THE FATE OF HYDROCORTISONE-4-C^{14} IN MAN [1]

By LEON HELLMAN, H. LEON BRADLOW, JEROME ADESMAN, DAVID K. FUKUSHIMA, J. LAWRENCE KULP, AND T. F. GALLAGHER

(From the Divisions of Physics and Biophysics, Steroid Biochemistry and Steroid Metabolism of the Sloan-Kettering Institute for Cancer Research, New York, New York and the Lamont Geological Observatory of Columbia University, New York, N. Y.).

(Submitted for publication March 1, 1954; accepted April 17, 1954)

This study deals with the dynamic behavior of hydrocortisone in man and the relationship of the metabolic fate of this principal adrenal hormone to the physiologic or pathologic state of the experimental subject. The period during which the hormone persists in the tissues, the rate of its excretion, and the relationship between the two have been measured by precise and discriminating methods. Furthermore, the details of the metabolic transformations and the rate at which these occur have been discussed and correlated with some known biologic effects of the hormone. These studies have been possible as a result of the availability of ring A labeled hydrocortisone through a cooperative effort sponsored by the Endocrinology Study Section of the U. S. Public Health Service (1).

Previous work in our laboratories employing radioactive cortisone labeled with tritium yielded information concerning the metabolites of cortisone and the routes by which they are eliminated. Exact quantitative evaluation of the total metabolites in terms of the amount given was difficult because of errors inherent in the methods employed for the analysis of tritium in very crude extracts. Other studies with C^{14} labeled testosterone, estrone, estradiol, progesterone, and desoxycorticosterone have been reported, and in general, these experiments showed a rapid elimination of the hormonal metabolites as conjugated substances in the urine (2). These analyses were possible because of the greater facility and accuracy of carbon-14 measurement. From the experience gained in these studies, it was possible to refine the experimental design so as to supply information concerning the following aspects of hydrocortisone metabolism.

First, the amount, the rate, and the route of excretion were established. Second, the problem of "tissue demand" as evidenced by the alteration in these excretory processes was explored in the complete absence of endogenously produced hormone. Third, these findings were compared with those obtained when the tissues were saturated with hormone. Fourth, the constancy of these processes was studied in different subjects with alterations in their physiological state as a result of disease. Fifth, the influence of the amount of hormone administered on these aspects of metabolism was defined. Sixth, the question of hormone breakdown ("utilization"), as indicated by the loss of C^{14} from ring A, was measured by the most delicate method available.

EXPERIMENTAL

The subjects in this study were patients on the metabolic ward of the James Ewing Hospital Unit of Memorial Center. Pertinent clinical information is summarized in Table I. With one exception, urine collections were made through a catheter to insure accuracy during the early portion of each study. Complete collections of urine and feces were made for a period of five days after the administration of the labeled material. Thirty to fifty ml. samples of blood were taken into tubes containing heparin at several time intervals for the determination of the quantity of hormone and its metabolites in circulation. In one patient, a sample of spinal fluid was obtained at 8 hours.

Hydrocortisone-4-C^{14} was supplied as a crystalline material with a specific activity of 4.69 μc per mg. The purity of the product had been established by the conventional methods of column chromatography, sulfuric acid chromogens and paper chromatography together with radioautography. In all experiments, the hormone

[1] This investigation was carried out under contract AT(30-1)-910 with the United States Atomic Energy Commission and supported in part by research grants (C-440 and C-322) from the National Cancer Institute of the National Institutes of Health of the U. S. Public Health Service. The authors also gratefully acknowledge the assistance of grants from the American Cancer Society (on recommendation of the Committee on Growth of the National Research Council), the Anna Fuller Fund, the Damon Runyon Memorial Fund for Cancer Research and the Lillia Babbitt Hyde Foundation.

TABLE I

Description of subjects

Subject	Age	Sex	Diagnosis and therapy	Exp.	Hydrocortisone administered	Remarks
F1	63	F	Chronic lymphatic leukemia 7 yrs. Radiation 6 yrs. and 6 mos.	1	0.855 μc (0.25 mg.)	Methadone addition Liver function normal
F2	43	M	Multiple benign lipomata 4 yrs.	2	0.94 μc (0.25 mg.)	Good general condition Liver function normal
				3	0.954 μc (100 mg.)	
F3	69	F	Breast cancer 2 yrs. Radical mastectomy 2 yrs. Recurrent disease 6 mos. Radiation 4 mos.	4	0.948 μc (100 mg.)	Good general condition Liver function normal
F4	48	F	Breast cancer 3 yrs. Radical mastectomy 3 yrs. Recurrent disease 2 yrs. Radiation 2 yrs. Castration 9 mos. Adrenalectomy 6 mos.	5	0.902 μc (0.25 mg.)	All hormone therapy stopped 48 hrs. before study. Usually maintained on oral cortisone
				6	0.790 μc (0.25 mg.)	Study performed while subject was receiving 100 mg. hydrocortisone i.v. each day

