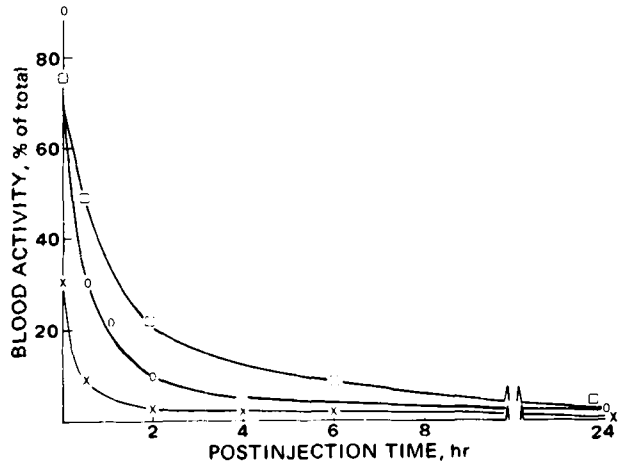

Figure 2—*Comparison of blood clearance rates of* ^{99m}*Tc-dimercaptosuccinic acid* (○), ^{99m}*Tc-dimercaptoglutaric acid* (×), *and* ^{99m}*Tc-dimercaptoadipic acid* (□) *formed at pH 2.5.*

kidney accumulation of V–VII *versus* postinjection time is shown in Fig. 1. Within a few hours after injection of the acidic complexes, V concentrated most highly in kidney tissue, plateauing at slightly above 30% of the total injected radioactivity. Five-carbon VI reached a maximum of 15%, and six-carbon VII concentrated least of all, plateauing at 8%. Paired *t* test analysis showed the curves to be significantly different ($p < 0.05$) for periods greater than 2 hr.

In contrast, the basic complexes showed an initial low kidney accumulation that radically dropped a short time after administration. Least sensitive of all to pH changes was VII, which showed very low kidney uptake of both acidic and basic preparations. In all cases, as the radioactivity in kidney tissue decreased, a marked increase in activity was found in the animal's excreta.

Figure 2 shows the average blood clearance of the three complexes formulated at pH 2.5. Slowest to clear was VII. The relative blood clearance rates were the same for complexes prepared at pH 7.5, but the individual clearance rates were faster. These complexes were excreted within 0.5 hr of injection.

Further studies indicated that low pH preparations were necessary for high kidney localization of the complexes. Complexes prepared at pH 2.5 and then raised to pH 7.5 by sodium bicarbonate addition prior to *in vivo* injection localized strongly in the kidney. However, complexes prepared at pH 7.5 and then lowered to pH 2.5 by hydrochloric acid addition still showed low kidney localization and were excreted rapidly. These observations are in agreement with those previously reported (9).

The ligand to tin ratio during complex formation and the injection time after complex preparation did not significantly affect the degree of complex localization in kidney tissue. During these studies, the ligand to tin ratio varied from 2:1 to 10:1, and the complexes were injected from 30 min to 6 hr after their preparation. If the complexes were not prepared using pure ligand and reagents and in an inert atmosphere, they rapidly decomposed into fragments that concentrated in the liver.

The nature of the technetium complexes formed is uncertain. The presence of two mercapto groups in the dicarboxylic acid molecules was necessary for good kidney uptake of the complexes. Comparative studies in this laboratory[6] using various monomercapto homologs such as ^{99m}Tc-mercaptoacetic acid and ^{99m}Tc-thiolactic acid showed that these complexes were more rapidly excreted and that their renal uptake was lower.

Reduction of pertechnetate with stannous chloride was used in this study because it was easy, rapid, and convenient. The complexes prepared by this method accumulated sufficiently in the kidney. However, reduction with hydrogen bromide, which can be used for a variety of mercaptan compounds, gave a higher deposition in the kidney with a considerable reduction in the excretion rate. Table II shows the distribution of V prepared by this method. The reason for the higher uptake is unknown. It may be due to the formation of a purer complex than that formed by tin reduction or to formation of an entirely different complex with higher specificity for kidney tissue.

Further studies are in progress to investigate the nature of complex formation as well as the chemical structure–kidney localization relationship.

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Hydrocortisone Stability in Human Feces

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Received July 31, 1978, from the *Clinical Pharmacokinetics Laboratory, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo,* and *Millard Fillmore Hospital, Buffalo, NY 14209.* Accepted for publication November 1, 1978.

