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Research Article

CORTISOL METABOLISM IN MAN: OBSERVATIONS OF PATHWAYS, POOL SIZES OF METABOLITES AND RATES OF FORMATION OF METABOLITES *

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Variations have been shown to occur in the quantitative urinary excretion pattern of the metabolites of cortisol in a variety of traumatic events such as surgery, bone fracture and burns (1). The compounds evaluated were THF, allo-THF and THE,[1] and it was demonstrated that the relative proportion of THF: THE excreted during and after a "traumatic event" was markedly increased. In addition almost complete absence of allo-THF was observed in a chronically ill individual and it reappeared upon convalescence. The *rate of secretion of cortisol* was one factor involved in producing the increase in urinary THF: THE, since following the administration of adrenocorticotropin (ACTH) to normal individuals the relative proportion of urinary THF as compared with THE was observed to rise (1–5). However, gross changes in the pattern of urinary metabolites of cortisol occurred in "traumatized" individuals during periods of normal or even declining adrenal activity (1). This suggested that factors other than the rate of adrenal secretion were involved in altering the rates of excretion of the numerous metabolites of cortisol. It became clear that knowledge concerning the *pathways* of metabolism, the *pool sizes* of the metabolites and the relative *rates of formation* of the individual metabolites from cortisol would prove valuable in interpreting the urinary excretion patterns of these compounds.[2] Consequently these studies were undertaken to determine the extent of the reaction THF $\rightleftharpoons$ THE in volunteer subjects with and without the administration of ACTH; the pathways for the formation of THF, allo-THF, THE and cortolone from cortisol; the miscible pool size of THF, allo-THF, THE and cortolone; and the possibility of determining the overall rates of formation of THF, allo-THF and THE. This work was performed in volunteer subjects with cortisol-4-C^{14} as a tracer.

METHODS

General plan. Tracer doses of cortisol-4-C^{14} (1 μc., about 250 μg.) were administered intravenously to normal volunteer subjects and urine samples were collected every 15 minutes. The specific activity of cortisol and its metabolites was determined as a function of time, and pool sizes and pathways were determined by isotope dilution procedures after the intravenous administration of small amounts of unlabeled cortisol metabolites. The rates of formation of these metabolites were computed in several instances using the criteria described by Zilversmit, Entenman and Fishler (6).

Subjects. All subjects were normal healthy volunteers between 20 and 35 years of age. With one exception, all were male. Several of the subjects received an intravenous infusion of ACTH beginning four hours prior to the injection of radioactive cortisol and continuing throughout the experiment. The ACTH dose was calculated as 25 I.U. per eight hours. The remaining indi-

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[1] The compounds referred to are cortisol or F; cortisone or E; 3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one, tetrahydrocortisol or THF; 3α,11β,17α,21-tetrahydroxy-5α-pregnan-20-one, allotetrahydrocortisol or allo-THF; 3α,17α,21-trihydroxy-5β-pregnan-11,20-dione, tetrahydrocortisone or THE; 3α,11β,17α,20β,21-pentahydroxy-5β-pregnane or cortol; 3α,17α,20β,21-tetrahydroxy-5β-pregnan-11-one or cortolone.

[2] The word *metabolism* as used denotes oxidation or reduction of the steroid molecule whereas *conjugation* is used to refer to the chemical binding of the steroid moiety with glucuronic acid.

viduals received no steroid or ACTH during the course of the experiment. Oral or intravenous fluids were used to maintain urine flow during the 15 minute collection periods.[3] For those not receiving ACTH the experiment was begun as soon as a satisfactory urine flow was obtained. All subjects were at bed rest for the duration of the experiment, except when they stood to void. An indwelling urethral catheter was employed in the female subject.

Steroids administered. In general, solutions of steroids for intravenous administration were prepared by weighing a known quantity of the compound into a clean vial, adding 1 to 2 ml. 70 per cent ethanol, capping the vial, and allowing it to stand overnight. The following morning 5 to 10 ml. sterile saline was added and the solution tested for sterility prior to use.

Cortisol-4-C^{14}. The cortisol-4-C^{14} was made available through the courtesy of the Endocrinology Study Section of the United States Public Health Service. One μc. of the compound corresponded to approximately 250 μg. of steroid and on chromatography, 92 to 94 per cent of the radioactivity migrated with cortisol while approximately 5 per cent of the radioactivity was more highly polar and about 2 to 3 per cent somewhat less polar than cortisol. Our analysis corresponds to that issued by the Endocrinology Study Section. The cortisol-4-C^{14} was used without further purification.

The THF and THE administered intravenously for the isotope dilution experiments were analyzed for several possible contaminants by chromatographing large quantities of the compounds and quantitatively analyzing specific contamination areas.[4] No significant amounts (less than 3 per cent) of THE or "20-hydroxylated compounds" were found in the THF and less than 1 per cent of THF or "20-hydroxylated compounds" was found in the THE. The β-cortolone was used directly.[5]

Analytic methods. Plasma and urines were extracted three times with double volumes of chloroform, the extract washed quickly twice with 1:20 volume 0.01 N sodium hydroxide and once with 1:20 volume of water. The solvent was dried with sodium sulfate and evaporated *in vacuo*. The extract containing the "free" (nonconjugated) F was chromatographed on paper overnight in the modified Bush (7) system, 3 volumes toluene: 1 volume 75 per cent methanol in water at 32°C. Quantitative fluorescence analysis for cortisol was carried out according to Sweat (8) and Peterson and Wyngaarden (9). Radioactive determinations were performed in a Nuclear-Chicago, D-47 windowless flow counter, 40 per cent efficient, at infinite thinness. Background was 20 counts per minute (cpm) and all samples were counted in duplicate with a maximal probable error of 3 per cent. Minimal counts collected for each sample were 1,280. All specific activities are expressed as cpm per microgram of steroid.