was administered intravenously. A sterile solution for the study was prepared immediately prior to infusion by the following procedure. The steroid was transferred into sterile ampules so that each ampule contained approximately 1 μc. The contents of the ampule were dissolved in 2 ml. of absolute ethanol which was added with proper precautions to approximately 150 ml. of sterile isotonic sodium chloride solution. An additional 2 ml. of absolute ethanol, used to rinse the ampule, was added to the infusion solution. When non-radioactive carrier hormone was used, 100 mg. of hydrocortisone, especially purified for this purpose, was transferred in alcohol solution to the infusion vessel. A small portion of the infusion solution was removed for analysis of the radioactivity. The quantity of solution administered to the patient was determined by weighing the infusion bottle before and at the termination of the infusion. In every instance, the solution of the radioactive hormone either with or without carrier was introduced over a period of exactly 30 minutes.

All radioactivity measurements were carried out independently by two different procedures. In the one, (Method A) the material was plated on planchets of 4.9 cm^2. area containing lens paper. These were then counted in a windowless flow gas counter and the data obtained were corrected to infinite thickness. A portion of the infusion solution was diluted with saline and plated as described. The radioactivity of this solution was referred to a National Bureau of Standards C^{14} reference standard ($Na_2C^{14}O_3$)—plated and counted in the same manner. This provided an accurate determination of the amount given to each patient. In order to permit a direct comparison of the infusion solution with the urine samples, an "infusion standard" with the plating characteristics of urine was prepared by dilution of a portion of the original hydrocortisone-4-C^{14} infusion solution with urine. The urine samples, themselves, were directly plated without dilution, extraction or further manipulation. In the other method of radiocarbon measurement (Method B), the infusion solution, urine, or extracts in organic solvents were made to a known volume, small portions were accurately removed and transferred to planchets of 5, 10 or 18 cm^2. area. The amount spread on the planchet was in the infinitely thin range; radioactivity was measured in a windowless flow gas counter. Both methods of radiocarbon measurement were intercalibrated by means of the National Bureau of Standards C^{14} reference standard.

In Experiment 1, samples of expired air were collected by exhalation through a 0.5 N sodium hydroxide solution. Several collections obtained for 30-minute intervals at various times during the first two days of the experiment were combined; a control sample was collected in the same fashion prior to the administration of any radioactivity. The control and experimental carbon dioxide collections were then separately converted to elemental carbon for measurement of radiocarbon by the method of Anderson and Libby (3). This procedure is capable of detecting 10^{-4} per cent of the dose used in these experiments.

Blood and spinal fluid samples were delivered into 1:1 acetone-alcohol, the precipitate was thoroughly extracted with the organic solvent and these extracts were counted, in many instances by both procedures. For the measurement of unchanged, unconjugated hydrocortisone in blood by the method of isotopic dilution, the following procedure was used in Experiment 2 (Subject F2). A 63 ml. sample of blood was withdrawn 15 minutes after the termination, *i.e.*, 45 minutes after the initiation of the infusion. The blood was extracted as above and to the extract was added 30.2 mg. of highly purified non-radioactive hydrocortisone. A total of 4,000 disinte-

grations per minute (DPM per min.) was present in the extract. The mixture was acetylated with an excess of acetic anhydride in pyridine at room temperature for 48 hours and the hydrocortisone acetate was extracted with ethyl acetate; the solution was washed with dilute alkali, dilute acid and with sodium chloride solution. The solvent was removed and the neutral extract contained 2,100 DPM per min. (54 per cent of the total). The extract was chromatographed on a silica gel-ethanol partition-type column (4) using alcohol and methylene chloride as the mobile phase. Most of the radioactivity was removed from the partition column before hydrocortisone acetate was eluted. The eluates containing hydrocortisone acetate were combined and recrystallized from acetone; the product melted 206 to 210°; specific activity = 15 DPM per min. per mg. After a second recrystallization, the m.p. was 214 to 217° and the specific activity was unchanged. The amount of material remaining was insufficient for further recrystallization.

The extraction of "free" steroids from urine was made by a minor modification of the procedure of deCourcy, Bush, Gray, and Lunnon (5). The urine was extracted four times, each with half its volume of chloroform, and twice with similar quantities of ether. The combined chloroform extracts were then washed three times with ⅛ volume of 5 per cent sodium hydroxide solution containing 5 per cent sodium chloride, followed by three washes with ⅛ volume of 25 per cent sodium chloride solution. The alkaline and saline extracts were backwashed with the two ether extracts. The combined chloroform and ether solutions were dried over sodium sulfate, the solvent was removed and the residue was diluted to volume with ethanol.

