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Rectal Absorption of 6-Alpha-C^{14}-H_3-Prednisolone.* (25417)

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Administration of adrenocortical steroids per rectum in treatment of ulcerative colitis has produced encouraging results(1-11). In general, the primary mode of action of this form of therapy has been attributed to a local effect, as in the topical treatment of certain dermatologic conditions(2,5,6,10,14) though Liddle(13) and Nabarro *et al.*(4) have demonstrated absorption of some forms of hydrocortisone given by rectum. Our first experience with rectal administration of steroids was in Feb., 1958 during a preliminary evaluation of 6-methyl-prednisolone. The response was surprisingly gratifying. The subsequent availability of radioactive 6-methyl-prednisolone (6-alpha-C^{14}-H_3-prednisolone) prompted studies to (1) estimate amount of steroid absorbed from the rectum, (2) determine the rapidity with which it might be absorbed, and (3) length of time it remained within the body, if absorbed.

Methods. Administration: One milligram (3.2 microcuries) of 6-alpha-C^{14}-H_3-prednisolone, dissolved in one ml of 95% ethanol, was drawn into a 20 ml syringe. Forty mg of 6-methyl-prednisolone sodium succinate, dissolved in 1 ml of water, were rapidly drawn into the same syringe; (in case 4 this step was eliminated). Fifteen milliliters of tap water were drawn into the same syringe. The contents of the syringe were mixed rapidly and emptied by gravity into a soft rubber catheter, whose tip had been placed in the rectum of the patient lying on his left side. The catheter drained into the rectum completely within one minute and then was withdrawn; no patient evacuated the rectum in the succeeding 24 hours. *Analysis of urine*: Two successive 24 hour urine collections were made as separate voided specimens. The volume and time of each voided urine were recorded. Aliquots (0.5 ml) from each specimen were counted in a Packard Tri Carb Scintillation Counter as disintegrations per minute; total disintegrations/minute per voided specimen were calculated from these data. All voided specimens for each 24 hour period were pooled and mixed. Nine hundred ml aliquots from each 24 hour pool were extracted for the neutral lipid fraction. *Extraction of urinary corticosteroids*:† (12) The urinary pH was adjusted to 5. One-tenth volume of acetate buffer (pH 5.0) was added. Hydrolysis with 300 units of spleen beta-glucuronidase/ml progressed 5 days. The hydrolyzed urine was adjusted to pH 1 with sulfuric acid and continuously extracted with ether for 48 hours. The ether extract was washed 3 times with each 1/10 volume 2N sodium hydroxide and 1/10 volume of 5% sodium chloride. Each hydroxide or salt washing was back extracted with ether. Each "back extract" was added to the original ether extract. The total ether extract was evapo-

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† Modification of the method of Dobriner made by Attallah Kappas—personal communication.

TABLE I. Rectal Absorption of Radioactive 6-Methyl Prednisolone.

	Patient 1	Patient 2	Patient 3	Patient 4
Rectal dose (3.2 μc)				
Total counts/ml	10,870,000 DPM	10,870,000 DPM	10,870,000 DPM	10,870,000 DPM
Urine				
(1st 24 hr)	3,811,390	3,270,090	1,704,800	6,583,380
(2nd " ")	182,610	229,560	275,416	372,190
Total urinary radioactivity	3,994,000 DPM	3,499,650 DPM	1,980,216 DPM	6,955,570 DPM
% of rectal dose	37%	31%	18%	64%
Urinary ethereal extract				
(1st 24 hr)	2,111,787	1,297,399	993,378	3,338,048
(2nd " ")	90,372	Not done	153,868	157,016
Total	2,202,159 DPM		1,147,246 DPM	3,495,064 DPM
% of urinary radioactivity	55%		57%	50%
Urinary neutral lipid extract				
(1st 24 hr)	583,204	402,185	355,374	900,176
(2nd " ")	67,648	6,399	126,902	94,480
Total	650,852 DPM	408,584 DPM	482,276 DPM	994,656 DPM
% of urinary radioactivity	16%	12%	24%	14%
Residual urine				
(1st 24 hr)	1,197,990	892,080	326,981	40,493
(2nd " ")	97,640	Insufficient count	31,907	2,070
Total	1,295,630 DPM		358,888 DPM	42,563 DPM
% of urinary radioactivity	32%	25%	18%	0.6%

DPM = Disintegrations/min.

