

THE PHYSIOLOGICAL DISPOSITION AND METABOLIC FATE OF HYDROCORTISONE IN MAN

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The present report is concerned primarily with the physiological disposition and fate of hydrocortisone in man. Large doses of this steroid have been administered intravenously, and the rate of its disappearance from plasma has been determined in normal subjects and in patients with liver disease and various endocrinopathies. Trace quantities of hydrocortisone-4-C¹⁴ have been employed in certain studies, either alone or as a label for the larger doses. From these studies information has been obtained regarding rates of metabolic transformation, rates and routes of excretion of hydrocortisone and its metabolites, and to some extent the identity and amounts of these metabolites. Data on the rate and extent of absorption of hydrocortisone after oral administration have also been obtained. Since, in most respects, the results obtained upon administering large or trace quantities of hydrocortisone did not differ greatly, the findings are to a considerable extent also applicable to problems of endogenous hydrocortisone metabolism.

MATERIALS AND METHODS

Administration of steroid

Fifty to 500 mg. of hydrocortisone were dissolved in ethanol and added to 500 ml. of sterile 5 per cent dextrose in water, producing a final alcohol concentration of 2 to 3 per cent. This solution was given intravenously over a period of 20 to 30 minutes. Following the infusion, blood samples were drawn in heparinized syringes every 20 to 30 minutes for 2 to 3 hours. Urine samples were collected for 24 hours or longer in single or fractional specimens, and preserved by freezing.

Procedure for determination of plasma hydrocortisone

The following procedure is a modification of the recently published method of Silber and Porter (1):

Principle—Hydrocortisone is extracted from plasma into dichloromethane. The dichloromethane extract is washed with aqueous alkali to remove a considerable amount of "blank" material. The dichloromethane is then shaken with a sulfuric acid-ethanol reagent, containing

phenylhydrazine. The resulting colored product is measured in the acid phase spectrophotometrically at 410 $m\mu$. A correction for material in plasma reacting with sulfuric acid is made by treating an equal aliquot of dichloromethane extract of plasma with sulfuric acid-ethanol which contains no phenylhydrazine.

Reagents.

(a) *Dichloromethane*—This solvent is purified by passing through a bed of silical gel (average 100 mesh) in a 7 by 125 cm. column. Ten to twenty liters can be purified in 2 to 3 hours. The effectiveness of purification is determined by shaking an aliquot of solvent with the phenylhydrazine-sulfuric acid-ethanol reagent (f). No color should develop on standing overnight at room temperature. The solvent has remained free of impurities for many months at room temperature.

(b) *0.1 N Sodium Hydroxide.*

(c) *70 Per Cent Sulfuric Acid*—700 ml. acid C.P. grade sulfuric acid added to 300 ml. H₂O.

(d) *Ethanol*—This is purified by adding 7 gm. silver nitrate and 15 gm. potassium hydroxide, separately (each dissolved in 100 ml. ethanol) to 4 liters of absolute ethanol. These are mixed, allowed to stand overnight, and then distilled, using a Vigreux column. The first 700 ml. and the last 100 ml. portions are discarded. The effectiveness of purification is determined by reacting this ethanol with the phenylhydrazine-sulfuric acid (f). No color should develop on standing overnight at room temperature.

(e) *Blank Reagent*—Two parts (c) with one part (d).

(f) *Phenylhydrazine-Sulfuric Acid-Ethanol Reagent*—50 mg. of phenylhydrazine hydrochloride (recrystallized from ethanol and water) are dissolved in 50 ml. of the blank reagent (e).

(g) *Hydrocortisone Standard*—100 mg. of hydrocortisone are dissolved in 100 ml. absolute ethanol. A working standard is made by diluting one ml. to a volume of 100 ml. with water (10 micrograms per ml. water).

Procedure. (All glassware used *must be scrupulously cleaned* with concentrated sulfuric acid.)

1. *Extraction*—Carefully add 5 ml. plasma to 25 ml. dichloromethane in a 200-ml. Erlenmeyer flask and place on rotating table (Arthur H. Thomas No. 3623). Extract for 10 to 15 minutes with gentle rotation (this procedure extracts more than 98 per cent of the hydrocortisone, free and protein bound, in plasma). Gently transfer entire contents to a 25-ml. graduated cylinder.

2. *Washing*—After aspirating as much plasma as possible, add 2 ml. 0.1 N sodium hydroxide to dichloromethane extract, shake for 15 to 20 seconds, and then remove the alkali layer by aspiration.

3. *Aliquots*—Transfer two 10-ml. aliquots (for unknown and blank) and place in separate 15-ml. ground glass stoppered conical test tubes.

4. *Color Development*—For unknowns, add 0.2 ml. phenylhydrazine-sulfuric acid-ethanol reagent (f) to dichloromethane extract aliquot; for blanks, add 0.2 ml. blank reagent (e). Stopper the tubes, shake vigorously for 15 to 20 seconds, and allow to stand for 30 minutes or more. The supernatant dichloromethane phase is removed by aspiration and the sulfuric acid-ethanol solution allowed to stand at room temperature for 10 or more hours for maximum color development.

5. *Spectrophotometry*—Transfer the contents of the tubes to microcuvettes (Pyrocell, 1.5 by 10 by 15 mm.) and measure the absorbency of the colored product against a water blank at 410 $m\mu$ in Beckman DU spectrophotometer.

Note: 5 ml. water run through the entire procedure serves as reagent blank, and 5 ml. water containing 5 micrograms of hydrocortisone serves as standard.

Specificity of procedure for determination of hydrocortisone in plasma after administration of hydrocortisone—Specificity of the method was shown by counter-current distribution. Plasma drawn 90 minutes after the end of a 30-minute intravenous infusion of 200 mg. of hydrocortisone was extracted with dichloromethane, the

extract evaporated to dryness, and the apparent hydrocortisone subjected to an eight-transfer counter-current distribution. The solvents used were water (8 volumes) and chloroform (1 volume). This system gave a partition coefficient of about 0.1 for authentic hydrocortisone. A trace quantity of hydrocortisone-4- C^{14} was added before the counter-current distribution. After distribution, aliquots of both phases were analyzed for carbon 14 and by the phenylhydrazine procedure. Figure 1 demonstrates the distribution of the phenylhydrazine reacting material and the carbon 14 hydrocortisone. These data demonstrated that more than 90 per cent of the material assayed with phenylhydrazine was distributed as hydrocortisone.

The specificity of the analytical procedure employed was further demonstrated by subjecting dichloromethane extracts of plasma obtained 60 and 120 minutes following the end of a 30-minute infusion of 200 mg. of hydrocortisone to paper chromatography, using a modification of the Bush (2) type system. A small quantity of hydrocortisone-4- C^{14} was added to the dichloromethane extract and the specific activity of the phenylhydrazine reacting material was determined. The extract was then chromatographed at room temperature for 6 hours in a benzene-methanol-water (4:2:1) system. The hydrocortisone of the extract, running at the same rate as pure hydrocortisone, was located by ultraviolet scanning. This area was cut out and eluted with 3 ml. of 95 per cent ethanol, and the eluate evaporated to dryness under nitrogen. The residue was redissolved and the specific ac-

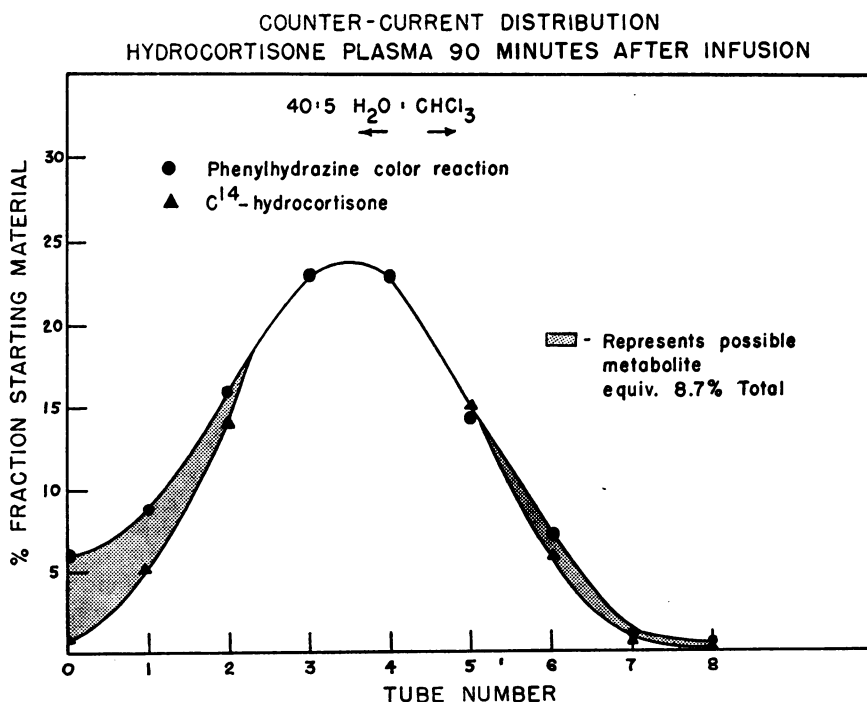


FIG. 1. COUNTER-CURRENT DISTRIBUTION ANALYSIS OF PHENYLHYDRAZINE REACTING MATERIAL IN PLASMA DICHLOROMETHANE EXTRACT 90 MINUTES FOLLOWING TERMINATION OF INFUSION OF 200 MG. HYDROCORTISONE TO NORMAL SUBJECT

tivity of the phenylhydrazine reacting material was again determined. Table I shows the specific activity of the material before and after chromatography. Portions of the steroid eluted from the paper were also assayed fluorometrically by a modification of the highly specific method described by Sweat (3). Other eluted portions were treated so as to convert the hydrocortisone to its acetate which was then chromatographed, eluted, and assayed. These procedures confirmed the data obtained by counter-current distribution studies.