RESULTS

Six individual studies of the metabolism of hydrocortisone-4-C^{14} were made in four subjects. Two subjects, one a man (F2) and the other a woman, (F1) each received a tracer quantity of hormone, approximately 0.25 mg.; on another occasion, the same amount of labeled hydrocortisone together with 100 mg. of carrier hormone was given to the same male subject, F2. This experiment permitted an evaluation of the effect of the quantity of hormone given on its subsequent fate in the same individual. A female subject (F3) also received 1 μc of hydrocortisone-4-C^{14} together with 100 mg. of carrier hormone. Two studies were carried out on the fourth subject (F4), a

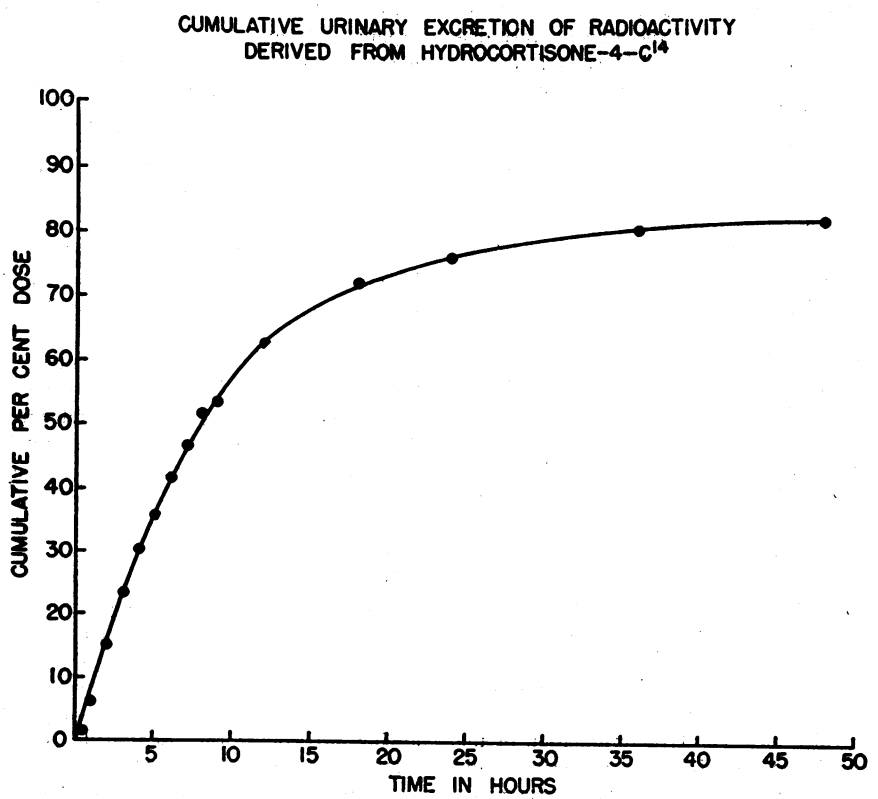

FIG. 1. THE CUMULATIVE URINARY EXCRETION OF RADIOACTIVITY IN EXPERIMENT 4 (SUBJECT F3) AFTER THE INTRAVENOUS ADMINISTRATION OF 100 MG. (0.948 μC) OF HYDROCORTISONE-4-C^{14} DURING 30 MINUTES

woman who was both oophorectomized and adrenalectomized. In the first of these, all hormone therapy was withdrawn for 48 hours before, and 24 hours after, the intravenous administration of a tracer amount of hydrocortisone. This procedure permitted an evaluation of the metabolism of the hormone when the tissues of the recipient were depleted of this substance. A second study on this same subject was carried out while she was receiving 100 mg. of hydrocortisone daily by continuous intravenous infusion. In this instance, the non-radioactive hydrocortisone infusion was interrupted for ½ hour while the tracer dose of the radioactive hormone was introduced. The infusion of the unlabeled hormone was then continued for the following 24 hours.

Data on the excretion of hydrocortisone metabolites in the urine are listed in Table II and a typical cumulative excretion curve (subject F3) is shown in Figure 1. In Experiment 1 (F1), 56 per cent was excreted in the urine at the end of 6 hours; 72 per cent was eliminated in one day and 80 per cent over three days. Virtually the same results were obtained in Experiment 2 with a tracer dose administered to the male subject, F2. In Experiment 3, when this same subject was given 100 mg. of carrier hydrocortisone together with radioactive hormone, the large additional quantity of hormone did not appreciably alter the excretory pattern. Fifty-four per cent was excreted in 6 hours, and 84 per cent in one day. The female subject, F3, who was also given 100 mg. of hydrocortisone containing 1 μc of C^{14}, showed an almost identical pattern of excretion in Experiment 4. In Experiment 5, conducted while the adrenalectomized woman, F4, was deprived of all replacement hormone therapy, the excretory pattern was also similar and no significant changes were observed when the same subject received 100 mg. of hydrocortisone daily by continuous intravenous infusion in Experiment 6.

