control animals repleted with vitamin D in an identical manner. Despite the effectiveness of vitamin D-repletion in leading to the formation of the Ca-BP, duodenal gut sacs from such animals were still defective in calcium transport.

Bioassay of vitamin D activity. The previous results indicate a disparity between the formation of the vitamin D–dependent Ca-BP by cortisone-treated animals and the transport of calcium even in response to vitamin D or 25-hydroxycholecalciferol. Studies concerned with bioassay of the levels of vitamin D activity in the small intestinal mucosa were therefore undertaken as described in Methods. As noted in Table XI, cortisone

TABLE IX

Effects of Vitamin D_3 and 25-Hydroxycholecalciferol on the Transport of Calcium in Duodenal Gut Sacs from Control and Cortisone-Treated Vitamin-D Depleted Rats

Experimental conditions	Number	Mean ^{45}Ca concentration ratio inside/outside	SE	P
Control, cottonseed oil:ethanol	8	2.5	0.1	>0.10
Cortisone, cottonseed oil:ethanol	8	2.2	0.2	
Control, vitamin D_3	8	7.6	0.6	<0.005
Cortisone, vitamin D_3	8	4.1	0.4	
Control, 25-hydroxycholecalciferol	8	7.5	0.8	<0.005
Cortisone, 25-hydroxycholecalciferol	8	4.0	0.2	

Gut sacs were filled with and incubated in the standard medium (see Methods) with the exception that the calcium concentration was 4.0×10^{-4} M. The sacs were incubated for 2 hr. The effects of vitamin D_3 and 25-hydroxycholecalciferol in the control animals were each significant at the level of $P < 0.005$. The effects of vitamin D_3 and 25-hydroxycholecalciferol in the cortisone-treated animals were also significant at the level of $P < 0.005$.

administration clearly diminished the vitamin D-dependent transport of calcium in the duodenal gut sacs. The mucosa from the cortisone-treated, vitamin D–repleted animals, however, had more vitamin D activity than that from the vitamin-repleted saline injected control animals. This finding is consistent with the slightly increased levels of Ca-BP and again suggests that the effects of cortisone on the transport of calcium may indeed be somewhat independent of a direct interaction with either vitamin D or its active metabolites.

Vitamin D metabolites. In view of the report by Avioli, Birge, and Lee (17) demonstrating an abnormality in the metabolism of vitamin D in humans treated with prednisone, it was of interest to study the effects of glucocorticoid administration on the metabolic transformation and tissue localization of tritiated vitamin D_3 in the rat. 45 hr after the intraperitoneal administration of 15 IU of vitamin D_3-^3H to groups of control and cortisone-treated vitamin D–deficient animals, the liver, intestinal mucosa 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 milliter of serum), and the distribution between the aqueous and chloroform phases were not altered by cortisone administration (Table XII). Furthermore, as shown in Table XIII, there was little difference in the proportion of the chromatographed radioactivity in the various peaks [2] from serum and tissues of control and cortisone-treated animals. The proportion of activity in these peaks which may be of importance in expressing the biological activity of vitamin D (peaks IV, V, and perhaps VI) (39–44), may even have been slightly higher in the mucosa from cortisone-treated animals than in the mucosa from the controls. Similar results were obtained with serum and tissues from control and cortisone-treated animals extracted 20 hr after the administration of vitamin D_3-^3H.

TABLE X

Effects of Cortisone and Vitamin D_3 Administration on Calcium-Binding Protein Activity

Experimental conditions	Calcium-binding protein activity [ratio compared to control]*
Experiment 1, animals on stock diet	
Control	1.00*
Cortisone	1.69 ±0.08‡
Experiment 2, animals on vitamin D–deficient diet	
Control, plus vitamin D	1.00*
Cortisone, plus vitamin D	1.34 (1.10–1.57)
Control, no vitamin D	0.20 (0.12–0.28)
Cortisone, no vitamin D	0.26 (0.18–0.37)

The preparation of the animals is described in Methods. The activity of the duodenal mucosal calcium-binding protein was measured as described (Reference 25 and Methods), employing pooled material from four to six animals in each group. Five experiments of type 1 and three experiments of type 2 were performed in an identical manner. While the results of the two types of experiments are presented together for clarity, they were conducted with different groups of animals on separate days with separate calcium-binding protein assays. Comparisons between groups in each type of experiment are valid, but direct comparisons between groups in the different types of experiments may not be justified.