Procedure for determination of hydrocortisone metabolites in urine—The method and reagents were the same as those described for plasma, except that to the urine (10 ml.) 2 ml. 0.5 M phosphate buffer (pH 6.2) and 1000 units Sigma bacterial glucuronidase in one ml. of water were added. Following incubation for 24 hours at 37° C., the mixture was extracted with 8 to 9 volumes of dichloromethane. After washing the dichloromethane extract with 1/20 volume 0.1 N NaOH, aliquots (40 ml.) were extracted with 3.0 ml. of the phenylhydrazine-sulfuric acid-ethanol, and the sulfuric acid-ethanol reagent as described for plasma. The resulting colored product was measured at 410 m μ .

Phenylhydrazine reacting materials extracted from urine either directly or after β -glucuronidase hydrolysis represent many different metabolites of hydrocortisone.

Studies with hydrocortisone-4-C¹⁴

Hydrocortisone-4-C¹⁴, specific activity 1.47 millicuries per millimole, was made available through the Endocrine Study Section of the National Institutes of Health. This compound was shown to be approximately 95 per cent pure by counter-current distribution, and isotope dilution analyses and paper chromatography. It was administered intravenously in trace quantities in solution in a small volume of 5 per cent ethanol in water over a period of 3 to 5 minutes, or mixed with non-isotopic hydrocortisone given in 500 ml. 3 per cent ethanol in 5 per cent dextrose in sterile distilled water. Similar solutions were administered orally in certain studies.

After intravenous or oral administration, samples of blood were taken at 30 to 60-minute intervals for 4 to 8 hours, and urine was collected every 2 hours for 12 hours, the following 12 hours, and for 24-hour periods until no additional radioactivity was demonstrable. Fecal samples were collected for 4 days, homogenized with water in a Waring blender, and an aliquot lyophilized.

Plasma samples were extracted directly with 5 volumes of dichloromethane. The dichloromethane extract was washed once with 1/20 volume 0.1 N sodium hydroxide, twice with 1/15 volume water, and then evaporated to dryness at 40° C. under nitrogen. The residue was then dissolved in methanol and an aliquot added to the planchet.

Biliary excretion of radiometabolites of hydrocortisone—Bile collections were made on a 53-year old male patient who gave a history of intermittent colicky pain of several years' duration with signs of recurrent obstructive jaundice for 8 months, and persistent signs of jaundice and RUQ pain for 3 weeks prior to hospitalization.

TABLE I
Comparison specific activities phenylhydrazine reacting material in plasma extracts following infusion of hydrocortisone

	Specific activities (cpm/ μ g.)	
	Before chromatography	After chromatography
<i>Expt. No. 1</i> (plasma 60 min. after infusion)		
1st chromatography	2,370	2,680
2nd chromatography		2,710
3rd chromatography*		2,500
<i>Expt. No. 2</i> (plasma 120 min. after infusion)		
1st chromatography	635	650†

* Converted to hydrocortisone acetate and rechromatographed.

† Six hundred and fifty cpm per microgram with phenylhydrazine and fluorometric assay.

Liver function tests were compatible with a diagnosis of obstructive jaundice. Surgical exploration¹ revealed the presence of cholecystitis and cholelithiasis, and in addition revealed an acute pancreatitis completely obstructing the common bile duct which was dilated to 3 to 4 times its normal size. No stones were found on exploration of the common bile duct. The sphincter of Oddi was cut. A T-tube was inserted in the common duct, and at its distal end was attached a small "Foley-type" catheter. These two tubes were led to the outside. Bile was permitted free access to the T-tube and to the duodenum through the common duct; however, the distal end of the common duct could be obstructed at will by inflating the Foley catheter bag with 1 to 2 ml. water, thereby draining all the bile through the T-tube. On the morning of the study, the bile duct was obstructed and the patient was given 1.5 μ c. of hydrocortisone-4-C¹⁴ intravenously. Bile samples were collected every two hours for 12 hours, and for the following 12 hours. Feces and urine were also collected.

Procedure for carbon¹⁴ analyses of samples—All analyses for carbon¹⁴ were carried out with a Robinson (4) gas-flow counter, using a gas mixture of nine parts argon and one part methane, and connected to a Nuclear Model 172 scaler. This apparatus gave a background of 3 to 4 cpm and an efficiency for carbon¹⁴ of slightly more than 53 per cent when counted at infinite thinness. Planchets of stainless steel with a surface area of 1.6 sq. cm. and a depth of 0.6 cm. were used. They were cleaned by boiling with alcoholic potassium hydroxide.

From 0.05 to 0.30 ml. of fresh urine was plated directly onto tared planchets. The urine was dried on a rotating platform with the aid of air and heat from an infrared

¹ We are indebted to Dr. Henry Doubilet of New York University College of Medicine, and to Dr. Robert Smith, National Cancer Institute, for the operative procedures involved in carrying out this study.

lamp, and the planchets were then placed in a desiccator until weighed and counted. A correction for self-absorption was made from a self-absorption curve of urine, and all samples were corrected to infinite thinness. Self-absorption curves run on different urines were not found to show a significant degree of variation. It was not necessary to correct any samples for self-absorption that weighed less than 0.1 mg. (infinite thinness). Results obtained using a double isotope dilution technique (5) were found to check with results obtained with the self-absorption correction curve data. All counting results were corrected for instrument variation by reference to a barium carbonate- C^{14} standard.

Untreated urine was extracted with 8 volumes of dichloromethane, an aliquot of the dichloromethane extract evaporated *in vacuo*, and the residue dissolved in methanol and plated. The remaining urine was then hydrolyzed with β -glucuronidase. This hydrolysate was then extracted with 8 to 9 volumes of dichloromethane, and an aliquot of the dried dichloromethane residue dissolved in methanol and plated. Aliquots of this extract and of the remaining aqueous residue were then applied to planchets.

An aliquot of the lyophilized feces was digested using the Peters and Gutmann (6) modification of the Van Slyke-Folch (7) procedure for carbon combustion. The carbon dioxide was collected in a solution of barium hy-

droxide, and titrated with acid. The barium carbonate precipitate was filtered dry on a small circle of filter paper, and this barium carbonate mount placed on a planchet, counted, and corrected to infinite thickness.

The bile samples were applied directly to planchets; a self-absorption correction factor obtained from a self-absorption curve for bile was employed.

RESULTS

Rate of disappearance of infused steroids from plasma

Rate of disappearance from plasma of hydrocortisone administered intravenously—When plasma hydrocortisone concentrations determined at various time intervals following infusion were plotted on semilogarithmic paper, a straight line was obtained. In Figure 2 are shown the plasma levels and the biological half-times obtained in a single normal subject following the infusion of quantities of hydrocortisone ranging from 50 to 500 mg. The half-times are virtually identical, indicating that the rate of disappearance of hydrocortisone from plasma is proportional to concentration over a wide range of plasma levels. This finding permitted a ready comparison of behavior of infused hydrocortisone in various subjects under various conditions. The biological half-life of hydrocortisone was compared in control subjects and in patients with liver disease, rheumatoid arthritis, hyperthyroidism, hypothyroidism, hypopituitarism, and adrenal cortical insufficiency (Figure 3). In 20 control subjects, representing primarily normal laboratory personnel, the mean biological half-time of hydrocortisone disappearance was 114 ± 6.5 minutes, with a range of 90 to 130 minutes. In 12 patients with mild to moderately severe cirrhosis, both alcoholic and post-hepatic types, the disappearance of hydrocortisone from plasma was considerably slower, and biological half-times ranging from 160 to 800 minutes were obtained. In some cases markedly abnormal half-times were obtained despite relatively little clinical and laboratory evidence of hepatic dysfunction; however, in all but two cases the diagnosis had been confirmed by liver biopsy. The range of serum bilirubin values in these subjects was 1.6 to 6.0 mg. per cent.