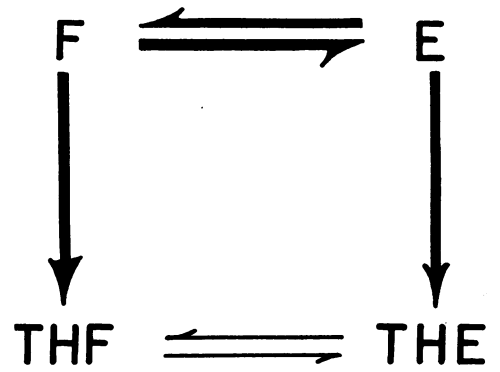


FIG. 1. SIMPLIFIED DIAGRAM OF ONE SEQUENCE IN METABOLISM OF CORTISOL IN THE HUMAN

To obtain the conjugated metabolites the urine samples were subsequently treated with β-glucuronidase,[6] at a final concentration of 200 Fishman units per ml. for three days at 37° C. at pH 5.0 or overnight at 47° C. and the same pH with 1,000 Fishman units per ml. The urine was extracted as described for the "free" fraction. The extract was chromatographed on paper for 39 hours in the toluene-methanol-water system. The THF, allo-THF and THE areas were located by dipping a 2 mm. wide center strip in alkaline blue tetrazolium. The peaks in blue formazan stain coincided with the location of radioactive peaks (1). The THF, allo-THF and THE areas were eluted with ethanol and their specific activities determined. The latter steroids were evaluated by the Porter-Silber reaction (10) using THF and THE as standards. The cortolone area[7] was located by eluting 1 cm. strips in the portion of the chromatogram corresponding to the migration of authentic β-cortolone. Aliquots of these eluates were counted and the radioactive peak was pooled, oxidized with periodic acid (11) and chromatographed for eight hours at 32° C. in a system composed of equal volumes of 67 per cent ligroin in toluene and 75 per cent methanol in water. The radioactive peak corresponding to the migration of 11-ketoetiocholanolone was eluted and its specific activity determined by using the Zimmermann reaction for the quantitative estimation

[3] At times large variations in the 15 minute urine output occurred even though fluid intake was regulated. However these variations did *not* alter the 15 minute excretion of metabolite. As an extreme example, in one individual 15 minute urine volumes showed a 10-fold variation from 12 ml. to 137 ml. while allo-THF excretion was 25 μg. and 27 μg. per 15 minutes, respectively.

[4] Quantitative determinations of THF and THE were carried out by the Porter-Silber reaction. The Zimmermann reaction, after periodic acid oxidation, was employed to evaluate "20-hydroxylated compounds."

[5] The β-cortolone was graciously supplied by David K. Fukushima and Thomas F. Gallagher of the Sloan-Kettering Institute for Cancer Research, New York.

[6] Ketodase®, a product of the Warner-Chilcott Laboratories, Warner-Lambert Pharmaceutical Co., New York, N. Y., was the β-glucuronidase preparation used.

[7] The α- and β-cortolone are not separated by this chromatographic system.

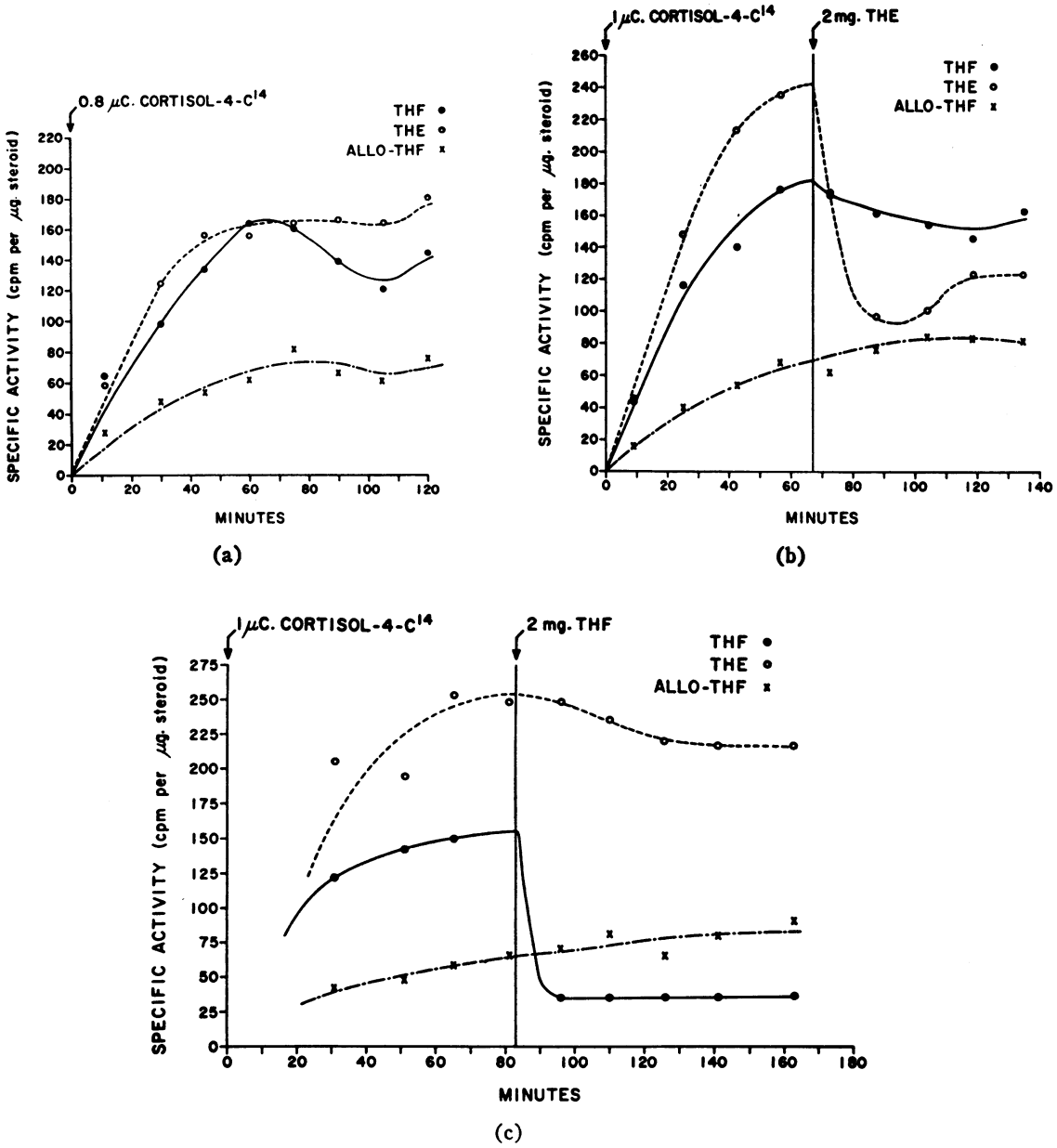


FIG. 2. SPECIFIC ACTIVITIES OF SEVERAL URINARY CORTISOL METABOLITES AFTER RAPID ADMINISTRATION OF TRACE QUANTITIES OF CORTISOL-4-C^{14} TO NORMAL MALE VOLUNTEERS RECEIVING NO EXOGENOUS ACTH

