Effects of Cortisone Administration on the Metabolism and Localization of 25-Hydroxycholecalciferol in the Rat

MURRAY J. FAVUS, DANIEL V. KIMBERG, GAIL N. MILLAR, and ELAINE GERSHON

From the Department of Medicine, Harvard Medical School, and the Gastrointestinal Unit of the Department of Medicine, Beth Israel Hospital, Boston, Massachusetts 02215

ABSTRACT Glucocorticoid administration is known to decrease calcium absorption in vivo and the vitamin D-dependent active transport of calcium by rat duodenum in vitro. The basis for this antivitamin D-like effect of glucocorticoids is unclear.

Previous studies in the rat failed to demonstrate an effect of glucocorticoid treatment on the hepatic conversion of the parent vitamin to 25-hydroxycholecalciferol (25-HCC). Moreover, pharmacologic doses of 25-HCC did not restore intestinal calcium transport to normal. The results of these experiments suggested that if indeed glucocorticoids interfere with the metabolism of vitamin D, the step involved must be subsequent to 25-hydroxylation.

The present studies demonstrate that the administration of cortisone to vitamin D-deficient rats does not affect the rate of conversion of a physiologic dose of [^{3}H]25-HCC to the biologically important metabolite, 1,25-dihydroxycholecalciferol (1,25-DHCC). Furthermore, pretreatment with glucocorticoids affects neither the tissue distribution nor the subcellular localization on or in intestinal mucosal cell nuclei of 1,25-DHCC. Of note is the fact that 1,25-DHCC is currently considered to be the "tissue-active" form of the vitamin in the intestine. Whereas tissues from cortisone-treated animals had increased concentrations of the biologically less active 24,25-DHCC, the physiologic significance of this observation remains unclear.

The results of the present studies strongly support the concept that the antivitamin D-like effects of glucocorticoids in the intestine are due to hormonal influences on the biochemical reactions responsible for calcium transport. While the effects of these hormones are opposite in direction to those of vitamin D, they occur by a mechanism that is independent of a direct interaction with either the vitamin or its biologically active metabolites.

INTRODUCTION

The administration of glucocorticoids is known to interfere with the absorption of calcium in vivo as well as with the active transport of calcium by the small intestine in vitro (1–5). While the apparent antagonism between the effects of these steroid hormones and vitamin D has been recognized clinically and experimentally for a number of years, the basis for this glucocorticoid effect remains incompletely explained.

In previous studies in vitro with duodenum from cortisone-treated rats, Kimberg, Baerg, Gershon, and Graudusius (5) demonstrated a decrease in the vitamin D-dependent active transport of calcium. This decrease in calcium transport could not be reversed by the administration of large doses of either cholecalciferol (vitamin D_3) or 25-hydroxycholecalciferol (25-HCC).[1] In these earlier studies, 45 h after the administration of a physiologic dose of [^{3}H]cholecalciferol to groups of control and cortisone-treated vitamin D-deficient animals, there were no differences in the amounts of cholecalciferol and 25-HCC in the plasma, intestinal mucosa, and liver from the control and treated animals. The results suggested that if indeed glucocorticoid administration induced a defect in the metabolism of cholecalciferol, it was *not* at the step involved in the side-chain hydroxylation of the parent vitamin to the 25-hydroxyl form, a reaction known to occur in the liver (6).

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[1] *Abbreviations used in this paper:* 25-HCC, 25-hydroxycholecalciferol; 1,25-DHCC, 1,25-dihydroxycholecalciferol; [^{3}H]25-DHCC, [^{3}H]25-dihydroxycholecalciferol.

Previous experiments did not exclude the possibility that cortisone treatment might influence the conversion of 25-HCC to the more polar forms of the vitamin (7–9). One of these metabolites, recently shown to be 1,25-dihydroxycholecalciferol (1,25-DHCC) (10–12), represents the predominant form of the vitamin in the intestine (8, 9), and it is believed that 1,25-DHCC is indeed the end-organ active metabolite in this tissue. 1,25-DHCC is biologically more active and more rapid in the onset of its intestinal effects than either cholecalciferol or 25-HCC (13, 14). Current evidence would indicate that the kidney is the major, if not the only organ capable of converting 25-HCC to 1,25-DHCC (15-17).