In 8 patients with rheumatoid arthritis, half-time values ranging from 68 to 164 minutes were obtained. Essentially normal values were found in

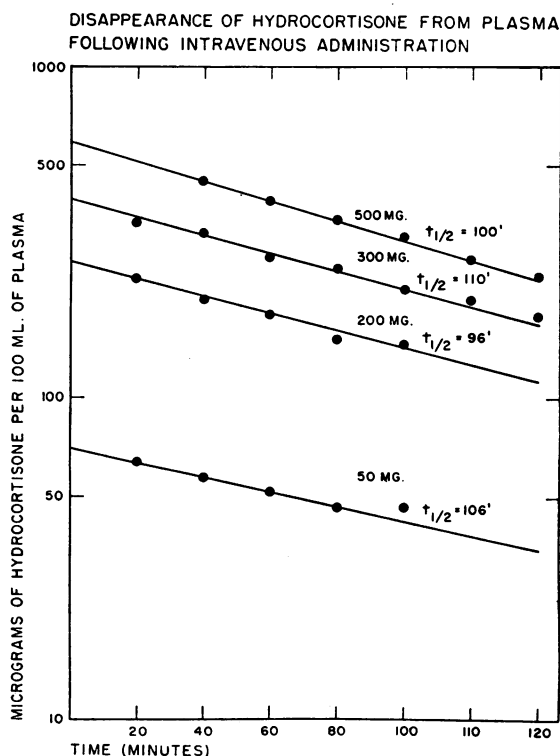


FIG. 2. COMPARATIVE RATES OF DISAPPEARANCE OF HYDROCORTISONE FROM PLASMA FOLLOWING INTRAVENOUS ADMINISTRATION TO A NORMAL SUBJECT

BIOLOGICAL HALF-TIMES OF INTRAVENOUSLY ADMINISTERED HYDROCORTISONE

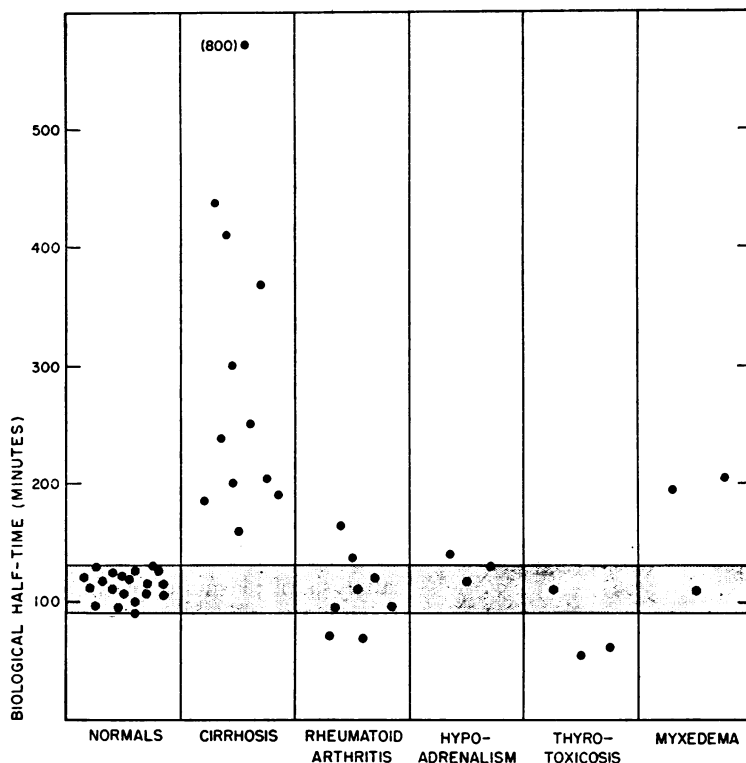


FIG. 3. BIOLOGICAL HALF-TIMES OF INTRAVENOUSLY ADMINISTERED HYDROCORTISONE IN NORMAL SUBJECTS AND IN PATIENTS WITH VARIOUS DISEASES

3 patients with hypoadrenalism. In 3 subjects with hyperthyroidism (BMR in +25 to +35 range) somewhat increased rates of hydrocortisone disappearances were found in 2, whereas a normal rate was found in one. In this subject hepatomegaly was present at the time of the study, and later resolved during antithyroid therapy. In 3 subjects with hypothyroidism (BMR in -40 to -25 range) delayed rates of hydrocortisone disappearances were observed in 2 and a normal rate in one.

No studies were carried out on patients with hyperadrenalcorticism; however, the rate of disappearance of hydrocortisone from the plasma of a patient with rheumatoid arthritis who had been maintained on 100 mg. of hydrocortisone for 6 weeks, and of another who had been taking 75 mg. a day for more than a year, was within the normal range.

Comparative rates of disappearance from plasma of steroids structurally related to hydrocortisone—Figure 4 demonstrates the rate of disappearance

from the plasma in a normal subject of six different steroids² structurally related to hydrocortisone, including one with physiologically similar actions (cortisone). Figure 5 demonstrates the disappearance from the plasma of the same steroids in a patient with cirrhosis of the liver. With the exception of hydrocortisone, whose disappearance was greatly delayed in the subject with liver dis-

² The cortisone, 11 α -hydrocortisone, and tetrahydrocortisone were assayed by the phenylhydrazine procedure as described in the Methods section. The corticosterone, 11 α -corticosterone, and 20 β -ol-hydrocortisone were assayed by a modification of the fluorometric method of Sweat (3). This method involves exactly the same extraction procedure as described for the phenylhydrazine method. However, the steroid was extracted from the washed dichloromethane extract with one ml. of a reagent containing 7 parts by volume concentrated sulfuric acid and 3 parts by volume redistilled ethanol. The fluorescence of this solution was then determined by using a Farrand Fluorimeter with a primary filter made up of a 470 m μ interference and Corning No. 5113 filter, and a secondary filter containing a 540 m μ interference and Corning No. 3486 filter.

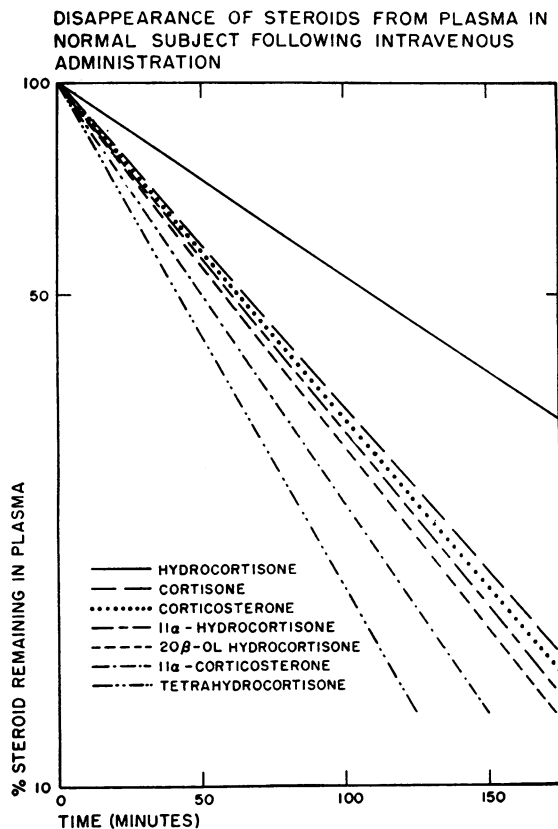


FIG. 4. DISAPPEARANCE OF INTRAVENOUSLY ADMINISTERED STEROIDS FROM PLASMA IN NORMAL SUBJECT

ease, the rate of disappearance of each steroid tested was not strikingly different in the normal and in the cirrhotic subject. Furthermore, all of these steroids disappeared at rates faster than hydrocortisone in both subjects.³

Disappearance of radioactive hydrocortisone from plasma—Following intravenous infusion of tracer quantities of hydrocortisone-4-C¹⁴, the concentration in plasma of isotopic substances extractable with dichloromethane decreased logarithmically with time (Figure 6). Half-times of 60 to 90 minutes (10 subjects) were obtained. Not all the extracted material represented unaltered hydrocortisone, however, and in 3 subjects the results of chromatographic analyses of plasma ex-

³ The method described for phenylhydrazine reacting steroids in plasma following their infusion, has been found not to be specific for cortisone (8). Approximately 50 per cent of the material reacting with phenylhydrazine 60 minutes after termination of the infusion is not cortisone. The specificities of the methods used for the other steroids have not been determined.

tracts obtained from 40 minutes to 4 hours after infusion indicated that from 40 to 60 per cent of the radioactivity of this fraction was attributable to the presence of other products. In a given subject the fraction of unconjugated steroid that was hydrocortisone was constant over this time span, however. The identity of the other labeled products was not established, although a reasonable guess as to their nature may be made from the analyses of the freely extractable group of labeled urinary metabolites described in this paper. In addition to the unconjugated metabolites in plasma, there is present a fraction of labeled metabolites that is rendered dichloromethane soluble following β -glucuronidase hydrolysis. Such a class of conjugated plasma corticoid constituents has previously been demonstrated, using chromatographic (9) and colorimetric (10) methods of analysis of plasma extracts. The data of Bongiovanni, Eberlein, Grumbach, VanWyk, and Clay-