Abstract □ Hydrocortisone stability in human feces was studied under various conditions to determine whether stability accounts for the variable effects of hydrocortisone enemas. Recovery from feces and assay specificity were assured using dual isotopes, TLC separation, and liquid scintillation counting. Hydrocortisone degraded slightly from 7 to 26% in 24 hr when incubated in fresh human feces at 37°. Less than 7% degradation occurred in feces stored at 10°, and negligible degradation occurred with hydrocortisone in water at 37°. Fecal bacteria may account for the observed degradation. Hydrocortisone stability in feces may contribute to local persistence and may account partly for its efficacy in ulcerative colitis treatment.

Keyphrases □ Hydrocortisone—degradation, human feces, various rectal dosage forms □ Dosage forms—hydrocortisone, various rectal dosage forms, degradation in human feces □ Stability—hydrocortisone in human feces, various rectal dosage forms

Rectally administered hydrocortisone (cortisol) is an established mode of long-term ulcerative colitis therapy. It has the proposed advantage of direct drug delivery without excessive systemic absorption and the attendant

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Table I—Extraction of Hydrocortisone from Feces: Percent Recovery of Added Radiochemical

Sample	4-^{14}C-Hydrocortisone	1,2-^{3}H-Hydrocortisone
1	69.2	70.7
2	74.7	74.7
3	76.8	72.6
4	74.2	78.7
5	70.2	72.9
6	66.8	67.5
Mean	72.0	72.9
CV, %	5.3	5.2

risk of side effects (1–3).

Reported systemic hydrocortisone absorption following rectal administration is 0–50% of that seen after oral administration (1, 3). The reason for incomplete rectal absorption is uncertain. In particular, hydrocortisone stability in the colo-rectal environment has never been demonstrated. Fecal bacteria are capable of 21-dehydroxylating certain corticoids (4, 5) and may degrade hydrocortisone in feces.

This paper describes the analysis and *in vitro* stability of hydrocortisone in human feces incubated at 37°.

EXPERIMENTAL

Materials—1,2-^{3}H-Hydrocortisone [250 μCi/0.002 mg in 0.25 ml of benzene–ethanol (9:1)] and 4-^{14}C-hydrocortisone [50 μCi/0.33 mg in 2.5 ml of benzene–ethanol (9:1)] were received[1] in sealed ampuls. The ^{3}H-hydrocortisone was diluted to 15 ml with benzene–ethanol (9:1).

Hydrocortisone as the free alcohol[2] and two commercial hydrocortisone rectal enema suspensions[3] were used as received. Acetone, ethanol, trichloroacetic acid, benzene, methylene chloride, sodium chloride, and sodium hydroxide were reagent grade. Reagents and standards were prepared with distilled and deionized water.

Fecal samples were collected in 1-liter polytef-lined[4] metal paint cans[5]. Hydrocortisone was extracted from fecal homogenate using 50-ml conical polyethylene tubes[6], and extracts were separated on silica gel 60 F-254 precoated TLC plates[7]. Radioactive samples and quenched standards were prepared with a universal liquid scintillation cocktail[8].

Apparatus—Hydrocortisone on TLC plates was visualized using a short wavelength (254 nm) UV visualizing cabinet[9]. Dual-labeled samples were counted with a liquid scintillation spectrometer[10].

Radiochemical Standards—Dilute stock solutions of ^{3}H- and ^{14}C-hydrocortisone were prepared. Aliquots (0.3 ml) of ^{3}H-hydrocortisone (16.7 μCi/ml) and ^{14}C-hydrocortisone (20 μCi/ml) were transferred to separate 50-ml volumetric flasks and diluted to volume with benzene–ethanol (9:1). These solutions were stored in amber, glass-stoppered bottles at 10°.

Ethanolic radioactive hydrocortisone standard solutions were prepared as needed by evaporating small volumes (3–5 ml) of the stock solutions under nitrogen and reconstituting immediately with equal volumes of pure ethanol. These solutions were used for no longer than 72 hr and were stored in darkness at 10°.

Quenched Standards—A set of quenched standards was prepared for ^{3}H- and ^{14}C-hydrocortisone to facilitate counting efficiency determination of each isotope.