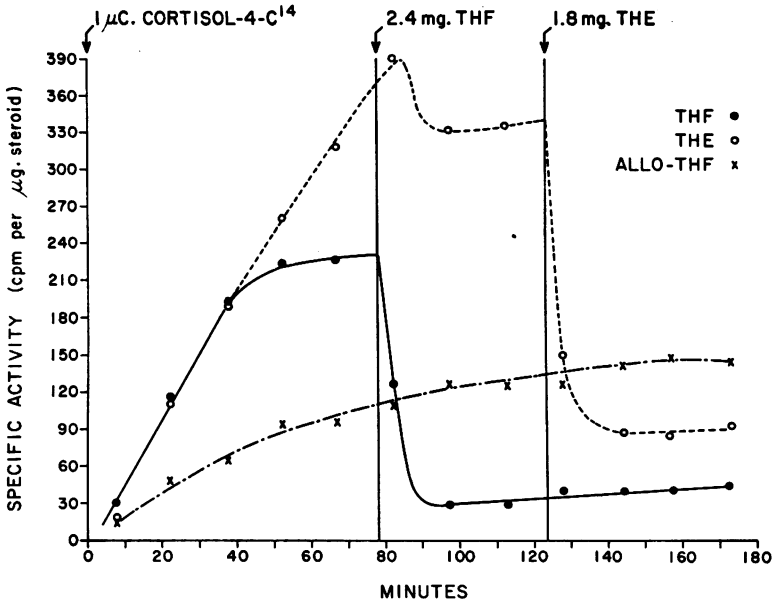
The data illustrate the small extent of the interconversion of the metabolites THF and THE.[1]

(a) D.B., age 22 years; no unlabeled metabolite given. The dip in specific activities at 100 minutes may be the result of a small increase in the rate of secretion of cortisol by the adrenal.

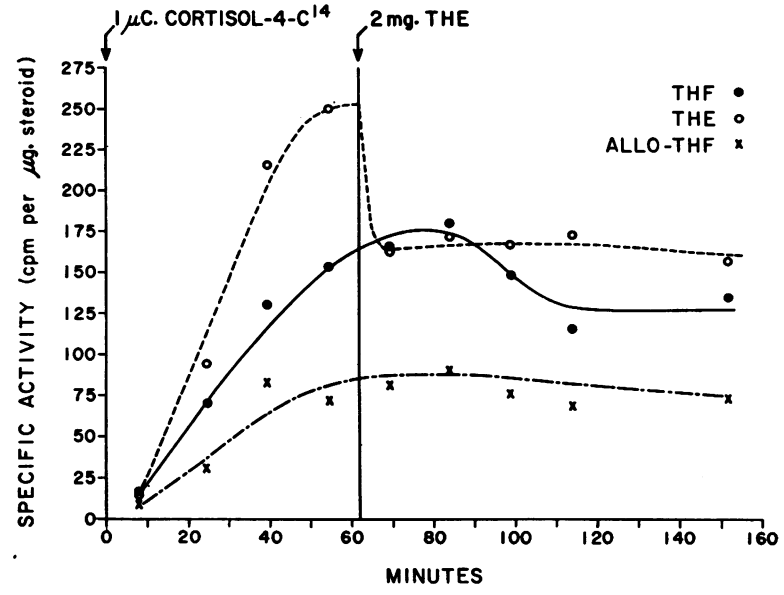
(b) D.B., age 22 years; unlabeled THE administered. Note the *minor* fall in specific activity of THF after the THE is administered. This suggests little conversion of THE to THF. Compare with (a).

(c) J.L., age 22 years; unlabeled THF administered. Note the negligible fall in specific activity of THE.

Figure 2 continued on following page



(d)



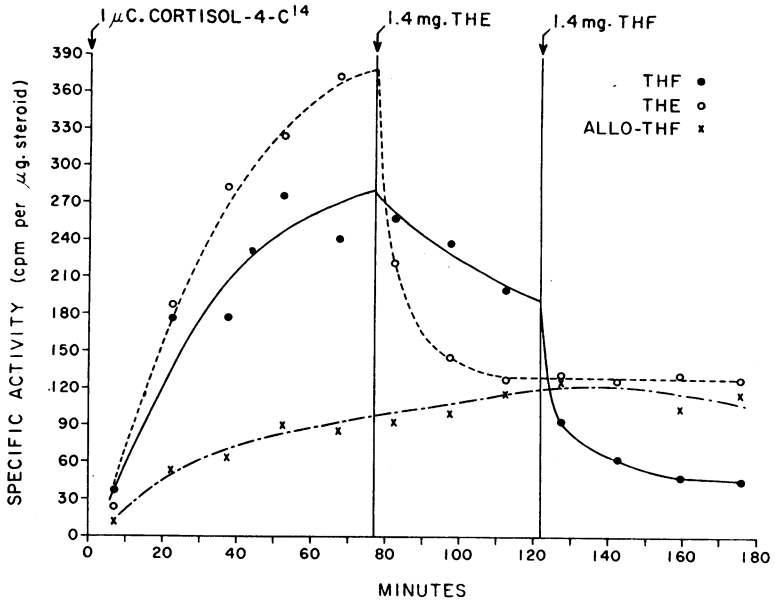
(e)

FIG. 2—*Continued*

(d) J.C., age 26 years; unlabeled THF and THE administered. Note some conversion of THF to THE.

(e) W.S., age 35 years; unlabeled THE administered. Note the relative lack of conversion of THE to THF.

Figure 2 continued on following page



(f)

FIG. 2—*Continued*

(f) E.K., age 21 years; unlabeled THE and THF administered. Note some conversion of THE to THF.

and expressing the micrograms of steroid as cortolone. Although authentic β-cortol separated distinctly from cortolone, no significant quantities of the cortols were found in the urine samples of Figures 5 and 8.

RESULTS

"Interconversion" of THF and THE

The reaction 11-hydroxy ⇌ 11-ketone occurs extensively after the administration of either cortisol or cortisone to man. The isolation of similar urinary metabolites after either cortisol or cortisone administration (12–14) strongly supports this contention. However, even though the chemical difference between THF and THE involves the same oxygen grouping at position 11 of the steroid nucleus as in compounds F and E, the two metabolites do *not* readily interconvert.