*In type 1 experiments, the calcium-binding protein activity (cpm bound/mg protein) of the controls is represented as 1.00 and the results of cortisone treatment are expressed as the ratio (mean ±1 SEM) compared to the control. In type 2 experiments, the calcium binding protein activity (cpm bound/mg protein) of the control animals given supplementary vitamin D is expressed as 1.00 and the other results as the ratio (mean and ranges) compared to the control.

‡ The effect of cortisone treatment in this experiment is significant, $P < 0.005$.

TABLE XI

Effect of Cortisone Administration on Calcium Transport and the Bioassay of Vitamin D–Activity in the Intestinal Mucosa

Treatment	Mean ^{45}Ca concentration ratio inside/outside	Vitamin D–activity
		IU per g mucosal protein
Control	7.7	31.6
Cortisone	4.8	40.7

Gut sacs were prepared from the proximal 5 cm of duodenum and were filled with and incubated in the standard medium with the exception that the calcium concentration was 4.0×10^{-4} M. The sacs were incubated for 2 hr. The mean I/O ratios, in the non-vitamin D–repleted control and cortisone-treated groups, were 2.5 and 2.1 respectively (significant at the level of $P < 0.05$). The amount of vitamin D–activity in the mucosa from the next most distal 15 cm of the control and cortisone-treated, vitamin D–deficient animals, varied between 10.5 and 13.1 IU per g of mucosal protein, and there was no significant difference between the two groups. The values given in Table XI for both the I/O ratios and vitamin D activity in the vitamin D-repleted control and cortisone-treated animals, are significantly different with a P value of <0.005 in each case. The results represent the mean values obtained in four separate experiments.

[2] The chromatographic numbering system is after that of DeLuca and his co-workers (32, 39).

TABLE XII

Radioactivity in Serum and Tissue 45 hr after Injection of 15 IU of Vitamin D_3-^3H to Vitamin D–Deficient Control and Cortisone-Treated Rats

Sample	DPM/mg protein or DPM/ml plasma		Chloroform soluble		Aqueous soluble	
	Control	Treated	Control	Treated	Control	Treated
			%		%	
Mucosa	13.8	14.5	81.9	80.5	18.1	19.5
Liver	24.4	25.3	84.2	81.8	15.8	18.2
Plasma	3478	3390	97.9	97.9	2.1	2.1

The data represent the values from pooled samples of 25 control and 26 cortisone-treated animals (see Methods).

DISCUSSION

Vitamin D clearly plays an essential role in the intestinal absorption of calcium. While there is abundant clinical and experimental evidence that glucocorticoids may antagonize the effects of vitamin D on calcium absorption and transport, there has been uncertainty regarding the mechanism whereby these hormones exert their effect on the vitamin D-dependent processes.

In 1962, Sallis and Holdsworth concluded that the apparent antagonism between cortisol and vitamin D was due to interference with the conversion of vitamin D_3 to an active form in the adrenal cortex (3). The observed failure of adrenalectomy in rats to influence serum calcium levels, or the vitamin D–related increase in intestinal calcium transport (45), as well as recent information concerning the sites and nature of the metabolic conversion of vitamin D (31, 32, 39, 40) make this possibility rather unlikely. While many of the effects of glucocorticoid administration on intestinal transport resemble those of hypophysectomy (34), it is unlikely that the cortisone-induced decrease in the intestinal calcium transport can be related to a decrease in the endogenous secretion of either ACTH or growth hormone. Finkelstein and Schachter (34) noted that in the rat, cortisol administration inhibited calcium transport even in hypophysectomized animals. Furthermore, in the present studies, the daily administration of large amounts of bovine growth hormone along with cortisone, failed to influence the steroid effect on calcium transport in the intestine.

As a result of numerous recent studies, it has become apparent that after the administration of tritiated vitamin D, radioactive metabolites with biologic potency appear in the plasma of man as well as in the plasma, bone, intestine, liver, and kidney of the rat (31, 32, 39, 40). While the liver is undoubtedly the most important, if not the exclusive site for the metabolic transformation of vitamin D_3 to 25-hydroxycholecalciferol (32, 39), further metabolism of 25-hydroxycholecalciferol to other products which may be active seems to occur (40).