To each of six scintillation vials were added 10 ml of scintillation cocktail, 3 ml of water, and 100 μl of either ^{3}H- or ^{14}C-hydrocortisone stock solution. Acetone was added as a quenching agent so that one vial in each set contained 0, 0.1, 0.3, 0.5, 0.7, or 0.9 ml.

Standard curves were prepared by plotting the external standards ratio (an index of quench) *versus* the counting efficiency of each label in two preset energy channels.

[1] New England Nuclear, Boston, Mass.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] I-Rectoid, Pharmacia; II-Cortenema, Rowell Laboratories.
[4] Teflon, du Pont.
[5] Glidden Paint Co., Buffalo, N.Y.
[6] Becton, Dickinson and Co., Cockeysville, Md.
[7] E. Merck, Darmstadt, West Germany.
[8] Scinti-Verse, Fisher Scientific Co., Springfield, N.J.
[9] Ultra-Violet Products, San Gabriel, Calif.
[10] Packard Instrument Co., Downers Grove, Ill.

Stability Protocol—Hydrocortisone stability was tested as three different formulations in biological specimens and under various conditions:

1. Incubation in feces at 37°—hydrocortisone enema I, three subjects; hydrocortisone enema II, one subject; and hydrocortisone free alcohol, one subject.
2. Incubation in feces at 10°—hydrocortisone enema I, one subject.
3. Incubation in water at 37°—hydrocortisone enema II, one subject.

Fecal Collection—Fresh fecal samples were collected from three healthy volunteers in clean dry-weighed metal cans. The cans were weighed again, an equal weight of water was added, and the contents were shaken vigorously for 1 hr.

Incubation—Each test series consisted of 1-g aliquots of fecal homogenate or water in six 50-ml plastic tubes. To each set of tubes was added hydrocortisone enema I, II, or aqueous hydrocortisone free alcohol so that the resulting hydrocortisone concentration in each sample was 0.25 mg/g.

Freshly prepared ethanolic ^{14}C-hydrocortisone solution (150 μl, ~39,960 dpm) was added to each tube, and the contents were vortexed for 30 sec. The tubes were sealed, covered with foil, and placed in either a constant-temperature water bath at 37° or a refrigerator at 10°.

Samples from each test series were removed from incubation at 0, 3, 6, 11, 14, and 24 hr. Freshly prepared ^{3}H-hydrocortisone in ethanol (150 μl, ~33,300 dpm) was added immediately to each tube to serve as an internal recovery standard and to account for losses during extraction. Six milliliters of trichloroacetic acid (6%) was added, and the contents were vortexed for 1 min. Following centrifugation at 3000 rpm for 10 min, the supernate was transferred to a clean plastic tube and stored at 10°.

Analytical Procedure—*Extraction*—To the supernate were added 4 ml of saturated sodium chloride solution and 35 ml of methylene chloride. After shaking vigorously for 30 min, the mixture was centrifuged at 3000 rpm for 5 min. The aqueous layer was aspirated and discarded. Then 10 ml of 0.1 *N* NaOH was added, and the mixture was vortexed for 15 sec. Following centrifugation at 3000 rpm for 5 min, the aqueous layer was again aspirated and discarded. The resultant methylene chloride phase was evaporated to dryness in the same tube under nitrogen with gentle heat (55°) provided by a water bath.

TLC—The residue was reconstituted with 100 μl of methylene chloride. Approximately 20 μl of this solution was spotted on the TLC plate with a 5-μl capillary tube. A known hydrocortisone standard in methylene chloride (0.5 mg/ml) was also spotted on the plate. Plates were developed in benzene–acetone (50:50) for the entire length of the plate (18 cm). The steroid R_f was 0.4. The hydrocortisone in each sample was visualized under UV light at 254 nm and compared to the hydrocortisone standard. Degradation products were observed but were not identified.

Liquid Scintillation Spectrometry—The silica was scraped from sections of the plate corresponding to unchanged hydrocortisone and placed in a scintillation vial containing 10 ml of scintillation cocktail and 3 ml of water. Cocktails were prepared to quantitate the actual disintegrations per minute in the ethanol solutions of ^{3}H- and ^{14}C-hydrocortisone that were added to these samples.

All samples were run with the two quenched standards sets and counted for approximately 10 min or 10,000 cpm under preset dual-label counting conditions.

Calculations—Unchanged ^{14}C-hydrocortisone radioactivity (disintegrations per minute) was analyzed as a function of time in all test groups. The amount of unchanged ^{14}C-hydrocortisone in each sample before extraction was determined as follows.