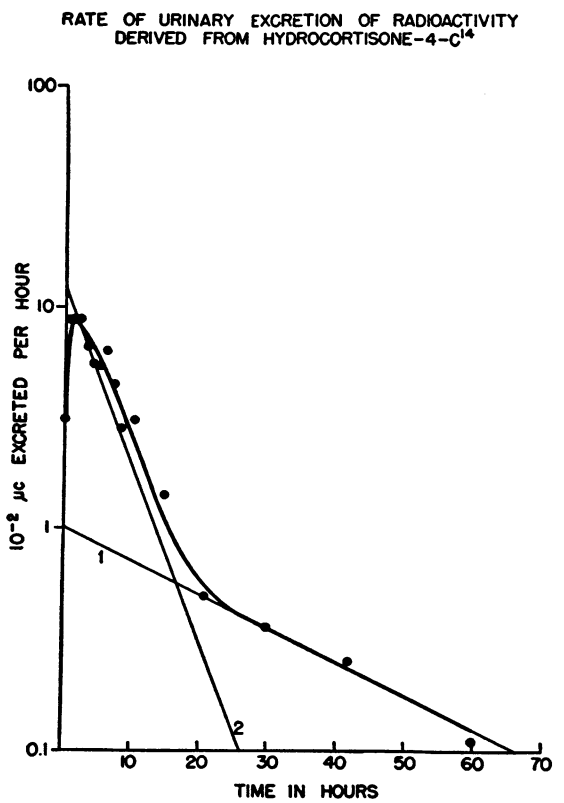

FIG. 2. THE EXCRETION OF RADIOACTIVITY IN EXPERIMENT 4 (SUBJECT F3) AFTER THE INTRAVENOUS ADMINISTRATION OF 100 MG. (0.948 μc) OF HYDROCORTISONE-4-C^{14} DURING 30 MINUTES

The curve has been analyzed for the two principal component rates, 1) with T/½ = 22 hours and 2) with T/½ = 4 hours.

In order to define the rate of excretion of the radioactive products derived from the administered hydrocortisone-4-C^{14}, the radioactivity in the urine was plotted semi-logarithmically against time for a typical experiment (Figure 2). During the first 24 hours after the dose, the data group about a straight line and suggest that the excretion approximates a first-order reaction. The rate of excretion of the products derived from the metabolism of 80 per cent of administered hydrocortisone during the first 24 hours can be characterized by an average half-life (T ½) of 3.6 hours in all subjects studied.

In the four days following hormone administration in subject F3, 9 per cent of the radioactivity was found in an acetone extract of the feces. In view of the high recovery of radioactivity in the urine, it appeared that similar results would be obtained in the other patients; further studies of the fecal metabolites are in progress.

The radioactivity found in the blood at various time intervals in the different subjects is shown in Table III. In a blood sample obtained in Experiment 2, 15 minutes after the end of infusion, a maximum of 13 per cent of the circulating radioactivity was present as unaltered hydrocortisone

TABLE II

Urinary excretion of radioactivity after hydrocortisone-4-C^{14}

Experiment 1 Subject: F-1 Dose: 0.855 μc (0.25 mg.)				Experiment 2 Subject: F-2 Dose: 0.940 μc (0.25 mg.)				Experiment 3 Subject: F-2 Dose: 0.954 μc (100 mg.)			
Time* *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose	Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose	Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose
0–0.5	0.8	1.0	1.0	0–0.5	1.1	1.2	1.2	0–0.5	3.6	4.6	4.3
0.5–1	3.5	3.8	4.3	0.5–1	4.6	4.6	4.9	0.5–1	5.0	5.6	5.6
1–2	13.7	12.5	15.3	1–2	10.6	11.2	11.1	1–2	10.4	11.8	11.2
2–3	8.6	8.6	10.0	2–3	8.4	9.5	9.5	2–3	9.4	10.5	10.4
3–4	8.6	9.9	10.8	3–4	6.8	8.2	8.0	3–4	9.2	9.2	9.6
4–5	9.1	9.9	11.0	4–5	6.6	7.2	7.4	4–5	6.5	6.2	6.5
5–6	3.8	3.1	4.0	5–6	4.9	6.0	5.8	5–6	6.8	5.0	6.2
6–12	7.2	7.2	8.4	6–12	24.4	21.7	24.6	6–12	12.3	13.2	13.3
12–18	3.2	3.2	3.8	12–18	7.0	7.2	7.6	12–18	10.1	12.5	11.8
18–24	3.2	3.1	3.7	18–24	3.5	2.6	3.3	18–24	4.7	2.8	3.9
24–36	2.1	—	2.5	24–36	3.3	—	3.5	24–36	—	3.8	4.0
36–48	1.7	—	2.0	36–48	1.0	—	1.0	36–48	4.7	—	4.9
Total: 24 hours				Total: 24 hours				Total: 24 hours			
	61.7	62.3	72.3		77.9	79.4	83.4		78.0	81.4	82.8

* Elapsed time in hours following start of infiltration.