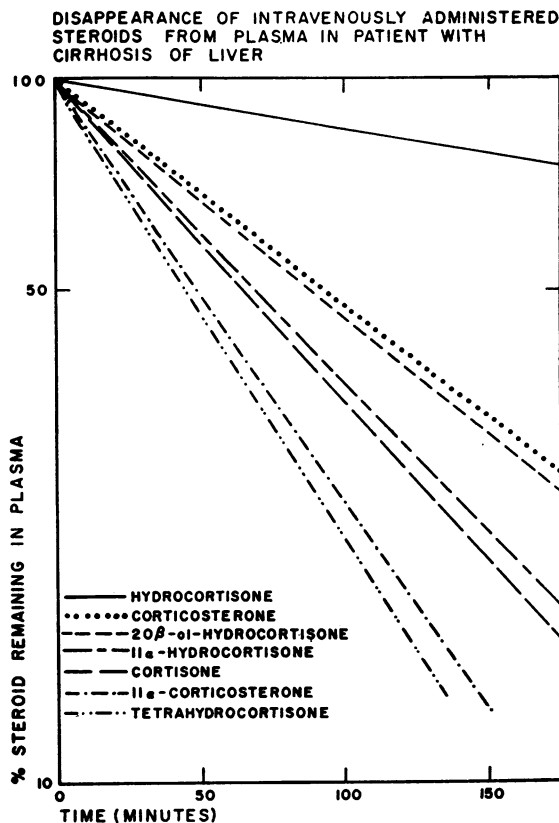


FIG. 5. DISAPPEARANCE OF INTRAVENOUSLY ADMINISTERED STEROIDS FROM PLASMA IN PATIENT WITH CIRRHOSIS

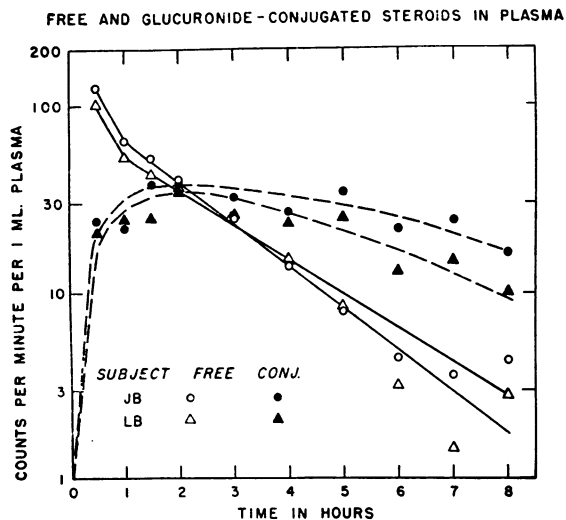


FIG. 6. DIRECT DICHLOROMETHANE EXTRACTABLE ("FREE") AND GLUCURONIDE CONJUGATED RADIOACTIVE STEROIDS IN PLASMA FOLLOWING INFUSION OF TRACER QUANTITIES OF HYDROCORTISONE-4-C¹⁴ TO NORMAL SUBJECTS

ton (10) suggest that quantitatively the plasma glucuronide fraction of conjugated adrenal hormone metabolites is approximately equal to the concentration of unconjugated phenylhydrazine reactive corticoids which normally consist predominantly of hydrocortisone (11). In the present study, the rate of appearance of labeled metabolites of hydrocortisone in conjugated form in plasma has also been followed (Figure 6). As free steroid disappears from plasma, conjugated derivatives hydrolyzable with β -glucuronidase appear, and reach a maximum at about 2 hours, at which time the free and conjugated fractions are of equal magnitude. Thereafter, the conjugated fraction greatly exceeds the free fraction of labeled steroids. Following extraction of both free and conjugated labeled products, the residual plasma was analyzed for radioactivity, and none was detected. However, self-absorption factors were large so that small quantities of labeled substances may have escaped detection.

Distribution of hydrocortisone in body fluids

Plasma protein binding of hydrocortisone—Fresh human plasma obtained from four normal donors, and isosmotic phosphate buffer of pH 7.4 (0.1 M buffer in M/15 sodium chloride) were equilibrated across a cellophane membrane for 18

hours at 37° C. with gentle continuous motion. To one or both phases, graded amounts of hydrocortisone, labeled with a tracer quantity of radioactive hormone, were added. At the end of the equilibration period, aliquots of both phases were extracted with dichloromethane, and the extract reduced in volume *in vacuo*. The concentrated extracts were then analyzed for hydrocortisone by phenylhydrazine and radioactivity assay procedures. Over the concentration range of 2 to 1,000 micrograms per 100 ml., 75 per cent of the hydrocortisone present in plasma was bound to the non-diffusible elements of plasma.

Uptake of hydrocortisone by erythrocytes—Three *in vitro* and one *in vivo* experiments were performed to evaluate the extent of diffusion of hydrocortisone into the erythrocyte. Fresh heparinized whole blood drawn from one subject was incubated with a tracer quantity of labeled hydrocortisone at 25° C. and 37° C. for one and two hours, with gentle agitation. After the incubation period, aliquots of whole blood, of separated plasma, and of packed cells (hemolyzed in water) were extracted with dichloromethane, and radioactivity measurements made on the extracts. The

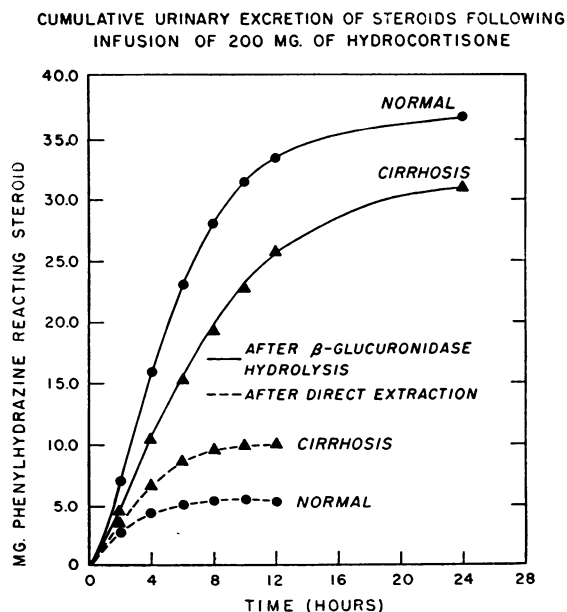


FIG. 7. COMPARATIVE RATES OF URINARY EXCRETION (CUMULATIVE) OF PHENYLHYDRAZINE REACTING STEROIDS FOLLOWING INFUSION OF 200 MG. HYDROCORTISONE TO NORMAL SUBJECT AND PATIENT WITH CIRRHOSIS (ALL ANALYSES MADE ON DICHLOROMETHANE EXTRACTS)

counting values obtained by analysis of the hemolyzed erythrocytes were compared with values calculated for erythrocytes from whole blood and plasma radioactivity concentration data and hematocrit measurements. A similar study was made on whole blood drawn from a normal subject 10 minutes following the intravenous injection of 2.5 μ c. of labeled hydrocortisone over a 3-minute period. One fraction of blood was kept at 37° C. for one hour, and another at 25° C. for one hour prior to extraction with dichloromethane. The results of all experiments were similar and indicated that erythrocytes took up hydrocortisone. Solution of hydrocortisone in red cell water appeared to account for only about one-half of the total. Intracellular binding was calculated to account for 49 to 66 per cent of the hydrocortisone present.

Metabolism of the hydrocortisone by whole blood *in vitro* was considered unlikely since varying the time or temperature of incubation did not alter the results. Furthermore, hydrocortisone was not metabolized to a measurable extent in

plasma *in vitro* at 25° C. for 72 hours, as judged by chromatography and radioautography of the dichloromethane extract in which the added steroid was recovered quantitatively.

From the above data, it can be calculated that 75 to 80 per cent of the hydrocortisone in a given volume of whole blood (hematocrit, 40 per cent) was in the plasma, and 20 to 25 per cent in the red cells, as calculated by the difference between whole blood and plasma counting values.