The disintegrations per minute of carbon 14 in a sample was determined by:

$$^{14}\text{C dpm} = {}^{14}\text{C cpm/efficiency of } {}^{14}\text{C in } {}^{14}\text{C channel} \quad \text{(Eq. 1)}$$

The counts per minute of hydrogen 3 in each sample was corrected for its efficiency in the hydrogen 3 window as well as the efficiency of carbon 14 in the hydrogen 3 window:

$$^{3}\text{H dpm} = \frac{{}^{3}\text{H cpm} - ({}^{14}\text{C dpm} \times \text{efficiency of } {}^{14}\text{C in } {}^{3}\text{H window})}{\text{efficiency of } {}^{3}\text{H in } {}^{3}\text{H window}} \quad \text{(Eq. 2)}$$

The internal standard (^{3}H-hydrocortisone) recovery efficiency was determined by:

$$\text{recovery efficiency} = \frac{{}^{3}\text{H dpm}}{\text{known } {}^{3}\text{H dpm added to sample}} \quad \text{(Eq. 3)}$$

The unchanged ^{14}C-hydrocortisone present in the sample before extraction was then calculated:

$$\text{actual } ^{14}\text{C dpm} = \frac{^{14}\text{C dpm calculated}}{\text{recovery efficiency}} \qquad \text{(Eq. 4)}$$

Extraction Efficiency and Reproducibility—^{14}C-Hydrocortisone and ^{3}H-hydrocortisone were added to six 1-g fecal homogenate samples, and the extraction procedure was performed. Following extraction, the methylene chloride phase was transferred to an empty scintillation vial and dried under nitrogen with gentle heat. Scintillation cocktail and water were added to the vials, and they were counted using the dual-label setting. The mean recovery of each labeled compound from feces was determined as well as the variability in recovery.

Added Radioactivity Recovery—The appearance of ^{14}C-hydrocortisone degradation products with time was quantitated for one incubated fecal sample set. After the regions corresponding to unchanged hydrocortisone had been scraped from the TLC plates, the remaining silica gel for the 0-, 11-, and 24-hr samples was scraped into separate vials and counted. Added ^{14}C-hydrocortisone recovery (unchanged and degraded) was calculated for each sample.

RESULTS

Extraction Technique—The efficiency and reproducibility of the technique are shown in Table I. The similarity in recoveries of the two labeled compounds in each sample indicates that either compound may be used as an internal recovery standard for the other. The extraction procedure reproducibility was good with a variation coefficient of about 5%.

Stability Studies—Hydrocortisone degradation over time under various conditions is presented in Fig. 1. Hydrocortisone enema I degradation in the feces of three different subjects ranged from 4.4 to 12.3% in 6 hr and from 7 to 26% in 24 hr (Fig. 1A). This same formulation showed negligible degradation when incubated at 37° in water (Fig. 1B). A study comparing hydrocortisone degradation in the feces of one subject under different temperature conditions (Fig. 1C) showed only 6.5% degradation at 10° as compared to 26% at 37° in 24 hr.

The degradation of three hydrocortisone formulations in the incubated feces of one subject (Fig. 1D) ranged from 13.6 to 26%, with the least degradation occurring with hydrocortisone free alcohol.

Added Radioactivity Recovery—At 0, 11, and 24 hr, the total carbon 14 percent present on the TLC plate as degradation product was 3.5, 9.6, and 15.6%, respectively. The added carbon 14 percent recovery, as both unchanged and degraded hydrocortisone, was 102, 89.3, and 83.1 for the 0-, 11-, and 24-hr samples, respectively. Inability to extract degradation products from feces as efficiently as unchanged hydrocortisone may account for the observed decrease in total radioactivity recovery with time. These results might also be explained by increased binding of unchanged hydrocortisone to incubated feces over time.

DISCUSSION

Hydrocortisone incubation in human feces resulted in as much as 26% degradation over 24 hr. Hydrocortisone given as an enema, however, is rarely retained in the rectum and colon longer than 6 hr, and the maximum observed degradation was 12% at this time.