TABLE II—*Continued*

Experiment 4 Subject: F-3 Dose: 0.948 μc (100 mg.)				Experiment 5 Subject: F-4 Dose: 0.902 μc (0.25 mg.)				Experiment 6 Subject: F-4 Dose: 0.790 μc (0.25 mg.)			
Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose	Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose	Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose
0–0.5	1.6	1.4	1.6	0–0.5	—	—	0.05	0–0.5	1.0	1.1	1.3
0.5–1	4.4	4.3	4.6	0.5–1	7.2	6.6	7.6	0.5–1	5.3	6.0	7.2
1–2	8.8	7.9	8.8	1–2	8.0	7.2	8.4	1–2	10.2	11.2	13.5
2–3	8.8	7.6	8.7	2–3	7.2	5.9	7.2	2–3	8.1	7.2	9.8
3–4	6.7	5.9	6.7	3–4	8.6	7.9	9.2	3–4	4.7	5.1	6.2
4–5	5.6	5.2	5.7	4–6	8.2	7.2	8.5	4–5	5.5	5.9	7.2
5–6	5.4	5.3	5.6	6–7	8.4	7.9	9.0	5–6	5.2	5.1	6.5
6–7	6.4	3.6	5.3	7–8	5.1	5.1	5.6	6–9	5.0	3.9	5.6
7–8	4.5	4.8	4.9	8–12	7.5	7.5	8.3	9–12	4.0	3.5	4.7
8–9	2.8	2.9	3.0	12–18	6.8	4.5	6.3	12–18	3.7	3.4	4.5
9–12	9.5	7.9	9.2	18–24	2.7	2.0	2.6	18–24	3.5	2.7	3.9
12–18	8.5	9.2	9.3	24–36	2.5	1.8	2.4	24–36	1.5	—	1.9
18–24	3.0	4.9	4.1	36–48	1.3	1.4	1.5	36–48	1.0	—	1.3
24–36	4.4	4.1	4.5	48–72	2.0	—	2.2	48–72	0.8	—	1.0
36–48	3.0	0.9	2.1	72–96	0.5	—	0.6	72–96	0.7	—	0.9
48–72	1.3	—	1.4								
Total: 24 hours				Total: 24 hours				Total: 24 hours			
	75.9	70.9	77.5		69.7	61.9	72.6		56.3	55.1	70.5

TABLE III

Radioactivity in blood during and after intravenous hydrocortisone-4-C^{14}

Exp. 1		2		3		4		5		6	
Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.
15	4.0	15	2.1	15	1.6	30	6.0	30	7.6	30	4.8
30	3.3	30	5.1	30	2.1	60	3.3	75	3.8	75	1.9
120	1.2	45	2.7	50	1.8	105	2.6	130	3.4	130	2.9
		150	1.8	135	1.3						
		180	1.5	250	1.5						

TABLE IV

Conjugated urinary radioactivity and exponential rates of excretion in all subjects

Experiment	Conjugation		Rate of excretion	
	Time (*hours*)	% Conjugated	Component	T 1/2
1	1–2	89	2	2.5
	12–18	99	1	37
2	1–2	96	2	4.5
	6–12	99	1	—
3	1–2	89	2	4
	6–12	98	1	—
4	1–2	81	2	4
	6–7	93	1	22
5	1–2	93	2	3.5
	12–18	96	1	20
6	1–2	81	2	3
	9–12	93	1	20

as demonstrated by the isotopic dilution described. In this short time, most of the hormone had therefore undergone metabolic transformation.

The results obtained by separating the urinary metabolites into "free" and conjugated fractions are shown in Table IV. The data indicate that from 4 to 19 per cent or an average of 12 per cent of the radioactive hormone products excreted during a single hour 30 minutes after completion of the infusion, was present in the "free" or nonconjugated form. After the sixth hour, only 1 to 7 per cent or an average of 3 per cent of the excreted material was still unconjugated.

No radioactivity was detected in a single 10 ml. sample of spinal fluid (subject F 1) obtained 8 hours after the infusion was completed. Analysis of the C^{14} content of the control carbon dioxide sample from subject F1, Experiment 1, gave a net activity of 15.1 ± 0.2 DPM per gram of carbon, a value identical with that of contemporary carbon. After the administration of 0.855 μc of hydrocortisone-4-C^{14}, the pooled carbon dioxide collections contained 20 ± 1 DPM per gram of carbon. This increase of 5 DPM per gram of carbon in the expired air represents excreta of radioactivity equivalent to 0.05 per cent of the administered hydrocortisone per day. It is unlikely that degradation of the A ring or, by implication, the entire steroid nucleus exceeds this amount.

DISCUSSION

The results of these studies reveal several new aspects of transport, distribution, and fate of the most important adrenal hormone in human subjects. It would be well to emphasize that the "tracer" dose, without carrier hormone, was well within the physiological range and in no sense an excessive or abnormal addition to the endogenous, glandular production of hydrocortisone. The "tracer" dose was 0.25 mg., introduced into the blood stream during a half-hour period. From the quantity of C_{19}-11 oxygenated steroids in normal urine and from the amount of these same compounds that can be recovered after the administration of exogenous hormone, it can be inferred that the human adrenal supplies about 30 mg. of hydrocortisone or similar steroids per day. The credibility of this estimate is supported by the fact that a totally adrenalectomized human, with minor supportive therapy such as desoxycorticosterone, can be maintained on approximately 25 mg. of hydrocortisone given by continuous intravenous infusion. Therefore, approximately 1 mg. of steroid per hour would be delivered into the circulation. The "tracer" dose of 0.25 mg. then represented approximately half the subject's hormone production during 30 minutes in which the hormone was administered, and was well within acceptable physiological limits. In the experiments where 100 mg. of carrier hydrocortisone was administered together with the labeled compound, the amount injected represented a relative excess of approximately 200 times the daily hormone requirement for that interval; the total amount administered was 400 times the tracer dose of the same hormone.