Excretion of hydrocortisone and metabolites

Urinary excretion of hydrocortisone and its metabolites—Following intravenous administration of hydrocortisone, 20 to 30 per cent of the administered dose was recovered in the dichloromethane extract of glucuronidase treated urine of the first 24 hours as phenylhydrazine reacting material (Figure 7). An additional 1 to 2 per cent was recovered during the following 24 hours. Approximately one-half of the recovered material appeared in the urine in the first 4 hours. Direct

COUNTER-CURRENT DISTRIBUTION PHENYLHYDRAZINE REACTING STEROIDS IN URINE AFTER INFUSION HYDROCORTISONE

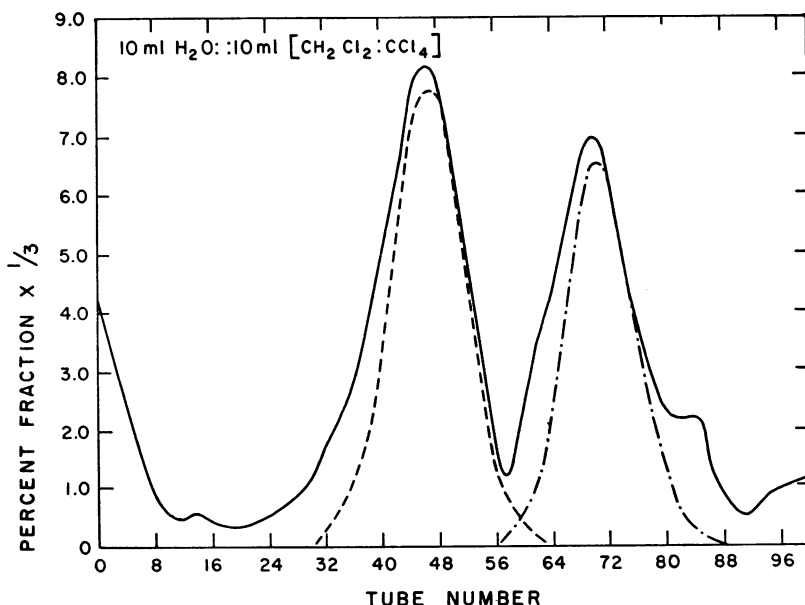


FIG. 8. COUNTER-CURRENT DISTRIBUTION ANALYSIS OF PHENYLHYDRAZINE REACTING MATERIAL IN GLUCURONIDASE HYDROLYZED URINE DICHLOROMETHANE EXTRACT AFTER INFUSION OF 200 MG. HYDROCORTISONE (FIRST 24-HOUR URINE)
 — Experimental curve; --- Calculated curve of tetrahydrocortisone ($K=0.94$); — · — Calculated curve of tetrahydrohydrocortisone ($K=3.50$).

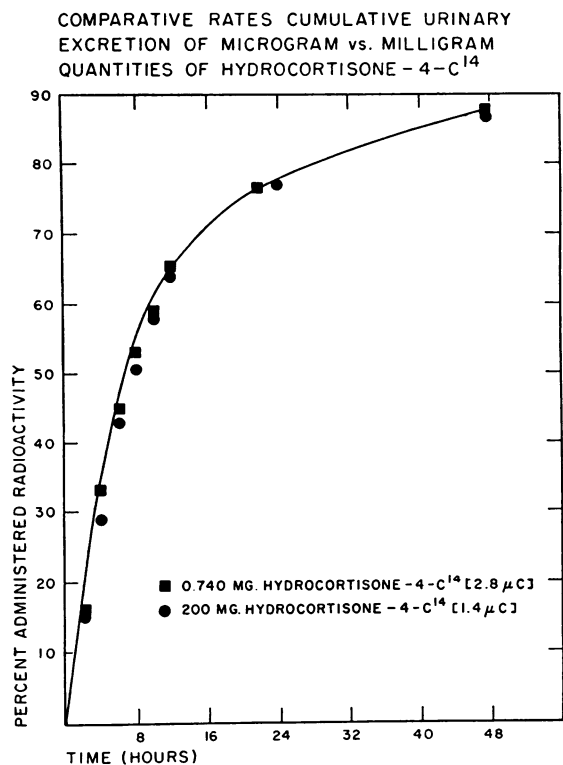


FIG. 9. COMPARATIVE RATES OF URINARY EXCRETION (CUMULATIVE) OF RADIOACTIVITY FOLLOWING ADMINISTRATION OF MICROGRAM vs. MILLIGRAM QUANTITIES OF HYDROCORTISONE-4-C¹⁴

extraction of urine with dichloromethane recovered phenylhydrazine substances equivalent to only 2 to 3 per cent of the administered dose. (Most of the extractable steroid was excreted during the first 4 to 6 hours.) Most of the conjugated metabolites were relatively polar compounds as indicated by a counter-current distribution of a dichloromethane extract of a 24-hour urine in a 99-tube transfer analysis (Figure 8). Two pronounced areas appeared among the polar fractions; however, paper chromatographic fractionation of the material comprising these two areas showed at least two phenylhydrazine reacting compounds in the area on the left and three in the area on the right (more polar). The two components of the area on the left migrated on paper as hydrocortisone and tetrahydrocortisone. One of the compounds in the more polar area on the right migrated as the tetrahydro derivative of hydrocortisone. One of the compounds migrated slightly faster and one slightly slower than the tetrahydro derivative of hydrocortisone. All of

these substances (hydrocortisone, tetrahydrocortisone, tetrahydrohydrocortisone) gave the appropriate color reactions with phenylhydrazine and blue tetrazolium, and the anticipated absorption curves in sulfuric acid. In addition, when run as mixed chromatograms, both before and after conversion to the acetates, they behaved in the same manner as the authentic compounds.

Following the infusion of hydrocortisone into a patient with cirrhosis of the liver, the metabolites appeared in urine more slowly than in the normal subject (Figure 7). Also, the total excretion of freely extracted steroid was greater than that found in the normal subject.

Urinary excretion of radiometabolites of hydrocortisone—Figure 9 demonstrates the cumulative urinary excretion of labeled hydrocortisone and metabolites following intravenous administration of a tracer quantity of hydrocortisone-4-C¹⁴. Eighty per cent of the injected dose was excreted within the first 24 hours. A small additional quantity appeared during the second and third 24-hour periods, and by the fourth day no labeled urinary products could be demonstrated. In a total of five such studies, an average of more than 90 per cent of the radioactivity was accounted for through urinary excretion (Table II). The half-time of the initial excretion rate of these products averaged 4 hours. During the first several hours following the infusion of labeled hydrocortisone, there was excreted a urinary fraction which was extractable with dichloromethane without prior hydrolysis. This fraction may constitute 15 to 20 per cent of radiometabolites present in the first 2-hour fraction but after about 8 hours virtually

TABLE II

Urinary excretion of carbon¹⁴ labeled steroid following administration of hydrocortisone-4-C¹⁴ (200 to 800 µg.)

		% Excreted 24 hrs.	% Total excretion			
			72 hrs.	"Free"	Glucuronide t _{1/2} hrs.*	
L. B.	i.v.	79	92	4.0	60	4.0
J. B.	i.v.	81	89	4.0		4.2
L. B.	i.v.†	80	90	4.0	50	4.0
S. H.	p.o.	82	91	3.0	55	3.5
H. E.	p.o.†	70	76	8.0	45	4.8
R. U.	i.v.	77	89	3.3	60	
R. U.	i.v.	79	94			
W. R.	i.v.	74	86	3.0	50	3.8

* Determined as half-time of the initial rapid excretory rate.

† Plus 200 mg. carrier hydrocortisone.

all products were present in conjugated form. Analysis of a 4-day pool of feces collected following infusion of the steroid revealed an additional 2 to 4 per cent radioactive metabolites of hydrocortisone.

Following intravenous administration of labeled hydrocortisone plus an additional 200 mg. of non-isotopic hydrocortisone, a similar curve of cumulative excretion of hydrocortisone radiometabolites was obtained (Figure 9).

Nature of the hydrocortisone metabolites in the urine—Determination of the nature of the urinary metabolites excreted following intravenous administration of 200 mg. of hydrocortisone labeled with hydrocortisone-4-C¹⁴ yielded the following results (Figure 10): Direct dichloromethane extraction of the fresh untreated urine recovered 4 per cent of the infused radioactivity, and 3 per cent of the administered steroid as measured with phenylhydrazine. However, following glucuronidase hydrolysis and dichloromethane extraction of the urine, 50 per cent of the administered radioactivity⁴ was recovered, but only 17 per cent as measured by phenylhydrazine analysis. An additional 15 per cent of the administered radioactivity could be extracted with dichloromethane following hydrolysis with boiling 15 per cent hydrochloric acid for

⁴ An additional 2 to 3 per cent more radioactivity can be obtained by reextracting the urine residue with an additional 8 to 9 volumes of dichloromethane. Further treatment of the urine residue with glucuronidase and incubating for an additional 48 hours does not result in the release of an additional amount of radioactivity. Also, treatment of the urine residue with mammalian β -glucuronidase (Viobin 1000 units per ml. urine, or Ketodase 500 units per ml. urine) at pH 4.8, 37° C. for 5 additional days, did not release any more radioactive metabolites extractable into dichloromethane.

15 minutes. Direct extraction of the urine with 2 or 10 volumes of n-butanol saturated with water (pH 2.0) removed 80 per cent of the infused radioactivity. Treatment of the residual urine with either glucuronidase or boiling 15 per cent hydrochloric acid, and re-extracting with dichloromethane failed to release any additional radioactivity.