The purpose of this report is to present evidence that in the vitamin D-deficient rat the administration of cortisone does not influence the rate of conversion of 25-HCC to 1,25-DHCC. Furthermore, glucocorticoids do not alter the target tissue distribution or the subcellular localization in the intestinal mucosa of this biologically active dihydroxyl vitamin D metabolite. The results of the present study support the concept that the glucocorticoid-related effects on intestinal calcium transport occur independently of alterations in the metabolism and localization of vitamin D_3 or its biologically important metabolites.

METHODS

Animal preparation. 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.). The animals were housed in the dark and fed a vitamin D-deficient diet containing 0.8% calcium and 0.4% phosphorous (General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio). After 4–5 wk on the vitamin D-deficient diet the animals were randomized into groups of 9–15, each group matched for weight. During each of the 7 days before sacrifice, steroid-treated animals received cortisone acetate (cortisone acetate, Merck Sharp & Dohme, West Point, Pa.), 3.0 mg subcutaneously per 150 g body wt; control animals received daily subcutaneous injections of 0.3 ml of isotonic sodium chloride. This treatment program with cortisone has been shown to cause a profound defect in the active transport of calcium in the duodenum (5).

Animals from the paired control and cortisone-treated groups were weighed and lightly anesthetized with ether. Each animal then received an intrajugular injection of a physiologic dose of 26,27-[^{3}H]25-hydroxycholecalciferol, ([^{3}H]25-HCC), 400 mCi/mmol, in approximately 50 μl of 95% (vol/vol) ethanol. The individual dose was selected according to body weight such that a 150 g animal received 10 IU (i.e., each animal received 1 IU/15 g body wt). At the time of injection, the control animals weighed 173±19.9 g (mean ±1 SEM), whereas the cortisone-treated animals weighed 134±19.6 g (mean ±1 SEM). Labeled 25-HCC was purchased from New England Nuclear, Boston, Mass., and the unlabeled compound was generously provided by Dr. John C. Babcock of The Upjohn Co., Kalamazoo, Mich. After injection the animals were maintained on the vitamin D-deficient diet, and they continued to receive either cortisone acetate or isotonic saline daily until the day of sacrifice.

Extraction of radioactivity. At intervals of 6, 12, 24, and 48 h after the administration of [^{3}H]25-HCC, groups of control and cortisone-treated animals were sacrificed by concussion followed by exsanguination. Blood was collected in heparinized tubes, and plasma was subsequently prepared. The entire small intestine was removed, everted over chilled glass rods, and rinsed in ice-cold isotonic saline. The mucosa was separated from the underlying coats by scraping on a chilled glass plate with a glass microscope slide. Kidneys and liver were removed, rinsed in ice-cold 0.25 M sucrose, and frozen on a dry ice-acetone bath. Both hind femurs were removed, chilled, freed from soft tissue, and split in order that the marrow could be scraped away from the cortex. Those tissues not subjected to immediate extraction were stored at −20°C in an atmosphere of nitrogen.

The tissues and plasma obtained from each group of animals (control vs. cortisone-treated) at each point in time were pooled. Homogenates of intestinal mucosa (10%), liver (20%), and kidney (15%) were prepared in 0.25 M sucrose using a Waring blendor (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.) at medium speed for 30 s. Portions of the homogenates and plasma were analyzed for total tritium content after combustion (18). Determinations of protein and DNA content were performed by previously described methods (19–21). Bone was triturated with an equal volume of dry ice in a mortar and pestle and then stirred overnight at 4°C in 200 ml of chloroform: methanol, 1:2 (vol/vol). Tissue lipids were extracted by the method of Bligh and Dyer (22) as modified by Lund and DeLuca (23). The chloroform phases were concentrated by evaporation in a stream of nitrogen and dissolved in a small volume of *n*-hexane for silicic acid column chromatography or in chloroform: hexane, 65: 35 (vol/vol) for Sephadex LH-20 column chromatography (see below).