In the simplified diagram of Figure 1, which illustrates one major sequence in the metabolism of cortisol in the human, the formation of THF and THE are shown to proceed via F and E, respectively, while the interconversion of THF and THE is indicated as being minimal. Evidence that the interconversion of THF and THE is a minor pathway was obtained in several experiments in which the specific activities of these metabolites were determined as a function of time after the administration of 1 μc. cortisol-4-C^{14} (see Methods). The specific activity of each metabolite reaches a peak and then slowly declines in characteristic fashion (15). Such specific activity curves in a normal male (D.B., age 22 years) who received no exogenous ACTH during the course of the experiment is illustrated in Figure 2(a). This figure should be compared with Figure 2(b) which shows the data from the same individual using the identical protocol except that 2 mg. unlabeled THE was administered during the course of the experiment. In the latter figure there is a sharp decrease in the specific activity within the THE pool following the administration of the unlabeled THE, but there is little or no drop in the specific activity of THF as a consequence of the conversion of "cold" THE to THF. In the same type of experiment, Figure 2(c), unlabeled THF was administered intravenously to a normal male (J.L., age 22 years). This subject also did not receive exogenous ACTH. The decline in specific activity of the THF pool is not accompanied by any observable decrease in the THE specific

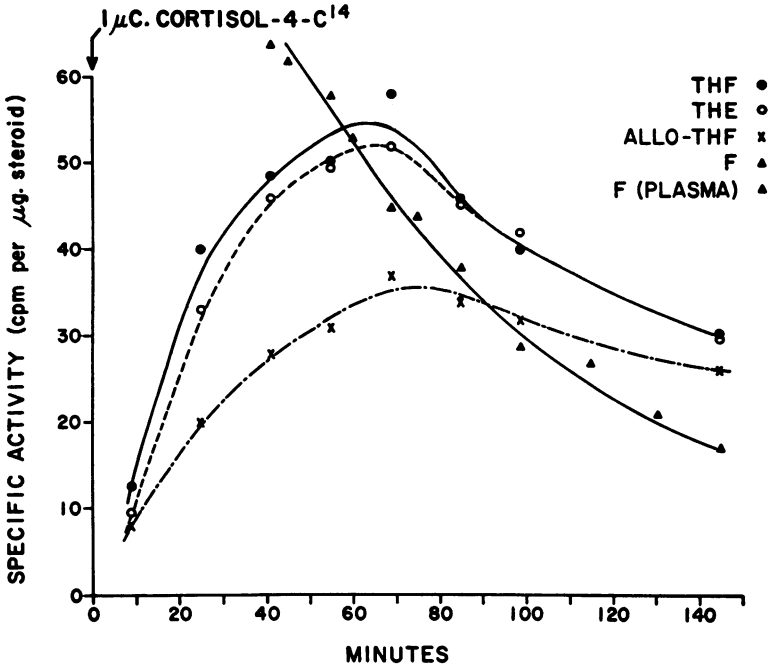


FIG. 3. SPECIFIC ACTIVITIES OF URINARY CORTISOL METABOLITES AFTER RAPID ADMINISTRATION OF 4-C^{14}-CORTISOL TO A NORMAL MALE, J.C., AGE 26 YEARS, DURING CONTINUOUS ACTH INFUSION

Compare with Figures 5 and 8 for conversion of metabolites during ACTH administration.

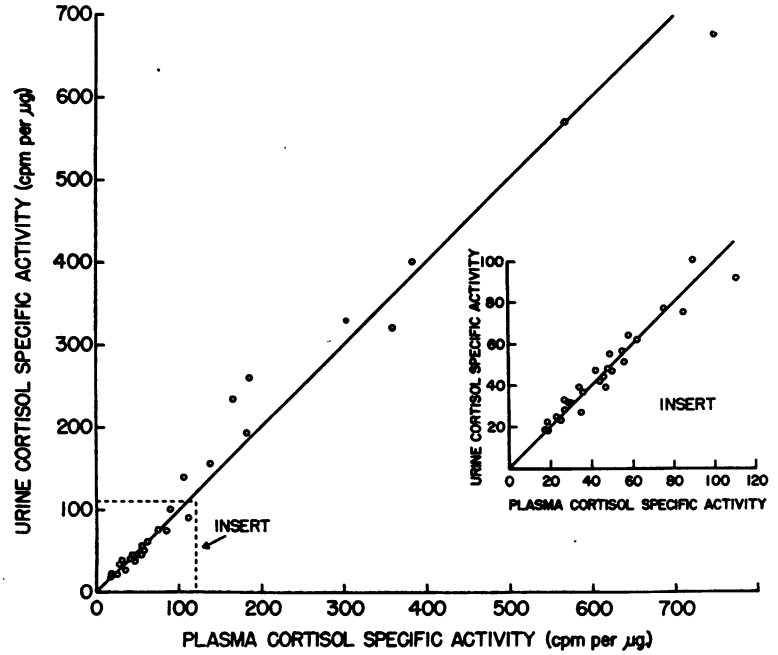


FIG. 4. RELATIONSHIP BETWEEN URINE AND PLASMA CORTISOL SPECIFIC ACTIVITIES

Data taken from six normal volunteer subjects. The straight line was drawn with a slope of 1.

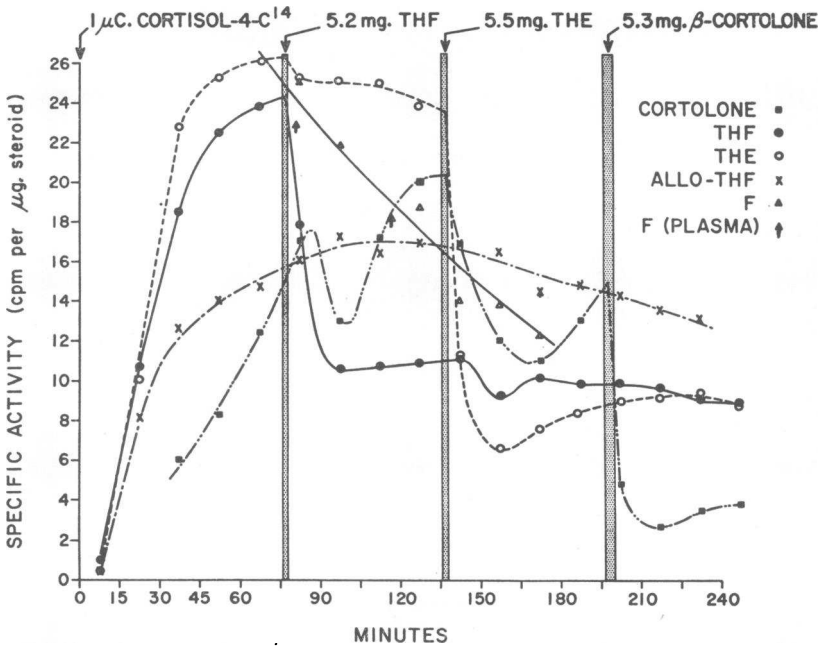


FIG. 5. SPECIFIC ACTIVITIES OF URINARY CORTISOL METABOLITES AFTER RAPID ADMINISTRATION OF 4-C^{14}-CORTISOL TO A NORMAL FEMALE, M.L.P., AGE 24 YEARS