With these considerations in mind, it is possible to arrive at certain conclusions about both "utilization" and "tissue demand" for the adrenocortical hormone. If it is assumed that the 80 per cent of the 0.25 mg. "tracer" dose excreted during the first day represented disposition of an excess of hormone, and if it is assumed that only the 50 γ *possibly* remaining in the tissues after the first day represented that portion of the dose actually utilized in physiological mechanisms, it might be expected that all but 50 γ of the 100 mg. dose would be similarly excreted during the first day. Since this was not the case and in fact an essentially identical fraction of both dose levels was excreted during the first day by the same as well as by different subjects, the conclusion is warranted that widely differing quantities of hormone are handled

in a similar fashion, by mechanisms independent of tissue requirements. In order to subject this conclusion to a critical test, a totally adrenalectomized and oophorectomized patient was deprived of all exogenous hormone for two days preceding the administration of a 0.25 mg. dose of hydrocortisone-4-C^{14}. It would be expected, and it can be strongly supported from the other results of this study, that the tissues of this patient were depleted of all residual hormone to the fullest extent obtainable in a human subject. In addition, the 250 γ dose of hormone represented only half the normal requirement for the period of the experiment and even less of the total daily need. If tissue requirements or "demand" influenced the excretion of hormone or its metabolites, retention of hormone should have been maximal in this patient. However, the rate and the total quantity excreted under these conditions was almost identical with those of the other subjects. Subsequently, when the tissue requirements were altered in this subject by the continuous daily infusion of 100 mg. of hydrocortisone, an identical pattern of excretion of the labeled hormone once more was observed. It is noteworthy also that in both experiments in this subject, the blood levels of radioactivity were in the same range. Therefore, by the criteria used, there was no evidence for tissue "demand" and no difference was observed in the "utilization" of a large as compared with a small dose. Indeed, the total excretion of radioactive products derived from the hormone, as well as the rate at which this occurred, was remarkably constant in all of the patients studied. This constant pattern under experimental conditions deliberately chosen to represent physiological, pharmacological, and pathological extremes, suggests that the same metabolic pattern will be found in all human subjects studied in this manner.

In the living organism, endogenously produced or artificially administered hormone is subjected to at least two potentially competitive processes. In the one, a hormone with its unique chemical characteristics is needed in a biological system for the initiation or regulation of a series of biochemical reactions that in turn lead to the well-known physiological effects of an endocrine secretion. The hormone may or may not undergo certain chemical alterations as a result of participation in these reactions. It could be suggested that, whether as a necessary or purely incidental consequence of reaction in a cellular process, the hormone attached to a cellular constituent undergoes transformation as a result of which it becomes readily dissociated from the complex in which it had performed its function. This altered product is rapidly conjugated or further metabolized and speedily eliminated from the body. Since the metabolites are invariably less effective hormonal agents than their precursors, and since the conjugated metabolites are practically devoid of biological activity, the chemical transformation and conjugation of the hormone may be the means by which the organs or tissues influence the level of homeostasis. While this series of events is in process with one fraction of the hormone, other reactions *may* be in operation simultaneously. In these, the hormone may be metabolized, conjugated and eliminated from the site of these reactions without having exerted any biochemical or biological actions despite the fact that cortisone or hydrocortisone has been shown to influence the dynamic behavior of practically every tissue in the body (6). It may, nevertheless, be viewed as a type of inactivation mechanism, designed perhaps to prevent sudden fluctuations in the highly integrated performance of the whole organism. The contribution of each of the processes to the sum of the hormonal metabolites cannot be evaluated since the fraction of the hormone used for a specific biological action and the questionable portion that may be "non-specifically" transformed and eliminated are unknown. Differences may yet be found in the metabolites excreted after a "tracer" dose and after a larger amount. The urinary metabolites of hydrocortisone-4-C^{14} under the several conditions studied are at present being examined in an attempt to clarify these problems.

The findings that the peak excretion of radioactive products derived from hydrocortisone-4-C^{14} occurred during the second hour after intravenous administration indicate that the hormone had been retained in one or another of these intermediate metabolic processes before it appeared in the urine. The excretion in the urine during the first few hours of the study certainly represents other mechanisms than direct renal clearance of the unaltered hormone. If this only were involved,