Preparation of intestinal nuclei. In a separate experiment, 30 weanling rats were raised in the dark and fed the vitamin D-deficient diet as described. During the 5th wk the animals were randomized into two groups, the steroid-treated group receiving cortisone acetate and the control group receiving isotonic saline subcutaneously every day for 7 days as described above. 24 h before sacrifice each animal received a physiological dose of [^{3}H]25-HCC intrajugularly as described. The rats were sacrificed by concussion and exsanguination, the entire small intestine was removed, the mucosa was scraped and pooled (see above), and nuclei were prepared by a technique modified after that described by Sporn, Wanko, and Dingman (24). All of the subsequent steps were performed at 0–4°C. A 10% mucosal homogenate was prepared in 0.32 M sucrose-0.001 M $MgCl_2$-0.0004 M K_2HPO_4-0.0004 M KH_2PO_4 (pH 6.7), using a Potter-Elvehjem homogenizer at 450 rpm for 14 complete passes. The homogenate was diluted to 2.5% with the above sucrose-containing solution, and it was then centrifuged at 800 *g* for 10 min in a Sorvall RC 3 centrifuge. The supernate was discarded, and the pellets were resuspended and washed three times in the sucrose-containing solution. During the final wash the pellets were sedimented at 10,000 *g* (Sorvall RC 2B, Ivan Sorvall, Inc., Norwalk, Conn.) for 10 min. The pellets were then resuspended in 0.32 M sucrose (final volume of 4.86 ml per g wet wt of mucosa). While stirring constantly, a dense sucrose solution composed of 2.39 M sucrose-0.001 M $MgCl_2$-0.0035 M K_2HPO_4 and 0.0007 M ATP was added to a final volume of 15.67 ml per g wet

wt of mucosa. The density of the suspensions was then adjusted to between 1.236 to 1.246. The suspensions were centrifuged in cellulose nitrate tubes at 50,000 *g* for 2 h in a Spinco SW 27 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The gelatinous film at the top of the tubes was removed with a spatula, the supernate discarded, and the nuclear pellets resuspended in isotonic sodium chloride (4.0 ml per g wet wt of mucosa). Portions of the original mucosal homogenates and of the final nuclear suspensions were removed for determination of protein (19), RNA (25), DNA (20, 21), and total radioactivity (18). The remaining nuclear suspension was extracted as described above, and the chloroform-soluble metabolites were chromatographed on columns containing Sephadex LH-20 (see below).

Column chromatography. Chloroform-soluble metabolites of [^{3}H]25-HCC in the various tissues obtained from control and cortisone-treated rats 6, 12, 24, and 48 h after administration of the labeled compound were subjected to chromatographic analysis on 58 × 1-cm columns of activated silicic acid employing the continuous gradient elution scheme described by Ponchon, Kennan, and DeLuca (6). Tritium present in the 10-ml fractions as well as that present in samples of the aqueous extracts was measured as previously described with an Intertechnique model SL-40 liquid scintillation spectrometer (Intertechnique Instruments, Inc., Dover, N. J.) (18). Disintegrations per minute in each sample were calculated after internal standardization with [^{3}H]toluene.

Chloroform extracts of various tissues from separate groups of control and cortisone-treated animals sacrified 24 h after [^{3}H]25-HCC administration, as well as the chloroform extracts of the nuclear suspensions described above, were subjected to chromatographic analysis using a 60 × 1.2 cm glass column containing 20 g of Sephadex LH-20 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) previously equilibrated in 70 ml of chloroform: hexane, 65:35 (vol/vol). The columns were eluted at room temperature with 345 ml of chloroform: hexane, 65:35 (vol/vol) and then stripped with 200 ml of chloroform: hexane, 70:30 (vol/vol) (26). Radioactivity in portions from each of the 3.0-ml fractions was determined as described above.

Periodate reaction. Fractions containing each of the peaks of radioactivity after Sephadex LH-20 chromatography were pooled, evaporated to dryness under N_2, and dissolved in 3.0 ml methanol, and 0.8 ml of 0.1 N paraperiodic acid was added. After 16 h at room temperature, the reaction mixture was poured into a separatory funnel and extracted with 10.0 ml chloroform and 3.0 ml water. The chloroform phase was removed and washed once with 3.0 ml of water, twice with 3.0 ml of 0.25 M $NaHCO_3$, and then twice again with 3.0 ml of water. Radioactivity was determined before and after periodation by counting portions of the original methanol solution as well as portions of the chloroform and aqueous phases following extraction. Generally, 95% of the radioactivity was recovered.