Subject received continuous ACTH infusion throughout the experiment. The data, which indicate the relative conversions of the metabolites, were used to compute pool sizes and rates of formation of metabolites. Refer to Figures 6 and 7 for additional data from this experiment.

activity. The results of the experiments of Figures 2(b) and 2(c) indicate that little THF is formed from THE and little THE is formed via THF. The experiments of Figures 2(d) through 2(f), also performed in normal individuals who did not receive exogenous ACTH, confirm the observations cited above. The conversion of a small amount of THF to THE is evident from the decline in THE specific activity upon the administration of unlabeled THF in Figure 2(d). Similarly a small conversion of THE to THF is noted in Figure 2(f) after the administration of the unlabeled THE. When volunteer subjects were maintained at a high steady adrenal secretory rate by continuous infusion of ACTH during the entire course of the experiment (Figures 3, 5 and 8) similar results relating to the interconversion of THF and THE were obtained. It can be concluded (see Figure 1) that in "normal" individuals the major pathway for the formation of THF is via F and the major pathway for the formation of THE is via E. Only minor interconversions of THF and THE occur (16, 17).

Further metabolite interconversions

In studies such as these one must assume that the specific activities of compounds obtained at any time from the urine represent a true sampling of the specific activities of the same compounds from the body pool. Fortunately, plasma and urine cortisol specific activities from six subjects do show a 1:1 correlation (Figure 4). Unfortunately, it has not been possible to demonstrate a similar correlation for each *metabolite* since their plasma concentrations are too low for accurate analysis.

More detailed studies are shown in Figures 5 and 8, which represent the "steady state" condition in two normal individuals during ACTH infusion. After the administration of unlabeled THF there is a sudden fall in the cortolone specific activity indicating the transformation of THF to cortolone. The subsequent rise in cortolone specific activity prior to the administration of unlabeled THE is due to the continued transformation of cortisol to cortolone via intermediates

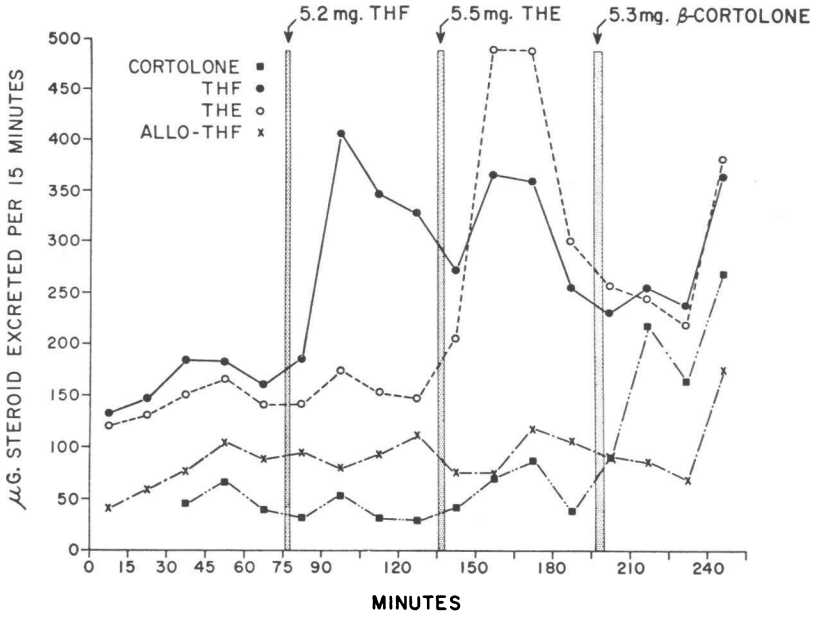


FIG. 6. QUANTITY OF URINARY METABOLITES EXCRETED PER 15 MINUTES IN EXPERIMENT OF FIGURE 5

other than THF. Following the administration of unlabeled THE there is another decrease in cortolone specific activity which is consistent with the observation (18, 19) that THE is also a precursor of cortolone. Quantitative values for the *extent* of conversion of THF and THE to cortolone are not deducible from Figures 5 and 8. The decreases in cortolone specific activity occur abruptly and rise sharply. There is no indication that simple isotope dilution calculations can be applied under these circumstances. Consequently the data do not make it clear whether THF proceeds to cortolone via THE or perhaps an unknown pathway. The rather sharp increase in cortolone specific ac-

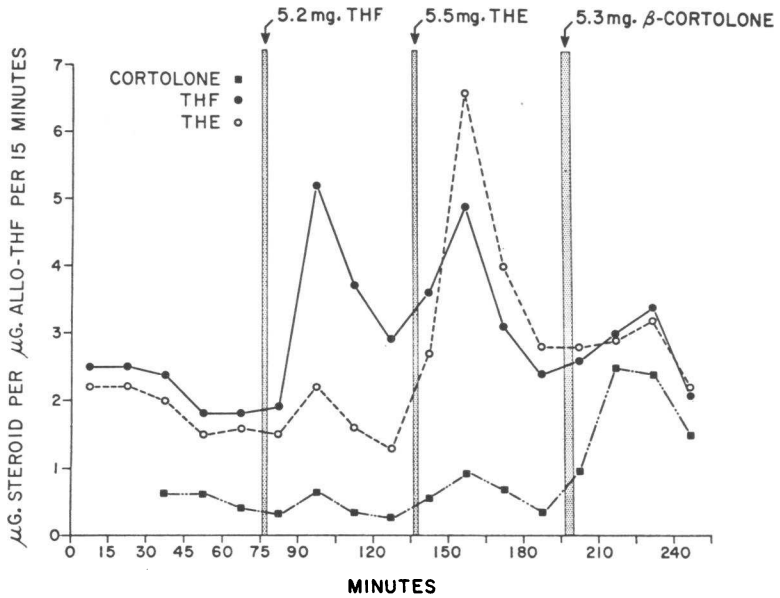


FIG. 7. QUANTITY OF URINARY METABOLITES EXCRETED PER 15 MINUTES IN EXPERIMENT OF FIGURE 5, EXPRESSED AS A FUNCTION OF ALLO-THF [1]

tivity, which occurs just prior to the intravenous addition of β-cortolone, suggests that there is yet another pathway of cortolone formation not involving either THF or THE as intermediate.

The figures clearly demonstrate that the allo-THF specific activity is not influenced by the addition of the unlabeled intermediates. Hence, once the double-bond in ring A has been reduced to the 5β configuration, as in THF, THE and cortolone, there is no subsequent unsaturation and formation of allo-THF that has the 5α configuration. Therefore allo-THF is not formed from either THF, THE or β-cortolone.