TABLE XIII

Serum and Tissue Chromatographic Fractions 45 hr after Injection of 15 IU of Vitamin D_3-^3H to Vitamin D–Deficient Control and Cortisone-Treated Rats

Sample	I	II_a	II	III	IV_a	IV	V_{a+b}	VI_{a+b}	VII_{a+b}
Plasma									
Control	1.1	0.1	0.8	67.0	4.7	18.4	4.2	3.1	0.6
Treated	1.4	0.1	1.2	65.5	3.4	19.6	3.3	4.1	1.4
Mucosa									
Control	3.3	2.1	2.4	74.3	2.3	4.6	2.7	4.3	4.0
Treated	0.6	1.4	2.4	67.7	2.9	6.2	3.2	7.5	8.1
Liver									
Control	4.5	5.2	3.1	71.6	3.4	4.4	2.0	2.8	3.0
Treated	8.5	4.1	3.0	68.4	3.6	3.9	2.2	2.8	3.5

The data are expressed as the per cent of the chromatographed radioactivity and represent values from pooled samples of 25 control and 26 cortisone-treated animals (see Methods). The chromatographic numbering system is after that of DeLuca and his coworkers (32, 39).

Much recent evidence suggests that 25-hydroxycholecalciferol or a more polar compound represents the end organ tissue-active form of the vitamin (39-44).

Recent studies by Avioli and his co-workers (17) have provided evidence suggesting that the glucocorticoid-induced decrease in the intestinal transport of calcium may be mediated by a steroid-related alteration in either the rate of synthesis or degradation of a biologically active vitamin D metabolite (peak IV), which was subsequently identified as 25-hydroxycholecalciferol (18). In these studies the administration of prednisone in man was associated with an abnormally rapid plasma turnover of vitamin D, a decrease in the amount of the biologically active peak IV metabolite in the plasma, and an overall decrease in the amount of biologically active substance in the circulation. Unfortunately, the plasma metabolites were studied at only one point in time (24 hr), and the study periods lasted only 5 days, during what may really represent the first phase of a biphasic semilogarithmic disappearance curve with the half-life of the second phase measured in weeks (46). It is difficult to conclude, on the basis of these studies of plasma, that steroid-induced alterations in calcium absorption are related to alterations in vitamin D metabolism.

The results of the present studies suggest that steroid hormones may not diminish the rate of formation or increase the rate of degradation of biologically active vitamin D metabolites. Furthermore, these studies fail to support the possibility that the steroid hormones interfere with the localization of the parent vitamin or its metabolites in the end organs. Rather, the present experiments do suggest that the glucocorticoid-related alterations in the vitamin D-induced intestinal calcium transport may result in large measure from alterations in the cellular mechanisms for calcium transport, which, while opposite in direction to the effects of vitamin D, may be independent of any direct interaction with either the vitamin or its metabolites. If indeed a disorder in vitamin D metabolism is at all involved, it is at a step subsequent to 25-hydroxylation.

The lack of correlation between the transport of calcium and the levels of the vitamin D-induced calcium-binding protein in the present study suggests that the effect of cortisone administration may be unrelated either to alterations in the metabolism or localization of vitamin D. Additional evidence in support of this view may be derived from the results of studies concerned with the bioassay of vitamin D-like activity in the intestinal mucosa of control and cortisone-treated, vitamin D–repleted animals. In keeping with the increased levels of Ca-BP, there was more assayable vitamin D–activity in the mucosa from cortisone-treated animals than in their respective controls. While cortisone administration did not influence the overall tissue distribution of vitamin D and its metabolites, the proportion of biologically active or potentially active material in the mucosa from the steroid-treated group may have been slightly increased. The higher concentration of vitamin D–active material and Ca-BP may represent a biological adaptation in an attempt to compensate for the steroid-induced reduction in intestinal calcium transport. A similar effect of cortisone administration on the distribution of plasma metabolites of vitamin D_3-^3H in the rat has been noted by others.[3] Additional evidence of a defect in the cellular mechanisms for calcium transport which is somewhat independent of a direct interaction with vitamin D may be derived from the studies employing 25-hydroxycholecalciferol. Despite the direct administration of a substantial dose of this biologically active metabolite to cortisone-treated animals, a marked impairment in calcium transport did persist.