TABLE I

Radioactivity in Plasma and Tissue 6, 12, 24, and 48 h after Injection of Physiologic Dose of [^{3}H]25-HCC to Vitamin D-Deficient Control and Cortisone-Treated Rats

		dpm/mg protein or dpm/ml plasma		Chloroform soluble		Aqueous soluble	
Sample	Time	Control	Treated	Control	Treated	Control	Treated
	h			%		%	
Plasma	6	4247	6380	99.5	98.9	0.5	1.1
	12	7973	7624	92.4	91.4	7.6	8.6
	24	4392	5805	91.5	94.0	8.5	6.0
	48	3116	2364	97.6	97.2	2.4	2.8
Mucosa	6	8.8	13.6	70.0	65.8	30.0	34.2
	12	18.2	25.6	82.0	74.8	18.0	25.2
	24	12.7	11.9	79.0	77.5	21.0	22.5
	48	7.0	6.3	76.7	72.6	23.3	27.4
Liver	6	5.4	5.8	82.0	83.9	18.0	16.1
	12	9.2	9.2	81.1	81.4	18.9	18.6
	24	5.8	7.0	79.8	81.8	20.2	18.2
	48	4.8	2.9	82.6	75.3	17.4	24.7
Kidney	6	8.1	12.2	93.2	91.8	6.8	8.2
	12	13.8	15.7	90.3	85.8	9.7	14.2
	24	9.1	11.8	76.1	82.4	23.9	17.8
	48	7.8	7.0	77.8	75.6	22.2	24.4

The data represent the values from determinations performed on pooled samples obtained from 9 to 15 control and 9 to 15 cortisone-treated animals at each point in time (see Methods).

RESULTS

Distribution of radioactivity at varying times. The effects of cortisone administration on the tissue localization of radioactivity and on the distribution of radioactivity between the aqueous and chloroform phases at varying times after the administration of [^{3}H]25-HCC are shown in Table I. Maximum localization of radioactivity occurred at 12 h in all of the tissues examined from both the control and cortisone-treated animals, and it gradually declined thereafter. Of note is the fact that there were no consistent differences in the localization of radioactivity or in the distribution between the aqueous and chloroform phases in tissues from the control and steroid-treated groups.

Silicic acid column chromatography. Certain experiments were performed before the development of the more refined techniques presently available for the separation of polar metabolites of 25-HCC. With the silicic acid column chromatographic scheme employed in these experiments, peak IV has been shown to represent unaltered 25-HCC (6, 27). Recent reports have demonstrated that peak V obtained with this scheme is heterogeneous and that it is composed of a number of biologically active compounds (11, 14, 27–29). One of the predominant metabolites, 1,25-DHCC is thought to be the "tissue-active" form of the vitamin in the intestine (10–14). Another dihydroxyl metabolite eluted in the peak V region from silicic acid columns is 24,25-DHCC (30). This metabolite is said to have a marked action in mobilizing bone mineral, but only small effects on intestinal calcium transport and in the cure of rickets in rats (28). 25,26-DHCC also migrates in the peak V region, and this polar compound has been shown to have some intestinal calcium transport activity, but no activity in curing rickets or in causing bone mineral mobilization (29). Metabolites eluted in the peaks VI and VII region are of doubtful biological significance.

TABLE II

Plasma and Tissue Silicic Acid Chromatographic Fractions 6, 12, 24, and 48 h after Injection of Physiologic Dose of [^3H]25-HCC to Vitamin D-Deficient Control and Cortisone-Treated Rats

Sample	Peak	6 h		12 h		24 h		48 h	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
Plasma, *dpm/ml*	IV	4463	5111	5531	4611	2656	3332	2368	1337
	V	279	875	1160	1257	739	1038	614	602
	VI	103	134	179	242	119	205	53	80
	VII	50	0	49	128	69	64	18	70
Mucosa, *dpm/g protein*	IV	2929	3383	4294	4257	2086	2245	1168	893
	V	1844	2956	6245	8590	5920	4985	2068	1842
	VI	454	285	976	1041	389	510	211	200
	VII	87	270	245	602	229	256	121	95
Bone, *dpm/g wet wt*	IV	299	351	598	676	330	470	300	225
	V	43	95	306	379	190	365	63	132
	VI	27	30	91	51	63	80	22	11
	VII	6	8	185	16	27	29	9	8
Liver, *dpm/g protein*	IV	2781	2812	3775	3654	1841	2652	1316	779
	V	616	1040	2429	2111	1192	2291	529	399
	VI	330	139	598	412	217	452	107	87
	VII	108	98	37	227	177	333	55	34
Kidney, *dpm/g protein*	IV	5029	6992	6753	7165	3818	4936	2259	1863
	V	1234	2342	3534	3816	2262	3162	952	1045
	VI	125	425	407	179	266	432	126	138
	VII	136	290	338	475	339	303	109	150