Although a conversion of cortolone to THF and THE is not evident by isotope dilution after addition of unlabeled β-cortolone, urinary excretion data suggest that these latter conversions may occur. Figure 6 expresses the *amount* of each urinary metabolite excreted per 15 minutes for the subject of Figure 5. Small variations in the 15 minute excretions of the metabolites prevent a clear visualization of the conversion of one metabolite to another. As an exception, after the administration of cold THE the increased excretion of THF is evident and indicates the conversion of THE to THF. Because of possible variations in *a*) adrenal secretory rate, *b*) renal function, *c*) completeness of β-glucuronidase hydrolysis or *d*) the extent of recovery, the amount of each metabolite excreted per 15 minutes is expressed in Figure 7 as a fraction of the allo-THF whose quantity is assumed not to vary with the administration of the "cold" THF, THE and β-cortolone. This latter figure shows the small increases in excretion of several metabolites after the administration of the "cold" steroid and hence is consistent with the interconversions, although small, of THF, THE and cortolone.

Pool sizes

Pool size of cortisol. The pool size of cortisol was calculated by dividing the total radioactivity injected (corrected to 92 per cent purity) by the specific activity of the cortisol extrapolated to time zero. The latter value was obtained from a

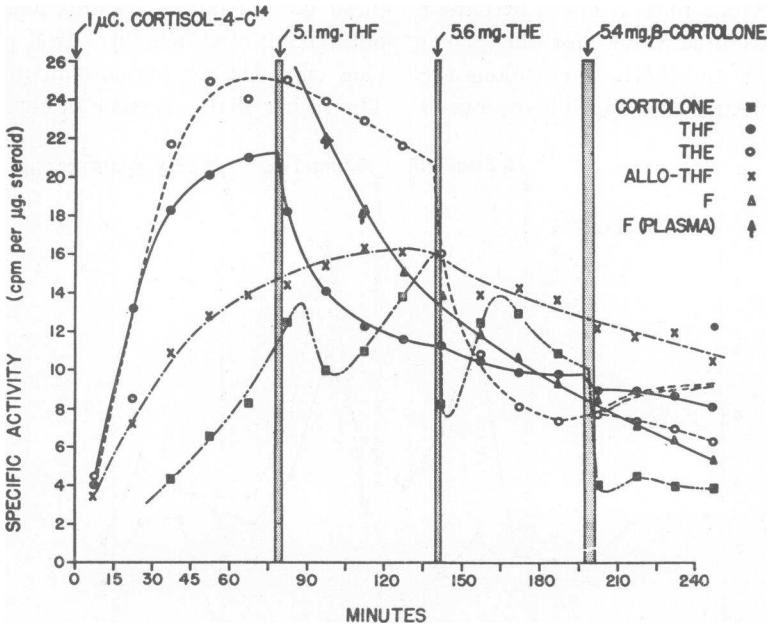


FIG. 8. SPECIFIC ACTIVITIES OF URINARY CORTISOL METABOLITES AFTER RAPID ADMINISTRATION OF 4-C^{14}-CORTISOL TO A NORMAL MALE, E.K., AGE 21 YEARS

Subject received continuous ACTH infusion throughout the experiment. Note similarity to Figure 5. The double dashed line is a crude extrapolation of the THE curve as it might appear if the added β-cortolone were not converted to THE (refer to Results, *Pool Sizes*).

TABLE I

*Readily miscible pool size and rates of formation of cortisol metabolites during adrenocorticotropin administration**

Patient (a)	Compound (b)	Pool size† (c)	Rate of formation* (d)	Metabolite ratio (e)	Ratio from calculated pools (f)	Ratio from urinary excretion (g)
		mg.	*mg. per 24 hr.*			
M. L. P.	THF	3.6	65	THF : THE	1.1	1.1
female	Allo-THF	2.1‡	18	THF : Allo-THF	1.7	1.9
24 years	THE	3.4	72	THF : Cortolone	3.3	3.7
	Cortolone	1.1§				
			Sum 155			
	F	17.5‖	195			
E. K.	THF	4.8	50	THF : THE	1.2	1.1
male	Allo-THF	4.4‡	33	THF : Allo-THF	1.1	1.1
21 years	THE	4.0	81	THF : Cortolone	4.0	4.7
	Cortolone	1.2‡§				
			Sum 164			
	F	16.6‖	211			

* Refer to Methods and Results for details. "Steady state" plasma cortisol concentrations, in micrograms per 100 ml. plasma, were 51 for M. L. P. and 37 for E. K.

† Calculated by isotope dilution unless otherwise indicated.

‡ It can be seen in Columns (f) and (g) that the relative amounts of the metabolites, THF and THE, excreted per 15 minutes as the glucuronides, are proportional to the size of the pools calculated by isotope dilution. Hence, "a" = kN, where "a" is the amount of metabolite excreted per 15 minutes, N is the pool size and k is a proportionality factor; k is 3.6×10^{-2} per 15 minutes for M. L. P. and 2.2×10^{-2} per 15 minutes for E. K. Knowing k and "a," pool sizes were calculated for allo-THF and cortolone.

§ Cortolone includes both 20α and 20β isomers.

‖ Total radioactivity injected divided by cortisol specific activity extrapolated to time zero. Cortisol half-life for M. L. P. is 88 minutes, for E. K., 78 minutes.

method of least squares plot of the log (cortisol specific activity) as a function of time.

Metabolite pool size, by isotope dilution. The fall in specific activity after the addition of nonlabeled metabolite can be used to determine the readily miscible pool size of a given cortisol metabolite by applying the following general isotope dilution formula:

$$N\ (s.a.)_0 = (N + n)(s.a.)_1$$

or

$$N = \frac{n\ (s.a.)_1}{(s.a.)_0 - (s.a.)_1}$$

where N = miscible pool size in milligrams prior to addition of diluent; n = milligrams unlabeled diluent added to pool (corrected for a small loss of diluent excreted and for conversion of unlabeled diluent to other metabolites); $(s.a.)_0$ = specific activity prior to addition of diluent; $(s.a.)_1$ = specific activity after addition of diluent.