Fecal material itself causes hydrocortisone degradation rather than temperature and hydrolysis (Fig. 1B). Relative hydrocortisone stability in water at 65° was previously reported (6) as a degradation $t_{1/2}$ of 25 days. A lower temperature apparently confers protection against degradation in feces (Fig. 1C). This phenomenon may be consistent with bacterial hydrocortisone degradation. Other investigators (4, 5) recently isolated and characterized in human feces bacterial organisms capable of metabolizing corticosteroids.

The small amount of hydrocortisone added to feces in the study (0.25 mg/g of fecal homogenate) was chosen to provide realistic exposure of hydrocortisone to fecal material, thus maximizing any possible degradation. The usual rectal hydrocortisone dose is 100 mg. Because the fecal samples were diluted twofold, the hydrocortisone concentration was actually 0.5 mg/g of feces. This concentration was selected assuming an average of 200 g of feces in the rectum at the time of hydrocortisone administration.

Because the greatest hydrocortisone loss was 26% in 24 hr at 0.5 mg/g of feces, only 6.5% of the drug should degrade in 6 hr if the process is first order. Extrapolation of these results to the clinical situation shows that even with a large fecal content in the rectum and thorough mixing of the

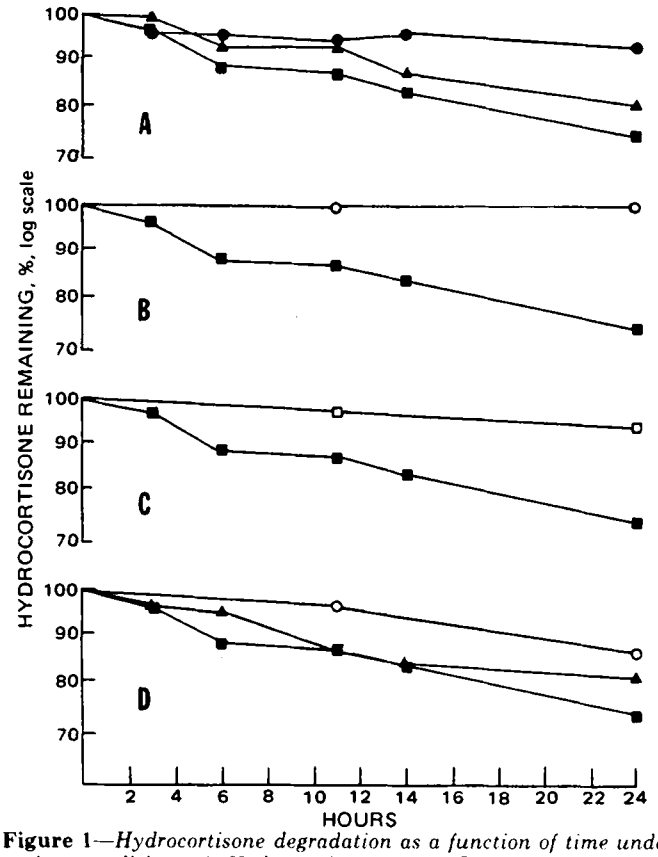

Figure 1—*Hydrocortisone degradation as a function of time under various conditions. A. Hydrocortisone enema I degradation in fecal samples from three human volunteers. Samples were incubated at 37°. B. Hydrocortisone enema I degradation in water (○) and human feces (■) at 37°. C. Hydrocortisone enema I degradation in fecal samples incubated at 37° (■) and refrigerated at 10° (□). D. Comparison of three hydrocortisone product degradations in human feces at 37°. Key: ○, hydrocortisone free alcohol in aqueous solution; ■, hydrocortisone enema I; and ▲, hydrocortisone enema II.*

administered hydrocortisone with rectal contents, little of the drug should actually be degraded during retention. For a 100-mg dose, only 6.5 mg should degrade in 6 hr.

It is widely assumed that the therapeutic benefit of hydrocortisone enemas is due in part to local action on the intestinal mucosa of ulcerative colitis patients. Previous studies (1, 3) suggested that hydrocortisone is incompletely absorbed through the intestinal mucosa and, therefore, persists in and acts upon the large intestine and rectum. A local effect could also result from lower GI tract exposure to the steroid during absorption. This demonstration of minimal hydrocortisone degradation in fecal material excludes one potential factor responsible for incomplete systemic availability of rectally administered hydrocortisone. Other factors that may account for incomplete systemic hydrocortisone availability include first-pass intestinal mucosa and/or liver metabolism and slow absorption and inadequate retention in the lower bowel.

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