The data are expressed as disintegrations/minute per milliliter (plasma), per gram wet weight (bone), or per gram protein (intestinal mucosa, liver, kidney) and represent values from determinations performed on pooled samples obtained from 9 to 15 control and 9 to 15 cortisone-treated animals at each point in time (see Methods). The chromatographic numbering system is after that of Ponchon, Kennan, and DeLuca (6).

Table II summarizes the results obtained when extracts of plasma and of various tissues from control and cortisone-treated rats were subjected to silicic acid column chromatography. 6 h after injection of labeled 25-HCC, peak IV, representing unaltered 25-HCC was still the major form of vitamin D metabolite in each of the tissues examined. By 12 h, considerable amounts of 25-HCC had been converted to more polar metabolites that were eluted in the peaks V–VII region. The intestinal mucosa was the only tissue studied in which peak V had become the dominant metabolites. Peaks VI and VII, which are of doubtful biological significance, contributed only a small percentage of the total radioactivity. Of interest is the fact that there were no striking effects of cortisone administration on the turnover of 25-HCC during the 48 h after its administration.

Sephadex LH-20 column chromatography. In view of the importance of 1,25-DHCC in mediating the intestinal response to vitamin D_3 and in view of the more recent availability of chromatographic methods to separate the components of silicic acid peak V, additional studies were undertaken. Vitamin D-deficient rats were treated with either cortisone acetate or saline for 7 days, given [^3H] 25-HCC and sacrificed 24 h later as described in Methods. Chloroform-soluble metabolites of 25-HCC extracted from various tissues were then chromatographed on columns containing Sephadex LH-20 (see Methods). Portions of each fraction were counted, and those fractions comprising the radioactive peaks were pooled and subjected to paraperiodic acid oxidation as described in Methods.

Oxidation using paraperiodic acid specifically cleaves carbon-carbon bonds at sites containing adjacent hydroxyl groups, thus leading to the formation of aldehydes and ketones. Since the [^3H]25-HCC was labeled at C-26 and C-27, cleavage at C-25–C-26 or C-25–C-27 would reduce total radioactivity by 50%, whereas cleavage at C-24–C-25 with loss of the C-25–27 chain as acetone would result in complete loss of radioactivity. Periodic acid oxidation of peak V*a* from plasma, intestinal mucosa, and kidney produced an 85–100% loss of radioactivity in both experimental groups. Oxidation of peak V from the same tissues of both control and cortisone-treated animals resulted in less than a 20% reduction in

TABLE III

Plasma and Tissue Sephadex LH-20 Chromatographic Fractions 24 h after Injection of Physiologic Dose of [^3H]25-HCC to Vitamin D-Deficient Control and Cortisone-Treated Rats

Sample	Peak	Tissue specific activity		Vitamin D metabolite concentration	
		Control	Treated	Control	Treated
Plasma	I + Ia	78	69	—	—
	IV	7473	8027	7.2	7.7
	Va	705	1580	0.65	1.45
	V	199	224	0.18	0.20
	Strip	59	79	—	—
Intestinal mucosa	I + Ia	4260	3430	—	—
	IV	96100	95080	93.62	104.53
	Va	9860	15510	9.11	16.33
	V	20791	24810	19.46	26.30
	Strip	2000	1430	—	—
Liver	I + Ia	435	394	—	—
	IV	3081	2886	2.95	2.76
	Va	358	586	0.33	0.53
	V	159	119	0.15	0.12
	Strip	91	26	—	—
Kidney	I + Ia	533	645	—	—
	IV	11322	11298	10.85	10.82
	Va	1406	2429	1.30	2.24
	V	595	666	0.55	0.60
	Strip	43	107	—	—
Bone	I + Ia	0	0	—	—
	IV	516	596	0.49	0.57
	Va	38	98	0.04	0.09
	V	16	29	0.02	0.03
	Strip	2	5	—	—
Intestinal nuclei	I + Ia	313	568	—	—
	IV	369	599	0.33	0.55
	Va	155	374	0.14	0.34
	V	1320	1485	1.22	1.38
	Strip	155	204	—	—