the greatest excretion would have occurred immediately after the compound was given when the circulating levels were at a maximum. From the results, it is clear that hormone is removed from the circulation by processes which operate at a more rapid rate than excretion by the kidney. Analysis of blood specimens taken during the infusion when 50 per cent of the total dose of the hormone had been delivered into the circulation as well as at the conclusion of the infusion when the entire dose had been given, indicated that a large portion of the homone had been removed from the blood and either distributed in extracellular fluids or deposited in tissues or both. Calculations of the approximate amount of radioactivity distributed in extracellular fluid were made from the average values for extracellular fluid and the concentration of radioactivity in blood. At the end of the infusion from 44 to 135 per cent with an average value of 93 per cent could have been in this compartment, if the assumption that equilibrium exists is correct. The large variation is due in part at least to the relatively low precision in measurement of radioactivity in blood. In earlier animal experiments with tritium labeled cortisone, it was found that 70 per cent of an intravenous dose of the hormone could be found in the liver within 5 minutes after administration (7). At later time intervals, the amount in the liver declined so that uptake by the liver is probably a transitory process. If the observations in mice may be applied to man, it is likely that most of the hydrocortisone-4-C^{14} was removed from the circulation by the liver, and later reintroduced into the blood stream, probably in a chemically altered form that could then be excreted by the kidney. Additional evidence for this viewpoint is obtained from the data concerning the fraction of hormone excreted in the free as compared to the conjugated form. In the second hour following the infusion, from 81 to 96 per cent of excreted material was already in a conjugated form and by 6 hours, from 92 to 99 per cent was conjugated. The hormone had clearly been subjected to certain chemical reactions and alterations in the short interval between its administration and excretion of products representing the majority of the original material.

Regardless of the mechanisms responsible for rapid removal of hydrocortisone from the blood, in order to arrive at an explanation of the constant pattern of excretion observed in these studies, the following considerations are pertinent. The systems concerned with the localization, transformation, and conjugation of the hormone have a high capacity so that large as well as small doses are handled with equal facility. The altered hormone or the hormone metabolites after discharge from the site of transformation attain a concentration in the body fluids, including blood, in direct proportion to the amount of material initially administered. Since the capacity of the systems for conversion are not rate limiting, the concentration of radioactive hormone metabolites in the circulation is achieved at the same time and in direct proportion to the dose administered. These are now excreted by a renal mechanism that is independent of all factors except the concentration of these compounds in the blood passing through the kidney. Kidney excretion, therefore, would always constitute a constant fraction of the dose administered and the identical characteristics of the excretion curve in all the patients could be explained.

Some other observations on the role of the kidney in the total metabolism of hydrocortisone are pertinent. The adrenalectomized patient, subject F4, who received a tracer amount of hydrocortisone while all hormone therapy was withdrawn, showed the renal excretory changes characteristic of adrenal insufficiency. The volumes of the hourly urine specimens obtained during the course of the experiment were extremely small, contained a great deal of salt, and there was delay in the excretion of a water load. Despite this marked alteration in renal function, the quantitative aspects of the excretion of hormone metabolites was precisely that exhibited by normal subjects as well as by this same adrenalectomized patient when she was receiving 100 mg. of hydrocortisone daily by continuous intravenous infusion as replacement therapy. Reduction in urine flow to the extent observed in this patient was therefore not a limiting factor in the biological life-time of the hormone and its metabolites. Renal clearance, calculated from the average amount of radioactivity excreted per minute at the end of 2 hours and the blood radioactivity measured at that time, indicates that from 30 to 140 ml. of blood per minute were cleared of radioactivity. Here again the low precision of blood C^{14} measurements makes the estimate only an approximation. Nevertheless the

values are less than the anticipated glomerular filtration rate and it can be suggested that tubular secretion is not an important excretory mechanism for the metabolites of hydrocortisone.

One of the most sensitive and prompt responses to hydrocortisone whether artificially introduced or secreted by the gland under stimulation with adrenocorticotrophic hormone is the fall in level of the circulating eosinophiles. This occurs after about 4 hours during constant intravenous infusion while the other manifestations of the hormone such as nitrogen excretion, glycogen deposition and the like are observed only after a longer time. It is clear from the results reported that even the onset of a biological response is seen at a time when a sensible proportion of the total amount of hormone given has been completely eliminated from the body. In addition to this, it is highly likely that a major portion of the hormone still resident in tissues or body fluids had already undergone chemical alteration. It seems evident therefore that the chain of reactions initiated by the hormone demands a continued supply of small amounts of the compound for the expression of a physiological or pharmacological response.

The possibility remains that a very small portion of the total hormone administered is localized in particular structures intimately associated with the true hormonal function. That this is an unlikely possibility is strongly suggested from the fact that 93 per cent of the administered radioactivity was recovered in the urine and feces and no more was recovered from the large dose than from the tracer amount of hydrocortisone. It seems more probable that a small additional quantity of hormone metabolites had reached the gastrointestinal tract and will eventually be found in the feces; in addition some of the radioactivity may have been eliminated through the skin. In view of the many uncertainties associated with the missing 5 to 10 per cent of the hormone, it seems more reasonable to focus attention upon the larger fraction that has been recovered rather than to speculate about the questionable significance of the minor quantity. Similar considerations might be applied to the small sustained urinary elimination observed after the first 24 hours following introduction of the hormone. The rate of excretion of radioactivity during the first 24 hours was essentially constant in all subjects and could be characterized by a half-life time of 3.6 hours. After the first day, the rate of elimination was entirely different from this value. It seems quite reasonable to suppose that this latter radioactivity represents hydrocortisone which has been eliminated into the gastrointestinal tract, reabsorbed, further metabolized and perhaps again subjected to similar cycles prior to its appearance in the urine.

