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Quantitative Determination of the Urinary Cortisolc Metabolites, "Tetrahydro F," "Allo-Tetrahydro F" and "Tetrahydro E": Effects of Adrenocorticotropin and Complex Trauma in the Human

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Research Article

QUANTITATIVE DETERMINATION OF THE URINARY CORTISOL METABOLITES, "TETRAHYDRO F," "ALLO-TETRAHYDRO F" AND "TETRAHYDRO E": EFFECTS OF ADRENOCORTICOTROPIN AND COMPLEX TRAUMA IN THE HUMAN [1]

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Methods for the quantitative estimation of plasma cortisol concentration have been devised by Nelson and Samuels (1), Silber and Porter (2), and Peterson and Wyngaarden (3) who used the sulfuric acid-induced fluorescence developed by Sweat (4). Together with urinary procedures (5, 6) these techniques have been employed to determine the secretion of cortisol by the adrenal in normal and pathological conditions (7–9) and to evaluate the factors involved in the "removal" of cortisol from the plasma in surgery (10–13) and liver disease (14–16).

Although a considerable amount of work has been done in isolating and identifying such urinary metabolites of cortisol as THF [3] and THE (17–22) and their interconversions (23–25), much less has been done to relate the quantitative transformations of these urinary metabolites from cortisol under normal and pathological conditions. In 1953, deCourcy, Bush, Gray, and Lunnon (26) reported daily excretion values for two chromatographically separated urinary steroids corresponding in migration rates to THF and THE, in 10 normal men. The mean values were 212 μg. per 24 hours for THF and 1.5 mg. per 24 hours for THE, giving a ratio for THF:THE of 0.14. Romanoff, Seelye, Rodriquez, and Pincus (27) have recently published quantitative data on the excretion of THF and THE combined with allo-THF (28, 29) in one group of normal men and in a group of schizophrenic men. There was no essential difference in the ratio of THF:(THE plus allo-THF) between the two groups of men, and the ratio was reported as approximately 1:2. Cope and Hurlock (30) published data on the excretion of urinary THF and THE in several normal and surgical cases. Their results indicated increased excretion of these metabolites after surgery but did not show any other definite pattern of alteration of the metabolism of cortisol, although they state that "a tendency can be observed for tetrahydro-compound F excretion to rise more than does tetrahydrocortisone." In a preliminary report, Gold, Macfarlane, and Moore (31) showed that the relative proportions of the two urinary cortisol metabolites, THF and THE, were related to situations of "stress" and adrenocorticotropin (ACTH) administration. It is the purpose of this current report to supply additional evidence for alterations in the excretion of certain cortisol metabolites in the human in a variety of "stress" situations, such as surgery, bone trauma, burns and prolonged illness, and to compare the proportion of metabolites in these cases with normal individuals and with the urine of individuals receiving ACTH.

METHODS

Analytical methods

Urines were collected without preservative and were frozen until used.

[1] This work was suported in part by a grant from the Atomic Energy Commission. It was sponsored and supported in part by the Subcommittee on Metabolism in Trauma, Advisory Committee on Metabolism, Office of the Surgeon General, Department of the Army, through a contract (DA-49-MD-472) with Harvard University. The assistance of Winthrop Laboratories, Inc., and The Upjohn Co. is gratefully acknowledged.

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[3] Compounds referred to are the following: THF, tetrahydrocortisol (3α,11β,17α,21-tetrahydroxypregnan-20-one); allo-THF (3α,11β,17α,21-tetrahydroxyallopregnan-20-one); THE, tetrahydrocortisone (3α,17α,21-trihydroxypregnan-11,20-dione); F, cortisol (11β,17α,21-trihydroxypregnan-3,20-dione); E, cortisone (17α,21-dihydroxypregnan-3,11,20-trione).