Tissue specific activities are expressed as disintegrations/minute per milliliter (plasma), per gram wet weight (bone), per gram DNA (intestinal mucosa, nuclei), or per gram protein (liver, kidney). Tissue concentrations of vitamin D metabolites are expressed as picomoles per milliliter (plasma), per gram wet weight (bone), per gram DNA (intestinal mucosa, nuclei), or per gram protein (liver, kidney). Peaks I and Ia are thought to be esters of 25-HCC; peak IV is 25-HCC; peak Va is 24,25-DHCC; peak V is 1,25-DHCC (Holick and DeLuca [26]). The nature of the metabolites eluted with chloroform: hexane 70:30 (strip) is unknown. The results of the experiment shown are representative of those obtained in two experiments performed in a similar manner.

radioactivity, whereas peak IV was not affected by the oxidation reaction. Based on the elution volume as well as the results of the periodate reaction, it can be assumed that peak IV represents 25-HCC, peak Va represents predominately 24,25-DHCC, and peak V represents predominantly 1,25-DHCC in both control and cortisone-treated tissue extracts.

The results shown in Table III demonstrate 25-HCC (Peak IV) to be the major form of radioactivity in all tissues examined. The initial peaks eluted (peaks I and Ia) are thought to represent esters of 25-HCC and are not considered of physiologic import (17). While 1,25-DHCC (peak V) was detected in each tissue, the greatest accumulation occurred in the intestinal mucosa. Of note is the fact that similar amounts of 1,25-DHCC were present in mucosa from the control and cortisone-treated groups (19.46 vs. 26.30 pmol per g mucosal DNA respectively in the experiment shown in Table III and 73.41 vs. 62.38 pmol per g mucosal DNA respectively in another experiment performed in a similar manner but with younger, more rapidly growing rats). Approximately twice as much 24,25-DHCC (peak Va) appeared in each of the tissues from cortisone-treated animals. Since the biological potency of this compound is relatively weak and its physiologic significance is unclear, it is difficult to attach great importance to this observation at the present time. Of special interest is the fact that the administration of cortisone in doses sufficient to depress intestinal calcium transport did not interfere with either the metabolic conversion of 25-HCC to 1,25-DHCC or the localization of the latter metabolite in the intestinal mucosa.

Nuclear localization of 1,25-DHCC. Myrtle, Haussler, and Norman (8, 31) and others (9) have demonstrated that a vitamin D metabolite subsequently identified as 1,25-DHCC preferentially localizes either within or on the intestinal mucosal cell nucleus. Furthermore, a number of steroid hormones undergo nuclear localization in target tissues (32). Since nuclear localization of a vitamin D metabolite may represent an important step required for the subsequent physiologic expression of the effect of the vitamin in the intestine, studies were undertaken to determine the influence, if any, of cortisone treatment on the intestinal cell nuclear localization of vitamin D metabolites.

Small intestinal mucosal cell nuclei from vitamin D-deficient control and cortisone-treated rats injected with [^{3}H]25-HCC 24 h before sacrifice were prepared as described in Methods. Employing the techniques described, nuclei could be prepared in relatively good yield (45–60%) and the preparations were shown to be fairly "clean." Phase contrast microscopy of the nuclear suspensions revealed the nuclei to be almost free of contaminating particles, and the nuclear membrane appeared to be intact. The protein: RNA: DNA ratios were 5.6: 0.22: 1.0, comparing favorably with those obtained in chick intestinal nuclei (33) and liver cell nuclei by other workers (34).

Nuclei prepared from the intestinal mucosa of control animals had 120 dpm per mg DNA, whereas those from cortisone-treated animals had 132 dpm per mg DNA. Between 71 and 84% of the total radioactivity was chloroform-extractable, and there were no significant differences between the control and steroid-treated animals in this regard. Fig. 1 and Table III