The striking analogy between the excretion of the metabolites of hydrocortisone and of testosterone is worthy of comment. Although the experimental procedure was not the same as that employed with the adrenal hormone, it was found that a similarly rapid excretion of the hormone metabolites occurred (8). Thus, 62 per cent of the total metabolites of testosterone recovered during the first day after an intravenous infusion lasting for 4 hours, was excreted during the period of infusion. Like the hydrocortisone metabolites, these urinary products were chemically altered and conjugated forms of the hormone administered. The similar fate of two steroid hormones with opposite biological properties presents a challenging problem in the correlation of urinary steroids with the metabolic processes under hormonal control.

Since negligible amounts of C^{14} appeared in the respiratory CO_2, it is apparent that no major destruction of the nucleus occurred. It is perhaps significant to note that a similar situation obtains with the nucleus of cholesterol (8) despite oxidative degradation of the side chain. Further studies of C-20 or C-21 labeled hydrocortisone would be of interest in this connection.

The urinary metabolites of the steroid hormones are used widely as a measure of adrenal function in man. While the facts and assumptions which underlie this measurement are almost certainly true, the inadequacy of the methods which have been applied to characterize adrenal function in man by these means must be emphasized. Many specific and non-specific determinations of certain functional groups in the cortisone and hydrocortisone molecule have been used as clinical indices of the performance of the adrenal gland in a variety of physiological or pathological conditions. A survey of these methods indicates that the majority account for but a fraction of the hormone administered. By inference they should account for only a fraction of the hormone produced by the

normally functioning adrenal gland. The present study clearly demonstrates that about 80 per cent of the hormone administered in either trace quantities or massive doses appeared in one form or another in the urine within the course of 24 hours. Indeed, most of the end products are excreted within a much shorter period of time. The characterization and definition of these various transformation products of the hormone in order to permit the development of satisfactory methods for their determination still remains a major task.

SUMMARY

The dynamics of the distribution and excretion of hydrocortisone-4-C^{14} has been studied in six experiments with four human subjects. Over 90 per cent of the radioactivity administered was excreted in the urine and feces in 72 hours. There was no evidence of *in vivo* breakdown of the hormone as determined from measurements of expired carbon dioxide. No radioactivity was found in the spinal fluid of one subject 8 hours after the intravenous injection of the hormone. Within 24 hours, from 70 to 80 per cent of the hormone was eliminated in the urine in the form of conjugated metabolites at a rate characterized by an average half-life of 3.6 hours. This rate and the cumulative excretion were independent of the amount of hormone injected and the physiologic or pathologic status of the subject. There was no apparent difference in the fate of a "tracer" dose administered to an adrenalectomized oophorectomized subject deprived of all therapy as compared with a "tracer" dose while the same subject was maintained on adequate intravenous hydrocortisone. The blood level of radioactivity was measured at various intervals during and after the infusion. The rapid metabolism of hydrocortisone was demonstrated by the finding that the unaltered hormone accounted for only 10 per cent of the blood radioactivity within 15 minutes after completion of the intravenous infusion.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Madeleine B. Stokem, Caterina Martinez, Hilda Y. Denman and Ruth S. Loevinger.

REFERENCES

1. Science, 1953, **118**, 239.
2. Gallagher, T. F., Bradlow, H. L., Fukushima, D. K., Beer, C. T., Kritchevsky, T. H., Stokem, M., Eidinoff, M. L., Hellman, L., and Dobriner, K., Studies of the metabolites of isotopic steroid hormones in man. Recent Progress in Hormone Research, 1954, **9**, 411.
3. Anderson, E. C., and Libby, W. F., World-wide distribution of natural radiocarbon. Physiol. Rev., 1951, **81**, 64.
4. Katzenellenbogen, E. R., Dobriner, K., and Kritchevsky, T. H., Separation of steroid hormones and their metabolites by partition type chromatography. J. Biol. Chem., 1954, **207**, 315.
5. deCourcy, C., Bush, I. E., Gray, C. H., and Lunnon, J. B., A chromatographic investigation of Δ4-3-ketosteroids and α-ketolic steroids in human urine. J. Endocrinol., 1953, **9**, 401.
6. Thorn, G. W., Jenkins, D., Laidlaw, J. C., Goetz, F. C., Dingman, J. F., Arons, W. L., Streeten, D. H. P., and McCracken, B. H., Pharmacologic aspects of adrenocortical steroids and ACTH in man. New England J. Med., 1953, **248**, 232, 284, 369, 414, 588, and 632.
7. Bradlow, H. L., Dobriner, K., and Gallagher, T. F., The fate of cortisone-*T* in mice. Endocrinology, 1954, In press.
8. Fukushima, D. K., Bradlow, H. L., Dobriner, K., and Gallagher, T. F., The fate of testosterone infused intravenously in man. J. Biol. Chem., 1954, **206**, 863.