Some information regarding the identity of metabolites comprising the free and conjugated fractions has been obtained. By paper chromatography, the direct dichloromethane extract was shown to contain several labeled products, consisting of hydrocortisone (equivalent to less than one-fourth the total of labeled metabolites of this fraction, or less than 1 per cent of the administered dose), 20 β -hydroxyhydrocortisone, 6 β -hydroxyhydrocortisone, tetrahydrohydrocortisone, and other unidentified products.⁵

By similar techniques, the metabolites released by β -glucuronidase hydrolysis and reacting with phenylhydrazine to form a yellow color were shown to consist predominantly of tetrahydrocortisone and tetrahydrohydrocortisone. The corresponding dihydro-derivatives were detected only in small amounts. Evidence for the presence of several additional more polar metabolites was also obtained by radioautography of these chromato-

⁵ These results have been based on a comparison of the rates of migration with the authentic pure compounds, by use of mixed chromatograms, and by conversion of unknown steroids to the acetates and comparing their chromatographic behavior with that of the authentic compounds. These substances also gave the appropriate reactions with phenylhydrazine and with blue tetrazolium, the appropriate sulfuric acid fluorescence results, and the characteristic spectral curves in sulfuric acid.

TABLE III

*Plasma levels phenylhydrazine reacting steroid after oral and intramuscular administration of 200 mg. hydrocortisone**

		Hours following administration								
		0	0.5	1	2	3	4	8	12	24
T. R.	p.o.	17	115	220	148	107	91	44	22	15
	i.m.	19		42	57		55	47	37	24
V. L.	p.o.	17		96	160		92	55	18	
	i.m.	16		33	37	30		27	21	15
T. L.	p.o.	25	100	150	190	120	70	25		23
	i.m.	25	26	40	56		56	46	35	28
R. H.	p.o.	13		100	260	255	190			
C. S.	p.o.	16	70	100	140	200	130			
W. R.	p.o.	15	180	260	180		86	18		

* Values are expressed as micrograms per 100 ml. plasma.

grams, but as yet none of these has been positively identified in our laboratory.

Biliary excretion of radiometabolites of hydrocortisone—Following intravenous administration of labeled hydrocortisone to the patient with the biliary cannula, the rate of accumulation of metabolites in the urine, and the total quantity appearing, were much the same as in the normals. The total quantity of metabolites appearing in the bile, however, was only 4 per cent of the injected dose.⁶ Analysis of the feces collected for 3 days after infusion of the steroid failed to reveal the presence of any radioactivity.

Direct dichloromethane extraction of the bile failed to recover any radioactive products. Direct extraction of the feces in the normal subjects demonstrated that none of the labeled substances was in the free form.

Absorption of hydrocortisone

Absorption of orally and intramuscularly administered hydrocortisone—With the oral administration of 200 mg. of hydrocortisone to fasting normal subjects, peak plasma levels were obtained in 1 to 2 hours (Table III), indicating rapid absorption of the hormone. The peak plasma steroid concentrations were of the same order of magnitude as the extrapolated initial concentrations obtained following intravenous administration of 200 mg. (Figure 2). The plasma concentration had returned to normal levels within 8 to 12 hours.

Following intramuscular administration of hydrocortisone, peak levels were reached more slowly than following oral administration, and were not nearly as high; however, the concentrations remained elevated for a slightly longer period of time (Table III).

Following oral administration, as with intravenous infusion of hydrocortisone, 20 to 30 per cent of the administered steroid was recovered in the urine as phenylhydrazine reacting metabolites of hydrocortisone. After intramuscular injection an equivalent quantity was recovered; however, it appeared in the urine at a slower rate.

⁶ A similar type of experiment revealed an excretion of 4 per cent of administered radioactivity in the bile after infusion of cortisone-4-C¹⁴, and 11 per cent after infusion of corticosterone-4-C¹⁴.

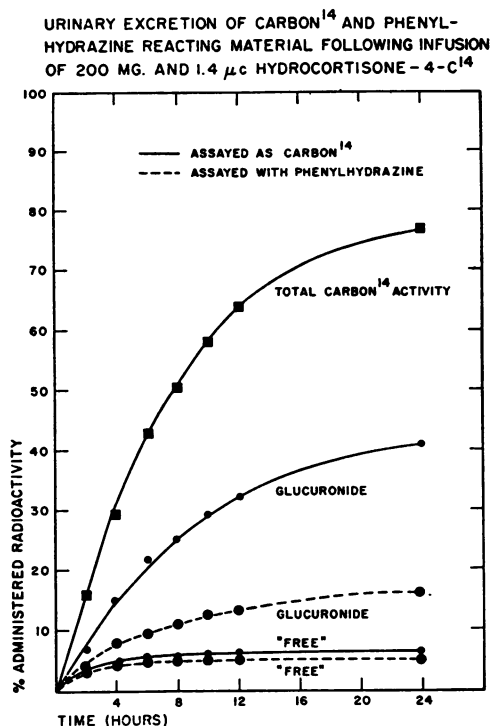


FIG. 10. URINARY EXCRETION (CUMULATIVE) OF RADIOACTIVITY AND PHENYLHYDRAZINE REACTING STEROID FOLLOWING INFUSION OF 1.4 μ C HYDROCORTISONE-4-C¹⁴ CONTAINING 200 MG. CARRIER HYDROCORTISONE

Following oral administration of a tracer quantity of hydrocortisone-4-C¹⁴, dissolved in 10 per cent ethanol, a cumulative rate of excretion of metabolites similar to that obtained after intravenous administration was found (Figure 11). Following oral administration of hydrocortisone-4-C¹⁴ with an additional 200 mg. of carrier steroid in solution in 10 per cent ethanol, a similar pattern of urinary excretion was demonstrated, but slightly less of the radioactivity could be accounted for in the urine. Four per cent appeared in the feces.

DISCUSSION

Disappearance of hydrocortisone from plasma

The rate of disappearance of hydrocortisone from plasma has the characteristics of a first order reaction. Since less than 1 per cent of administered hydrocortisone was excreted as unaltered hormone, the rate of disappearance of hydrocortisone from plasma is presumably also a measure of the rate of its metabolism. The slower disappear-

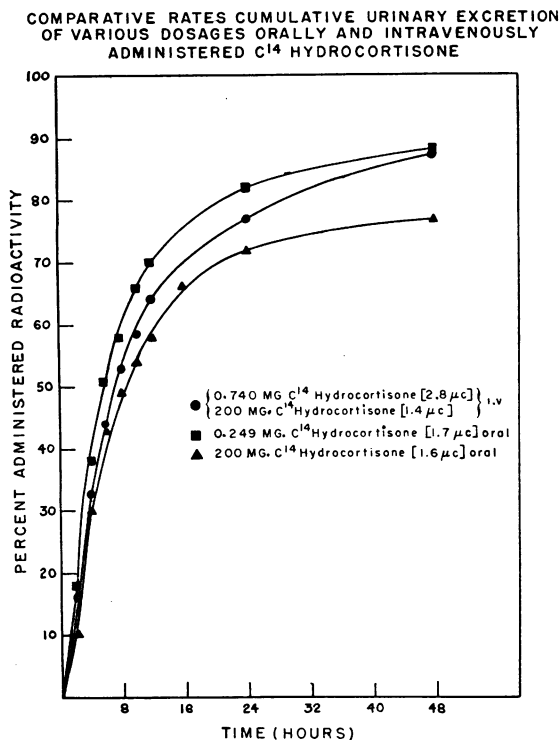


FIG. 11. COMPARATIVE RATES OF URINARY EXCRETION (CUMULATIVE) OF RADIOMETABOLITES FOLLOWING INTRAVENOUS OR ORAL ADMINISTRATION OF HYDROCORTISONE-4-C¹⁴

ance of infused hydrocortisone in patients with liver disease, also noted by Brown, Willardson, Samuels, and Tyler (12), is of particular interest. Since this defect is demonstrable in patients with relatively minor liver dysfunction, a test of a subject's ability to handle infused hydrocortisone may serve as a sensitive indicator of hepatic function.

In most cirrhotic patients studied, hydrocortisone was the only steroid for which a striking defect in rate of metabolism was demonstrable. Tomkins and Isselbacher (13, 14) have described a specific triphosphopyridine nucleotide-linked enzyme present in rat liver catalyzing the reduction of the Δ^4 bond of hydrocortisone, but without effect on the Δ^4 bond of other steroids. The product of hydrocortisone reduction is dihydrohydrocortisone, which is then further reduced by a second less specific enzyme (3-hydroxysteroid dehydrogenase [14]) to tetrahydrohydrocortisone. The first reaction is rate controlling, so that the intermediate product does not normally accumulate. Moreover, the dihydro-derivatives

do not appear in urine of man to any significant extent (15). These findings suggest that the rate-controlling step in the removal of hydrocortisone from plasma may be the reduction of the A ring of the hormone in the liver, and that it is the enzyme catalyzing this transformation that is deficient in hepatic diseases of the types studied. A finding consistent with this thesis is the observation that tetrahydrocortisone disappears from plasma at a normal rate in patients with liver disease (12) (Figure 5).

The ameliorating effects of jaundice (16) upon active rheumatoid arthritis may well be related to a defect in biological transformation of endogenous hydrocortisone resulting from liver dysfunction. The rate of disappearance of hydrocortisone was clearly delayed in only one of eight rheumatoid arthritics studied.