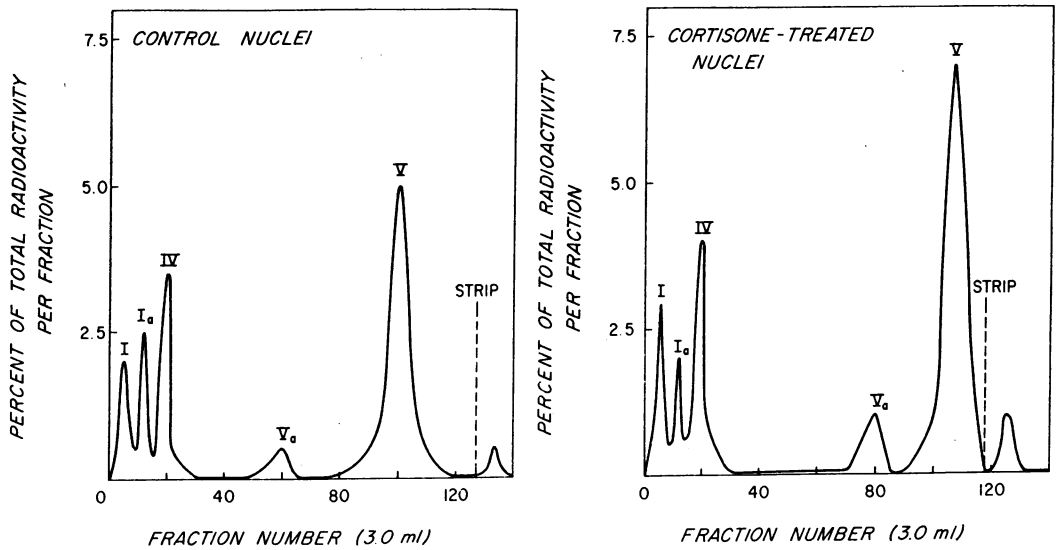

FIGURE 1 Comparison of Sephadex LH-20 chromatograms of radioactive metabolites extracted from intestinal mucosal nuclei prepared from control and cortisone-treated rats given a physiologic dose of [^{3}H]25-HCC 24 h before sacrifice. The results are expressed as the percent of the total radioactivity in each fraction. Nuclei from the control animals had a specific activity of 120 dpm per mg DNA, whereas nuclei from the cortisone-treated animals had a specific activity of 132 dpm per mg DNA. A total of 4982 dpm and 1928 dpm were chromatographed for the control and cortisone-treated groups, respectively.

present the results of Sephadex LH-20 column chromatography of the chloroform soluble metabolites extracted from the nuclear preparations of control and treated animals. In keeping with the results obtained by others, 1,25-DHCC (peak V) was found to be the predominant metabolite localized to the mucosal cell nucleus. Of importance is the observation that there were no striking differences in either the localization of radioactivity to the nuclei or in the amount of 1,25-DHCC present when one compares the results obtained with nuclei isolated from the mucosa of the control and cortisone-treated animals. Thus, although cortisone administration interferes with the intestinal transport of calcium, it does not influence the degree to which 1,25-DHCC, the presumed "tissue-active metabolite," localizes on or in the target cell nucleus. Once again, nuclei prepared from the mucosa of cortisone-treated animals may have greater amounts of 24,25-DHCC (peak V*a*).

DISCUSSION

The apparent antivitamin D-like effect of glucocorticoid administration has been demonstrated in the intestine of rats treated with pharmacologic doses of cortisone. Measurements of intestinal calcium transport in in vitro gut sac preparations (3–5) and in Ussing-type chambers (5) have revealed a decrease in net calcium absorption in animals maintained on adequate amounts of vitamin D. The mechanism whereby glucocorticoid administration interferes with the physiologic expression of vitamin D remains unknown. The possible explanations include: (*a*) cortisone induces an alteration in the metabolism of vitamin D; (*b*) cortisone interferes with the localization of vitamin D and/or its important metabolites in target tissues; (*c*) cortisone exerts biochemical effects in the intestine which interfere with the transport mechanism, quite independently of any direct interaction with the vitamin itself.

Avioli, Birge, and Lee (35) initially suggested that the antivitamin D-like action of glucocorticoids was due to an alteration in the metabolic conversion of the parent vitamin to a more polar, biologically active metabolite, subsequently shown to be 25-HCC. More recent studies in rats by Kimberg et al. (5) and by Schaefer, von Herrath, Koch, and Opitz (36) failed to substantiate the hypothesis that glucocorticoids alter the hydroxylation of the vitamin to the circulating-active form, 25-HCC. Furthermore, it has been shown that the administration of even pharmacologic doses of 25-HCC to cortisone-treated rats fails to restore intestinal calcium transport to normal (5).