Patient 1 (S.R.)—Normal. Patient 2 (M.F.)—Chronic ulcerative colitis under no therapy. Patient 3 (R.H.)—Chronic ulcerative colitis receiving prednisone, 40 mg daily. Patient 4 (I.B.)—Chronic ulcerative colitis receiving ACTH gel, 40 units intramusc., and prednisolone, 100 mg orally daily.

rated to dryness, then dissolved in 25 ml of purified ethanol. The ether extract, sodium hydroxide washing and sodium chloride washing were counted in a Packard Tri Carb Scintillation Counter.

Results. Unfortunately, studies in patients with ulcerative colitis involving urine and fecal collections are fraught with difficulties indigenous to the disease. Of 7 hospitalized patients in whom the radioactive 6-methyl-prednisolone was instilled, only 2 ulcerative colitis patients and one normal individual provided complete collections of urine. Athough a third ulcerative colitis patient (R.H.) maintains he supplied a complete collection, the low 24 hour volumes and low total radioactivity of his urine strongly suggest that the collections were not complete; nevertheless, this case is included.

The 4 patients in this study are: (1) S.R., a normal male 54 years of age; (2) M.F., a 25 year old male with chronic ulcerative colitis for 4 years who had not received steroid or corticotropin therapy for 16 months; (3) R. H., a 20 year old male with ileocolitis for 2 years and receiving prednisone, 40 mg orally at time of study; (4) I. B., a 34 year old male with ulcerative colitis for 5 years and receiving ACTH gel, 40 units intramuscularly and prednisolone, 100 mg orally each day during this study. All ulcerative colitis patients were under relatively similar control; averaging one bowel movement every one to 2 days. The stools of M.F. contained gross blood; the others were positive for occult blood (benzidine test). The rectal mucosa of M.F. was edematous, granular and slightly friable. In the other patients with ulcerative colitis, the rectal mucosa was edematous and granular but not friable. In spite of the limited number of patients, the preliminary findings appear of interest.

The pattern of excretion of radioactivity is depicted in Table I. The most striking observation is the general similarity of the curves of all patients. It also is apparent that half of the dose of total absorbed radioactive 6-methyl-prednisolone reflected in urinary ex-

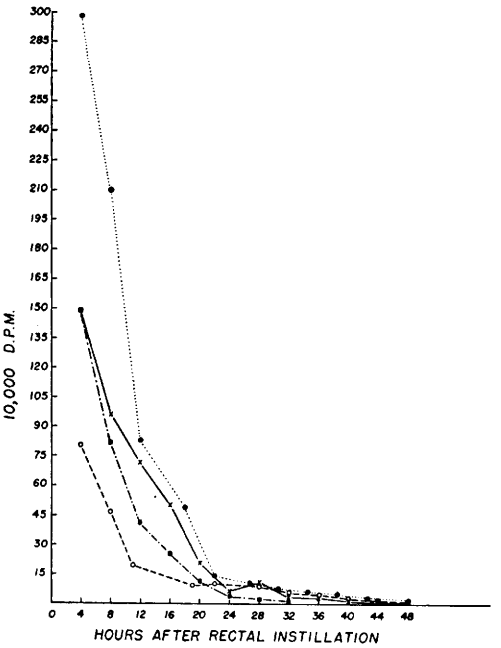

FIG. 1. Rectal absorption of 6-alpha-C^{14}-H_3-prednisolone. ×———×, Patient 1 (S. R.), normal; ■-·-·■, Patient 2 (M. F.), chronic ulcerative colitis with no therapy; ○- - -○, Patient 3 (R.H.), chronic ulcerative colitis receiving prednisone, 40 mg daily; ●·····●, Patient 4 (I. B.), chronic ulcerative colitis receiving ACTH gel, 40 units, and prednisolone, 100 mg daily.

cretion is excreted within 6 hours after rectal instillation, a trend observed in all 4 subjects. Two patients received tracer doses in the morning, 2 in the afternoon, there was no discernible diurnal variation in excretion.