The value for the specific activity of the metabolite *after mixing with the unlabeled diluent,* $(s.a.)_1$, is taken at the region in which the specific activity reaches a plateau. It is assumed that at this plateau the added metabolite is homogeneously mixed with all or the major portion of the metabolite pool. The latter is sometimes referred to as the "readily miscible pool." In some instances the plateau is clearly defined; in others it is not. As an example, in Figure 5, the THF plateau is apparent 30 minutes after the administration of "cold" THF, whereas the THE plateau is not reached until 75 minutes after the addition of unlabeled THE. Unfortunately, in the THE curve of Figure 8 the "plateau" after the administration of "cold" THE is not reached prior to the injection of β-cortolone. Our observations on the conversion of β-cortolone to THE would explain the failure of the THE curve to attain the expected plateau. Consequently, it was necessary to extrapolate roughly the expected THE "plateau." This is indicated by the double lines during the last period of Figure 8. In the future, increasing the time interval between additions of cold diluent will assist greatly in locating these plateaus without resorting to extrapolations.

Metabolite pool size calculated from urinary excretion data. It is difficult to determine pool size by isotope dilution procedures in those instances when metabolites are not available as diluents or when it is not possible to locate the plateau after dilution. In such situations it is possible to estimate pool size if the rate of excretion of urinary metabolites is known. In the "steady state" the rate of excretion of metabolites is relatively constant prior to the administration of diluents (Figure 6). If the metabolite pool exists mainly in the form of metabolite-glucuronidate, then the rate of excretion of each metabolite-glucuronidate should be proportional to the pool size. Hence, $a = kN$, where "a" is the amount of metabolite excreted per 15 minutes, k is a proportionality factor and N is the pool size. In each of the two experiments in Table I, k was found to be identical for THF and THE. For subject M.L.P., k was 3.6×10^{-2} per 15 minutes; for E.K., k was 2.2×10^{-2} per 15 minutes. That the same values of k were obtained for THF and THE merely implies that once conjugation with glucuronic acid has occurred the steroid-conjugate is so highly polar that small differences in the steroid moiety play little or no role in determining the rate of excretion of the steroid-conjugate. This implies that, in any given individual, k is identical for all cortisol-metabolite-glucuronidates.

By substituting the measured value of "a" and the value of k, previously calculated for THF and THE, N (the pool size) was determined for allo-THF in both subjects of Table I and for cortolone in Subject E.K. It can be seen, Column (g), that the relative proportions of the metabolites excreted per unit time, *are* equal to the ratio of the calculated pool sizes of the same compounds, Column (f). This is consistent with the assumptions made concerning the relationship of pool size to the rate of excretion of steroid-glucuronidate.

Rates of formation of metabolites. A method for computing the rate of formaton of a metabolite from its precursor in a biologic system has been clearly described by Zilversmit and associates (6). It is necessary to assume that the metabolite is in the steady state during the interval of the study, that is, its rate of appearance must equal its rate of disappearance and this rate must be constant. In addition, it is imperative that random treatment of all molecules occur. If these assumptions hold, then, in a graph of the specific activities of precursor (cortisol) and metabolite as functions of time, the specific activity curve of the precursor will intersect the specific activity curve of the metabolite at the maximum of the metabolite curve. The data of Figure 3, in which no unlabeled metabolites were administered, as well as the data of Figures 5 and 8, meet these criteria for THF, allo-THF and THE. Consequently Zilversmit's formulas were applied to calculate the rates of formation of THF, allo-THF and THE. The formulas are as follows:

$$p = \frac{N}{t_t} \quad \text{and} \quad t_t = \frac{A}{m}$$

where p = rate of formation of metabolite (amount formed per unit time); N = pool size of metabolite (amount); t_t = turnover time (time necessary to form an amount of metabolite equal to the pool size); A = area (determined graphically) *between* the specific activity curves (as a function of time) of the precursor and metabolite during an arbitrary time interval, $t_2 - t_1$. The units of A are specific activity $\times$ time; m = difference between the specific activities of the metabolite at t_2 and t_1.

The turnover, or rate of secretion, of cortisol, which in the steady state is equivalent to the amount of cortisol removed from the body pool per unit time either by conversion to metabolites or by excretion as "free" cortisol, was calculated as $\ln 2/t_{1/2} \times$ pool size where $t_{1/2}$ is the biological half-life of the isotopically-labeled cortisol (20). The biological half-life, or the time necessary to reduce the specific activity of the cortisol pool to one-half value, was obtained from the slope of the straight line, computed by the method of least squares, of a plot of the log of the specific activity of cortisol as a function of time.

The rates of formation, p, for the various metabolites are shown in Table I, Column (d), along with the rate of secretion of cortisol. It can be seen that the sum of the rates of formation of the three metabolites, THF, allo-THF and THE, represent approximately 80 per cent of the total turnover of the secreted cortisol. These metabolites, in turn, may be converted in part to other compounds. Data herein presented suggest that THF and THE may be transformed into cortolone. It seems logical that the former two metabolites may

also be converted to ketosteroids (21) and to the cortols (22). Since the size of the metabolite pool that exists during the steady state is the resultant of the rate of formation of this metabolite minus its rate of excretion plus transformation to other steroids, there may be considerable difference between the relative pool sizes of the metabolites and the relative rates of formation of the same compounds. For example, in Table I, it is clear that even though the ratio of the sizes of the THF and THE pools is approximately 1:1 for both subjects, the *rates of formation* of THF are significantly *lower* than the rates of formation of THE and hence the smaller pool size of THE is indicative of the transformation of this metabolite to other steroids.

The sum of the rates of formation of the metabolites THF, allo-THF and THE total about 50 mg. less than the turnover of cortisol. Hence certain of the metabolites of cortisol may not pass through the aforementioned three metabolites as intermediates. It may be inferred that this difference of approximately 50 mg. will include small amounts of cortisol itself, cortisone, a portion of the cortols and cortolones, 20α-hydroxy-cortisol (23, 24), 20β-hydroxycortisol (24, 25), 6β-hydroxycortisol (26), 11 ketoandrosterone (21) and a portion of 11β-hydroxyandrosterone (21).

DISCUSSION

Evidence presented in this study indicates that two of the major metabolites of cortisol, THF and THE, are derived essentially from cortisol and cortisone, respectively. Although there is some interconversion of THF and THE, this does not appear to be a major pathway for the formation of either THF or THE. It cannot be implied that enzyme systems capable of transforming THF $\rightleftharpoons$ THE are actually *deficient*. If conjugation with glucuronic acid prevents further transformation of the steroid then it is possible that the *rate of conjugation* of the cortisol metabolites may be an important factor in limiting the interconversion of certain of the metabolites. In addition, there is no perceptible conversion of THF, THE or β-cortolone to allo-THF, suggesting that once the double bond of ring A of the steroid nucleus has been reduced to form compounds such as THF and THE with the 5β configuration, there is no unsaturation with subsequent reduction of the double bond to the 5α or "allo-" configuration. Further evidence suggests the *in vivo* interconversion, to a small extent, of the compounds THF $\rightleftharpoons$ cortolone and THE $\rightleftharpoons$ cortolone.