In two of three patients with thyrotoxicosis, the rate of disappearance of infused hydrocortisone was increased. In two of three with myxedema, hydrocortisone disappearance was delayed. Since similar disturbances were noted after infusion of cortisone, these alterations presumably result from general disturbances of rates of metabolic processes associated with alterations of thyroid function.

In adrenal insufficiency, both primary and secondary, the rates of disappearance of hydrocortisone were normal. Also, in patients who had previously received large doses of hydrocortisone for long periods, the disappearance of infused hydrocortisone was normal. Hellman, Bradlow, Adesman, Fukushima, Kulp, and Gallagher (17) have found the rate of metabolism of hydrocortisone-4-C¹⁴ normal in adrenal insufficiency as judged by rates and extents of urinary excretion of the labeled metabolites. Apparently hepatic processes which metabolized hydrocortisone proceed without consideration of the peripheral requirements for hormone, being governed by the concentration of hydrocortisone in the circulating plasma, and by the level of enzyme activity in the liver.

Distribution of hydrocortisone in body fluids

The value of the apparent volume of distribution of hydrocortisone depends upon the basis selected for its calculation. Thus, if one utilizes the concentration of hydrocortisone in plasma at zero

time as obtained from an extrapolation of the semilogarithmic plot of plasma hydrocortisone concentrations (Figure 2), considered together with the quantity of hydrocortisone injected, an apparent volume of distribution of 50 to 90 liters can be calculated.⁷ (Average = 70 liters⁸ in 20 normal and 12 cirrhotic subjects given 50 to 500 mg. of hydrocortisone intravenously.)

In the strictest sense these calculations mean that at the moment of complete mixing it would take a volume of 70 liters to contain all the injected hydrocortisone in the concentration at which it exists in plasma. Since the figures obtained are in all cases close to total body weight, it follows that hydrocortisone is concentrated in some portions of the body at much higher levels than are present in plasma. Tissue localization has been demonstrated to occur in the red cell and in the non-diffusible elements of plasma, and probably occurs in other tissues as well.

When the hydrocortisone space is calculated from the value for the miscible pool as determined by conventional treatment of serial specific activity values of circulating hydrocortisone (18), and the plasma level of phenylhydrazine reacting steroid (largely hydrocortisone [11]), hydrocortisone spaces of 8 to 16 liters (12 subjects) are obtained. The method for determination of the miscible pool depends upon knowledge of the initial concentration of isotopic hydrocortisone in plasma. The addition of new unlabeled hormone will dilute the pool and change its isotope concentration; the removal of hormone from the pool will not. Therefore, only that hydrocortisone capable of mixing with circulating hydrocortisone will be determined by this method.

The discrepancy between values obtained by these two methods is at least in part attributable to the fact that in the estimation of a volume of distribution, equilibration of solute is assumed

to have occurred prior to any irreversible transformation or excretion of the test substance. This assumption cannot be valid in the case of hydrocortisone which is known to be metabolized rapidly. The hydrocortisone which is entering such irreversible fates undoubtedly makes a larger contribution to the volume of distribution when measured from hydrocortisone disappearance data than when measured from the isotope dilution data. The value arrived at from isotope dilution data, 8 to 16 liters, is perhaps a fairly accurate indication of the volume of water required to contain the miscible hydrocortisone at the concentration at which it occurs in plasma.

Excretory pathways of metabolites of hydrocortisone

The major route of excretion of labeled metabolites of hydrocortisone was *via* the urine, in which 86 to 94 per cent of the administered carbon¹⁴ appeared in 72 hours. These figures are in good agreement with those of Hellman and his associates (17) and Plager, Tyler, Hecht, and Samuels (19). Less than one per cent of the administered hormone was excreted as free hydrocortisone. These data suggest that no accumulation of hydrocortisone within the body would result from renal failure such as might be anticipated from hepatic failure.

Only a minor fraction, about 3 per cent, of the administered isotope was recovered in feces. This value is somewhat lower than those reported by Hellman (17) and by Plager and their coworkers (19). The quantity of labeled products appearing in feces was only slightly less than that excreted in bile, suggesting that the bile is the major and perhaps the sole source of fecal products. Furthermore, it appears unlikely that appreciable intestinal reabsorption of biliary metabolites of hydrocortisone occurs. These data are in striking contrast with those obtained in rats and guinea pigs in which 60 to 80 per cent of the administered hydrocortisone-4-C¹⁴ appeared in biliary metabolites, much of it destined subsequently to participate in an active entero-hepatic circulation in both species (20). These findings are also different from those obtained with progesterone-4-C¹⁴ in man, where 60 per cent of the radiometabolites appeared in bile (21).

⁷ Similar calculations made on the basis of the extrapolated value for the concentration of dichloromethane extractable radioactive substances present in plasma at zero time, after infusion of tracer quantities of hydrocortisone-4-C¹⁴ can have no significance, since it has been shown that a large fraction of these substances is not hydrocortisone.

⁸ This figure of 70 liters is based on the concentrations of free plus plasma protein bound steroid. By employing the free steroid concentration for the calculation, an apparent volume of 280 liters is obtained.

Hellman and his coworkers (17) have reported that less than 0.5 per cent of administered hydrocortisone appeared in the expired air as carbon¹⁴ dioxide. Similar results have been obtained in the guinea pig and rat (20). These findings indicate that the biotransformation of hydrocortisone is not associated with appreciable degradation of the ring structure of the steroid.

Excretion of metabolites in urine

Previous studies have shown that about 20 to 30 per cent of an administered dose of hydrocortisone is excreted as metabolites that are extractable with butanol (22), or become extractable with chloroform or similar solvents following β -glucuronidase hydrolysis (23, 24), and react with phenylhydrazine. Small additional quantities are detectable as 17-ketosteroids, but the sum of all classes of urinary metabolites recognizable by these various methods is less than 40 per cent of the administered dose.

In the present study, recoveries of metabolites in urine, as determined by the phenylhydrazine method after glucuronide hydrolysis agreed well with these earlier reports. However, both in the freely extractable and the glucuronidase hydrolyzed fractions, the percentage of the administered hormone detected by radioactivity analysis was considerably larger than that determined by colorimetric assay. The presence of 20 β -hydroxyhydrocortisone as a labeled metabolite of hydrocortisone-C¹⁴ offers a likely explanation for the discrepancy between results of carbon¹⁴ and phenylhydrazine analyses of the freely extractable fraction since this substance does not react with the phenylhydrazine reagent.

A similar explanation probably applies to the disparity between carbon¹⁴ and phenylhydrazine analyses of the β -glucuronidase-hydrolyzable fraction of conjugated metabolites. Metabolites equivalent to 50 to 60 per cent of administered hormone were released by enzymatic hydrolysis as determined by radioactivity analysis whereas this fraction was 20 to 30 per cent of infused steroid by the phenylhydrazine reaction. Recently, four new urinary metabolites of adrenal cortical hormone have been identified (25). These are derivatives of tetrahydrohydrocortisone and tetrahydrocortisone possessing a 20-ol group in either α or β con-

figuration, apparently present as glucuronides, presumably conjugated at the 3-ol group of each metabolite. These metabolites accounted for about 30 per cent of the neutral extractable steroid products found after the administration of labeled hydrocortisone. These 20-ol derivatives would not react with phenylhydrazine and their presence in urine may well explain the major portion of the difference (equivalent to about 30 per cent of the administered hormone) encountered between the results of the two analytical methods employed in this study.

An additional fraction of 10 to 20 per cent of the infused isotope could be extracted with n-butanol saturated with water, or following hydrolysis with boiling hydrochloric acid followed by dichloromethane extraction. Whether this fraction represents glucuronides resisting hydrolysis with bacterial enzyme, or sulfate or other conjugation products, remains to be determined, as does the nature of the metabolites in the residue completely resisting extraction.

Expressed in terms of the total radioactivity in the urine excreted during the first 24 hours following infusion of hydrocortisone-4-C¹⁴, 4 per cent is extracted directly with dichloromethane. An additional 60 to 70 per cent is released following glucuronidase hydrolysis and dichloromethane extraction, and an additional 15 per cent following either n-butanol extraction or dichloromethane extraction after treatment with boiling 15 per cent hydrochloric acid. This accounts for a total of about 85 per cent of the radioactivity present in the urine and leaves approximately 15 per cent unextractable.

Oral absorption

The presence of comparable peak plasma levels after oral and intravenous administration of hydrocortisone, and the similarities of recoveries and rates of excretion of metabolites, suggest that oral absorption of the hormone is both rapid and complete. The fact that the radiometabolites appeared in the urine did not of course exclude the possibility that some of the orally administered steroid was metabolically transformed in the gastrointestinal tract. However, studies carried out on the stability of hydrocortisone-4-C¹⁴ in fluids from various parts of the gastrointestinal tract

(26) have shown that in gastric juice (pH 1.0) and bile (pH 7.0) no transformation takes place for at least 36 hours (37° C.). In feces, however, after 4 hours all hydrocortisone had been metabolized.