Table II demonstrates amount of radioactivity from the rectal dose recovered in the urine. In the urine of the normal man 37% of the radioactivity in the rectal dose was recovered within 48 hours after instillation; 31% from the urine of the patient with ulcerative colitis receiving no other therapy. H.R., the patient receiving oral prednisone, excreted only 18% of the rectal dose in the urine, but, as stated previously, we suspect this low value reflects incomplete collection of urine, rather than a true decrease in absorption. I.B., the patient receiving ACTH intramuscularly and prednisolone orally, excreted 64% of the rectal dose in the urine. The increase in total absorption in this case may reflect an action of ACTH or prednisolone on the rectal mucosa, possibly increasing the absorptive capacity.

However, regardless of total amount of radioactivity excreted in urine, the various extractions of urine used to isolate the metabolites of 6-methyl-prednisolone contained approximately the same proportions of radioactivity in all cases. Between 50 and 75% of urinary radioactivity was contained within the metabolites extractable by ether. Between 12 and 24% was detected in the metabolites contained within the neutral lipid extract. This observation indicates that the major portion of steroid metabolites of 6-methyl-prednisolone are water soluble; an acceptable observation in relation to the known information concerning the metabolic products of prednisolone.

Discussion. Because the C^{14} atom is contained within the 6-methyl group, we have been concerned that bacterial action in the colon might split it off, permitting its absorption independently of the steroid nucleus. This may have occurred to the major portion of the 6-methyl-prednisolone instilled in the rectum. Nevertheless, the evidence indicates that a significant amount was absorbed unaltered. This result agrees with the findings of Nabarro *et al.*(4) and Liddle(5) who estimated that between 26 and 33% of hydrocortisone in alcohol was absorbed from the rectum. That plasma cortisol levels do not rise significantly following cortisol enemas(9) is not in disagreement with these findings. On the contrary, Schwartz *et al.* found that, although the plasma cortisol level was not altered measurably, the plasma content of conjugated steroids derived from cortisol was noticeably increased within the first hour after cortisol was given by rectum. These findings thus reflect the rapid metabolic turnover of adrenocortical steroids within the body.

The present observation that half of absorbed radioactivity excreted in urine leaves the body within 6 hours supports another observation of Schwartz *et al.*, that the elevated plasma content of conjugated steroids derived from radioactive cortisol given by rectum fell to non-detectable levels within 4 hours after the retention enema.

Although we do not know how much of the benefit of steroid retention enemas in treatment of ulcerative colitis is from local effect and how much from systemic absorption of the steroid, there is no doubt that a substantial amount of 6-methyl-prednisolone is absorbed. Evidence suggests that this drug is absorbed very rapidly, metabolized rapidly, and that half of the quantity absorbed and excreted in the urine is excreted in less than 6 hours.

Conclusion. 6-methyl-prednisolone is absorbed rapidly and in substantial amounts from the rectum in normal individuals and in patients with ulcerative colitis. Half of the 6-methyl-prednisolone absorbed from the rectum and excreted in the urine is eliminated within 6 hours. The beneficial action of 6-methyl-prednisolone retention enemas may be systemic as well as local.

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Action of Prolactin on Seminal Vesicles of Guinea Pig.* (25418)

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The function of prolactin (lactogenic hormone, luteotrophic hormone or LTH) in the male mammal is not well established. Pasqualini(1) reported a marked increase in secretions of seminal vesicles of castrate rats after administration of LTH for 3 consecutive days following priming dose of testosterone propionate. Influence of LTH on the prostate was not significant. Pasqualini considered that the action of LTH was directly on the seminal vesicles but manifested itself only when the wall of seminal vesicles was rendered reactive by action of testosterone. Chase *et al.*(2) observed minimal growth of seminal vesicles of castrate Sprague-Dawley rats with administration of LTH or LTH in combination with TP. Testosterone and STH together caused a significant increase in weight and proportion of glandular tissue of the ventral prostate and seminal vesicles. Their data suggest that LTH may act in a synergistic manner to stimulate accessories of the male rat. Everett(3,4) showed that pituitary autografts to the kidney capsule of female rats favored continuous secretion of LTH and maintained corpora lutea in a functional state at least 4 to 5 times the duration of pregnancy. Rennels (Univ. of Texas, personal communication) pointed out that the pituitary of male rat transplanted to kidney capsule of female rat was equally capable of producing LTH and maintained corpa lutea in a functional state. In view of these observations it became of interest to see if the autografted pitui-

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