Several laboratories have recently emphasized the biologic importance of metabolites of vitamin D which are even more polar than 25-HCC (8–17, 28–30). A number of these polar metabolites have been isolated, and those described to date are more polar by the addition of another hydroxyl group either at the 1 (10–12), 24

(30), or 26 (29) position. The precise role of each of these compounds in the physiologic expression of vitamin D action is not yet known. The potent biologic activity (13, 14), specific tissue localization (9, 31), and synthesis of picomole quantities of 1,25-DHCC by a closely regulated renal mitochondrial hydroxylase system (15–17) have suggested to some that this compound is both a "hormone" and the "tissue-active" form of the parent vitamin in the intestine (10, 14). The results of the present study demonstrate that further metabolism of 25-HCC to the important dihydroxyl metabolite, 1,25-DHCC, is not altered by cortisone administration. It seems reasonable, therefore, to conclude that cortisone exerts its antivitamin D-like effect by mechanism(s) other than interference with the required hydroxylations of the parent vitamin. The significance, if any, of the increased concentrations of 24,25-DHCC in the tissues of cortisone-treated animals remains to be determined.

The importance of intestinal mucosal nuclear localization of vitamin D metabolites in expressing the ultimate physiologic effects of the vitamin remains unclear. Haussler and Norman (37) have described a nonhistone protein in the chromatin fraction of intestinal mucosal cells that binds vitamin D compounds, especially 1,25-DHCC. The binding appears to be saturable and highly specific for 1,25-DHCC (31). A single injection of 0.125 mg of hydrocortisone in the chick did not interfere with the subsequent binding of tritiated vitamin D metabolites to the nuclear receptors (31). This small dose of hydrocortisone is, however, inadequate to produce a glucocorticoid effect on calcium transport (5). The demonstration in the present study that comparable amounts of 1,25-DHCC localize on or in intestinal mucosal cell nuclei prepared from control and cortisone-treated rats suggests that an alteration in mucosal nuclear localization of vitamin D metabolites is not a likely explanation for the steroid hormone-related inhibition of intestinal calcium transport.

Previous workers have been unable to obtain "pure" nuclei from rat intestine, presumably due to the presence of mucus (33). The technique used in the present study for the isolation of relatively uncontaminated rat intestinal mucosal cell nuclei is therefore, deserving of brief comment. The method was a modification of that described by Sporn, Wanko, and Dingman for the isolation of rat liver and brain cell nuclei (24). Dilution of the initial mucosal homogenate to 2.5%, centrifugation soon after homogenization, and final sedimentation through a sucrose-containing solution with a density of 1.240 (as opposed to a density of 1.270 for rat liver and brain nuclei), provided reasonable yields of relatively "clean" rat intestinal mucosal nuclei.

Cortisone administration is capable of inhibiting intestinal calcium transport despite its lack of an effect in preventing the synthesis of the vitamin D-dependent intestinal calcium-binding protein (5). Furthermore, glucocorticoid administration has been shown to influence the intestinal transport of iron and D-galactose, processes that are not dependent upon vitamin D (5). These observations suggested that the basis for the apparent antagonism between the effects of glucocorticoids and vitamin D may be independent of a direct interaction between the steroid hormones and the parent vitamin or its metabolites. Previous studies did indeed demonstrate that cortisone administration failed to interfere with the hepatic conversion of the parent vitamin D_3, cholecalciferol, to its 25-hydroxyl derivative (5, 36). This report presents evidence which indicates that glucocorticoids, administered in doses known to inhibit calcium transport, do not interfere with the subsequent conversion of 25-HCC to the "tissue-active" 1,25-dihydroxyl metabolite or with the cellular and subcellular localization of this metabolite in the intestinal target tissue. These results provide further support for the concept that the antivitamin D-like effect of glucocorticoids on intestinal calcium transport are due to hormone-related biochemical alterations in the mucosal cells containing the transport mechanism and that these alterations do not involve any direct interaction with vitamin D or its metabolites. Based on the results of the present study one would predict that vitamin D-deficient, cortisone-treated rats might be relatively resistant to the intestinal effects of physiologic doses of 1,25-DHCC. Studies designed to test this hypothesis are now being planned.

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