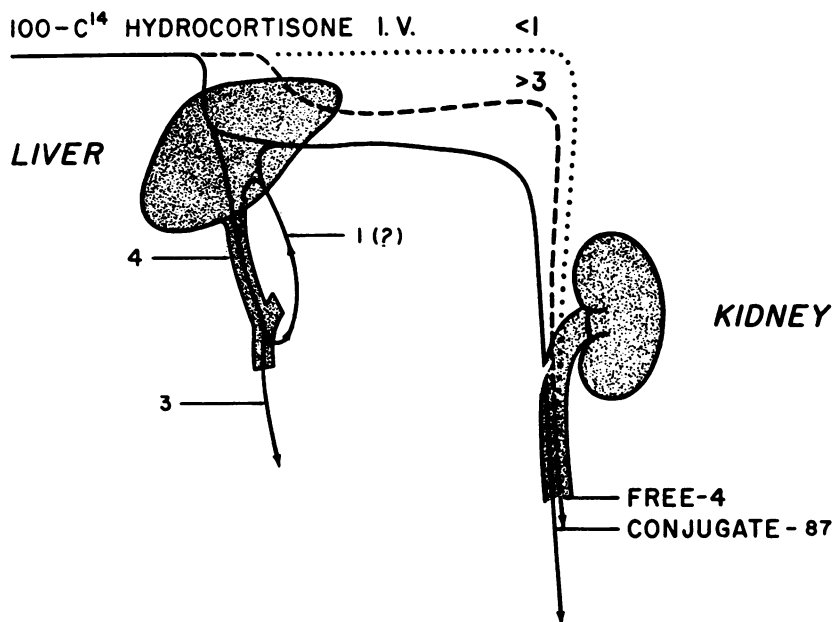
SUMMARY

The physiological disposition and metabolic fate of hydrocortisone have been studied in man following intravenous infusion of hormone in doses up to 500 mg. In normal subjects the half-life of infused hydrocortisone in plasma averaged 1.9 hours. Following the infusion of tracer quantities of hydrocortisone-4-C¹⁴, labeled conjugated metabolites appeared in plasma, and at 2 hours their concentration was equal to that of labeled unconjugated substances. Normal subjects excreted over 90 per cent of the administered hormone as urinary metabolites in 72 hours, as judged from excretion of carbon¹⁴ following infusion of tracer or labeled larger quantities of hormone. Feces of three normal subjects contained 2 to 4 per cent of the administered label, and in one sub-

ject with a biliary catheter, 4 per cent of the infused label appeared in the bile. About 4 per cent of the administered hormone was excreted in urine as unconjugated substances; unchanged hydrocortisone represented less than 1 per cent of the administered hormone. The routes of hydrocortisone disposition in man are schematically illustrated in Figure 12. About 60 per cent of the administered hydrocortisone was excreted as metabolites hydrolyzable with bacterial β -glucuronidase, although only about half of this fraction was detectable by the phenylhydrazine reaction.

In patients with liver disease disappearance of infused hydrocortisone was slower than normal. There was an associated increase in the fraction of infused hormone excreted in unconjugated form, and also a slower appearance of conjugated metabolites in urine. The defect in metabolism of hydrocortisone in patients with liver disease was discussed in terms of a low hepatic activity of the specific enzyme catalyzing the reduction of the Δ^4 bond of hydrocortisone.

The rates of disappearance of hydrocortisone



ROUTES OF HYDROCORTISONE DISPOSITION IN MAN

FIG. 12. ROUTES OF DISPOSITION OF HYDROCORTISONE IN MAN AFTER INTRAVENOUS ADMINISTRATION OF "100 UNITS" HYDROCORTISONE-4-C¹⁴

... Urinary excretion of unchanged hydrocortisone; --- Urinary excretion of hydrocortisone metabolized by the liver but not conjugated; — Urinary and biliary excretion of conjugated metabolites of hydrocortisone.

from plasma were increased in two of three patients with moderately severe thyrotoxicosis, and slowed in two of three with myxedema. Disappearance rates were normal in three subjects with hypoadrenalcorticism.

The absorption of tracer quantities of hydrocortisone from the gastrointestinal tract was shown to be rapid and complete. No destruction of hydrocortisone was detected after incubation of labeled hormone with gastric juice, bile, or serum, but extensive destruction occurred during incubation with feces.

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REFERENCES

- Silber, R. H., and Porter, C. C., The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma. *J. Biol. Chem.*, 1954, **210**, 923.
- Bush, I. E., Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues. *Biochem. J.*, 1952, **50**, 370.
- Sweat, M. L., Sulfuric acid-induced fluorescence of corticosteroids. *Anal. Chem.*, 1954, **26**, 773.
- Robinson, C. V., Windowless, flow type, proportional counter for counting C^{14} . *Science*, 1950, **112**, 198.
- Heard, R. D. H., Thompson, L., and Yates, C., A differential method for counting C^{14} in biological tissues and fluids. *Rev. canad. de biol.*, 1952, **11**, 66.
- Peters, J. H., and Gutmann, H. R., Micromethod for measurement of carbon-14-labeled material. *Anal. Chem.*, 1953, **25**, 987.
- Van Slyke, D. D., Plazin, J., and Weisiger, J. R., Reagents for the Van Slyke-Folch wet carbon combustion. *J. Biol. Chem.*, 1951, **191**, 299.
- Peterson, R. E., The physiological disposition and metabolism of cortisone in man. (In preparation.)
- Savard, K., Kolff, W. J., and Corcoran, A. C., Corticosteroids of peripheral blood. *Endocrinology*, 1952, **50**, 366.
- Bongiovanni, A. M., Eberlein, W. R., Grumbach, M. M., VanWyk, J. J., and Clayton, G., Conjugates of adrenal corticoids in human plasma. *Proc. Soc. Exper. Biol. & Med.*, 1954, **87**, 282.
- Peterson, R. E., A method for the determination of hydrocortisone in plasma, and an evaluation of its specificity. (In preparation.)
- Brown, H., Willardson, D. G., Samuels, L. T., and Tyler, F. H., 17-hydroxycorticosteroid metabolism in liver disease. *J. Clin. Invest.*, 1954, **33**, 1524.
- Tomkins, G., and Isselbacher, K. J., Enzymatic reduction of cortisone. *J. Am. Chem. Soc.*, 1954, **76**, 3100.
- Tomkins, G., A mammalian 3-hydroxysteroid dehydrogenase. *J. Biol. Chem.*, In press.
- Schneider, J. J., Further isolation of adrenocortical compounds from male urine. *J. Biol. Chem.*, 1952, **194**, 337.
- Hench, P. S., Effect of jaundice on chronic infectious (atrophic) arthritis and on primary fibrositis. *Arch. Int. Med.*, 1938, **61**, 451.
- Hellman, L., Bradlow, H. L., Adesman, J., Fukushima, D. K., Kulp, J. L., and Gallagher, T. F., The fate of hydrocortisone-4- C^{14} in man. *J. Clin. Invest.*, 1954, **33**, 1106.
- Peterson, R. E., and Wyngaarden, J. B., The miscible pool and turnover rate of endogenous hydrocortisone in man. (In preparation.)
- Plager, J. E., Tyler, F. H., Hecht, H. H., and Samuels, L. T., Metabolism and excretion of intravenously administered 4- C^{14} -17-hydroxycorticosterone. *J. Clin. Endocrinol. & Metab.*, 1954, **14**, 780.
- Wyngaarden, J. B., Peterson, R. E., and Wolff, A. R., Physiologic disposition of radiometabolites of hydrocortisone-4- C^{14} in the rat and guinea pig. *J. Biol. Chem.*, 1955, **212**, 963.
- Wiest, W. G., Fujimoto, G. I., and Sandberg, A. A., Metabolism of progesterone-4- C^{14} in man. *Federation Proc.*, 1955, **14**, 304.
- Reddy, W. J., Jenkins, D., and Thorn, G. W., Estimation of 17-hydroxycorticoids in urine. *Metabolism*, 1952, **1**, 511.
- Sandberg, A. A., Nelson, D. H., Glenn, E. M., Tyler, F. H., and Samuels, L. T., 17-hydroxycorticosteroids and 17-ketosteroids in urine of human subjects: Clinical application of a method employing β -glucuronidase hydrolysis. *J. Clin. Endocrinol. & Metab.*, 1953, **13**, 1445.
- Vestergaard, P., Investigations on the estimation of 17-hydroxycorticoids in urine using the Porter/Silber reaction. *Acta endocrinol.*, 1953, **13**, 241.
- Fukushima, D. K., Leeds, N. S., Bradlow, H. L., Kritchevsky, T. H., Stokem, M. B., and Gallagher, T. F., The characterization of four new metabolites of adrenocortical hormones. *J. Biol. Chem.*, 1955, **212**, 449.
- Peterson, R. E., Unpublished data.