The present studies indicate that the administration of steroid hormones affects both steps in the vitamin D–dependent transport mechanism: uptake at the mucosal surface and transfer to or towards the serosal medium (27, 28, 34). Furthermore, the hormonal effects on transport are not those which might result from an alteration in the simple diffusion of calcium at the luminal surface. The steroid effect was noted over a wide range of calcium concentrations and was not minimized even in the presence of high concentrations of calcium in the medium. Moreover, the results of the flux studies conducted in the chamber leave little doubt that cortisone administration affects the active component of the intestinal calcium transport system. The fact that the transmural electric potential differences during the steady state as well as the calcium fluxes noted in the present study were lower than those previously reported by others (26), may reflect the use of fructose rather than glucose as substrate and the presence of a higher (4.0 mM vs. 2.4 mM) phosphate concentration in the medium. Schachter and Britten (47) have reported lower transmural electric potential differences in duodenal gut sacs with fructose rather than glucose as the substrate. Walling and Rothman (26) noted lower calcium fluxes in the duodenum using a medium which contained 2.4 mM phosphate than in a phosphate free system.

The effects of glucocorticoid administration on intestinal transport mechanisms are complex and go beyond what might be anticipated solely on the basis of an anti-vitamin D–like effect. Previous studies (28) have demonstrated that the proximal duodenum transfers calcium to the serosal medium more readily when incubated in the presence of O_2 than under N_2, and that vitamin D enhances this O_2-dependent process. More distal segments on the other hand, appear to transfer calcium

[3] K. Schaefer. Personal communication.

more readily under N_2 than under O_2. While the rate of transfer of calcium across ileal sacs is enhanced by vitamin D when these sacs are incubated under O_2, the transfer is actually diminished by the vitamin when the sacs are incubated under N_2. It would appear that the mucosa of the distal small intestine is relatively impermeable to calcium, and the permeability barrier may be dependent upon oxidative metabolism. In the present studies, the administration of cortisone to vitamin D–repleted animals enhanced the transport of calcium and strontium in ileal gut sacs incubated under O_2 in vitro. These results suggest that cortisone treatment may interfere with oxidative metabolism in the intestinal mucosa, or perhaps with the coupling of energy to the divalent cation transport mechanism.

Further evidence in support of a more basic glucocorticoid-induced alteration in intestinal mucosal function may be derived from the results of the studies concerned with the transport of iron and D-galactose. The present studies demonstrate an impairment in iron transport in the intestine of cortisone-treated animals. While the transport of iron is clearly not influenced by vitamin D (28), calcium and iron may compete for one or more rate-limiting cellular constituents or reactions in transfer toward the serosal surface of gut sacs (48). The enhanced transport of D–glucose noted by others (35), and of D–galactose noted in the present study, again suggests that the alterations in intestinal transport mechanisms associated with glucocorticoid administration go beyond those processes that are simply sensitive to vitamin D. There is some specificity in this effect however, since the transport of L-leucine does not seem to be affected by cortisone treatment.

The precise basis for the apparent antagonism between the effects of vitamin D and of glucocorticoids on intestinal calcium transport still remains uncertain. The results of the present studies make it seem rather unlikely however, that glucocorticoids exert their effects by interfering either with the metabolism of vitamin D or the localization of its metabolites to the intestinal mucosa. The possibility of a defect in *subcellular* localization and metabolism within the intestinal mucosal cell must still be considered and this is currently under investigation. The presence of increased levels of vitamin D–induced calcium binding protein in cortisone-treated animals would tend to argue against such a possibility. These hormones may well influence any one of a number of vitamin D–dependent reactions within the intestinal mucosa. While such hormonal effects may be opposite in direction to those of vitamin D, they may be independent of any direct interaction with the vitamin. Furthermore, it seems likely that cortisone administration may also influence certain non-vitamin D–dependent mucosal cell reactions. A better understanding of the basis for the anti-vitamin D–like effects of cortisone awaits more detailed elucidation of those processes mediating transcellular calcium transport in the intestine.

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