The kinetic methods employed in this study involve isotope dilution procedures carried out within a complex body pool. The kinetics of the distribution of chemical substances within biological compartments have been described by numerous investigators (15, 20, 27). Certain aspects of the kinetics of distribution of cortisol in man have been discussed by Hellman, Bradlow, Frazell and Gallagher (28) and Peterson and associates (29), while other studies (30–32) have been concerned with measurements of the "half-life" of plasma cortisol in various physiological conditions. Implicit in these studies is the concept that if first-order kinetics prevail then half-lives can be computed. Unfortunately such studies do not yield any information concerning pool sizes of various steroids, rates of formation of individual metabolites or the various chemical pathways of metabolism that occur. However, the mathematical treatment applied by Zilversmit and co-workers (6) makes possible the calculation of the rate of formation of a substance from its immediate precursor. In reference to cortisol this makes feasible the calculation of the rates of formation of several of its metabolites such as THF, allo-THF and THE. Hence, one can add to the quantitative evaluation of urinary metabolites an additional rate parameter to aid in relating the metabolism of cortisol to the pathological condition of the individual. It cannot be emphasized too strongly, however, that such calculations attempt to describe the body pool as a *whole* and because of this gross treatment, changes in the kinetics of cortisol metabolism, which are small or slow in comparison to total changes, may be overlooked.

In the kinetic data derived for the two individuals receiving adrenocorticotropin (Table I), the criteria outlined by Zilversmit and associates (6) was met, and good agreement between pool size and urinary excretion data was obtained. This latter agreement is consistent with the concept that the miscible metabolite pools exist largely as glucuronides and that there is little or no discrimination by the kidney between the different metabolite-glucuronides.

The marked contrast between the type of data obtained from the *amounts of metabolite excreted per unit time* and from the *rates of formation* of metabolite is well illustrated for both patients in Table I. The relative proportion of THE: allo-THF *excreted* is 1.6 for M.L.P. and 0.91 for E.K., whereas the relative proportion of the *rates of formation* for THE: allo-THF is 4.0 for M.L.P. and 2.5 for E.K. It is obvious that an estimation of the relative rates of formation of metabolites based on their relative quantities in the urine can be grossly misleading.

Another interesting observation concerns the probable lack of chemical transformation of the steroid moiety once conjugation with glucuronic acid has taken place. The data of Figure 5, suggest that the steroid-glucuronic acid conjugate is not further altered at positions C-11 and C-20. In this figure it can be seen that upon the addition of unlabeled THF the specific activities of THE and cortolone are lowered. This is attributable to the formation of a small quantity of THE and cortolone from the sudden infusion of unconjugated, nonradioactive THF. Since the unconjugated THF is rapidly transformed into THF-glucuronide, the decreases in specific activity of THE and cortolone are abrupt. There is not a continuous fall in specific activity of THE and cortolone as one might expect if any substantial amount of conjugated THF of specific activity, 11 cpm per μg. (THF specific activity after administration of unlabeled THF) were *continuously* being transformed into THE and cortolone. After the administration of unlabeled THE similar results can be seen in the cortolone curve. Such observations are consistent with the concept that once conjugation with glucuronic acid has occurred no further alteration of the steroid metabolite occurs at the two positions, C-11 and C-20. In view of these considerations the *small* amount of conversion of one metabolite to another may be the result of the removal of metabolite by conjugation rather than the result of a lack of enzyme systems capable of performing the reversible reduction at position 11 or position 20.

The role of protein-binding in these studies is unknown. It would appear that if *in vivo* protein-binding of steroids is extensive (33, 34) its nature is such that it does not prevent either the ready mixing of cortisol and its metabolites or the rapid metabolism of cortisol.

Several investigators (1, 31, 35, 36) have suggested that the quantitative aspects of the metabolism of steroid hormones may aid in evaluating the role these hormones play in physiological processes. Hence, the results of these current studies are most encouraging. Further work correlating urinary excretion data, metabolite pool size, rates of formation of metabolites and metabolite interconversions in the steady state are in progress. It is necessary that extensive quantitative data, consistent in all aspects, be obtained to establish isotope dilution techniques for determining the rates of transformation of cortisol to specific metabolites in man.

SUMMARY

Several aspects of the kinetics of cortisol metabolism were investigated in normal adult volunteers after the rapid intravenous administration of trace amounts of cortisol-4-C^{14}. One group was maintained on a constant infusion of adrenocorticotropin begun four hours prior to the injection of cortisol-4-C^{14} and continued throughout the experiment.

The specific activities, counts per minute per microgram, of several cortisol metabolites were evaluated in urine samples collected every 15 minutes. Cortisol specific activities were determined in both plasma and urine. The metabolites evaluated were cortolone (3α, 11β, 17α, 20α or β, 21-pentahydroxy-5β-pregnane), tetrahydrocortisol (3α, 11β, 17α, 21-tetrahydroxy-5β-pregnan-20-one), allo-tetrahydrocortisol (3α, 11β, 17α, 21-tetrahydroxy-5α-pregnan-20-one) and tetrahydrocortisone (3α, 17α, 21-trihydroxy-5β-pregnan-11, 20-dione).

During the course of the experiments small amounts of nonlabeled metabolites were administered intravenously. From changes in specific activities of each metabolite and from the *amounts* of metabolite excreted per 15 minutes the following information was obtained:

Interconversion of metabolites. Two of the major metabolites of cortisol, tetrahydrocortisol and tetrahydrocortisone, are derived mainly from cortisol and cortisone, respectively. There is little interconversion of these two metabolites.

The specific activity of the metabolite, allo-tetrahydrocortisol, was not altered by the addition of either unlabeled tetrahydrocortisol, tetrahydrocortisone or β-cortolone. Hence, these metabolites are not converted to allo-tetrahydrocortisol. The implications of this lack of conversion are discussed.

The fall in cortolone specific activity after the addition of unlabeled tetrahydrocortisol and tetrahydrocortisone suggests that these two compounds may be, to some extent, precursors of cortolone.

Pool sizes of metabolites. "Readily miscible" pool sizes of metabolites were calculated from the isotope dilution data and from urinary excretion values.

"Rates of formation" of metabolites. In two studies performed during adrenocorticotropin administration, the "rates of formation" (milligram per 24 hours) of tetrahydrocortisol, allo-tetrahydrocortisol and tetrahydrocortisone were computed from the kinetic data. The sum of the "rates of formation" of these three metabolites accounted for approximately 80 per cent of the total turnover of cortisol.

It is suggested that additional similar studies may aid in correlating cortisol metabolism with altered physiology in man.

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