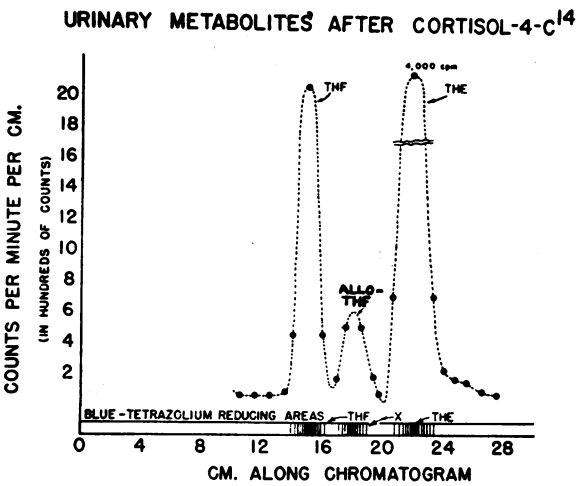

FIG. 1. THE DISTRIBUTION OF RADIOACTIVITY ON THE CHROMATOGRAM OF AN EXTRACT OF A URINE SAMPLE OBTAINED FROM A NORMAL INDIVIDUAL ADMINISTERED CORTISOL-4-C^{14}

The peaks of radioactivity correspond to the blue tetrazolium reducing steroid areas for both reference and urinary compounds. "X" is allo-THF.

Extraction procedure I. Thirty ml. of urine was adjusted to pH 5 with acetic acid and β-glucuronidase (Ketodase®) was added to a final concentration of 1,000 Fishman units per ml. The solution was incubated overnight at 47° C.

Extraction procedure II. Eight ml. or less of urine was treated as in Procedure I.

After incubation, the samples from either procedure were extracted with redistilled chloroform (3 × 20 ml.). The extracts were washed quickly with cold 0.5 N NaOH (2 × 5 ml.) and with distilled water (1 × 5 ml.). The combined aqueous washes were back-extracted with 15 ml. chloroform and the extract combined with the initial one. After drying with anhydrous Na_2SO_4 the chloroform solution was transferred to a 100 ml. round-bottom flask containing a 1 ml. well in its base. The chloroform was evaporated at 47° C. with the aid of a gentle stream of air and the sides of the flask were washed with chloroform to concentrate the residue in the well.

The residue obtained in Procedure I was made up to 0.5 ml. with ethanol and a suitable aliquot taken for paper chromatography. In Procedure II the entire residue was transferred to paper for chromatography.

Descending chromatography was a modification of one employed by Bush (32) and consisted of four volumes of toluene equilibrated with one volume of 75 per cent methanol in water. Chromatography was carried out at 32° C. using Whatman No. 1 filter paper. Although compounds THF and THE separated well in 6 hours, it was necessary to employ long "over-runs" of 16 to 39 hours to separate a third steroid, allo-THF, whose mobility was intermediate between THF and THE. The urinary allo-THF migrated with the same mobility as an authentic sample of the compound and after isolation of sufficient crystalline material from a urine pool, allo-THF was identified by its infrared spectrum.[4] This compound has recently been shown to be a normal component in human urine by Romanoff and co-workers (28) and Bush and Willoughby (29), and is apparently a metabolite of cortisol, as can be seen from Figure 1. Here the distribution of radioactivity was obtained by eluting, directly into planchets, 1 cm. strips from the chromatogram of an extract of a urine sample obtained from a normal individual administered 2 μc. of cortisol-4-C^{14} intravenously. Three peaks of radioactivity appear which correspond to the blue tetrazolium (BT[5]) reducing areas THF, allo-THF and THE.

Quantitative estimation of chromatographically separated steroids was achieved by spraying the chromatogram with a mixture of equal volumes of 0.2 per cent aqueous BT and 10 per cent sodium hydroxide. After remaining at room temperature for 10 minutes to assure full color development, the chromatogram was washed with tap water for 10 minutes and allowed to dry at room temperature. Blanks, standards and unknowns of equivalent areas were excised, cut into approximately one-fourth inch squares and shaken in closed vials for one hour with 1 to 3 ml. of the eluting agent, ethyl acetate: pyridine (1:4 by volume). Absorbances were read at 580 mμ in the Beckman spectrophotometer using microcuvettes.

Validation of analytical techniques

Mixtures of from 2 to 30 μg. each of THF, THE, F and E were chromatographed and estimated by the BT technique. The mean absorbances per μg. of steroid at 580 mμ plus or minus one standard error were, respectively, 0.036 ± 0.001; 0.038 ± 0.001; 0.035 ± 0.001; and 0.036 ± 0.004. Hence, there is adherence to the Lambert-Beer Law.

To demonstrate the recovery of steroid which has been subjected to chromatography, six samples of 21.4 μg. THE each were chromatographed along with reference standards to locate the THE areas. The former areas were eluted with ethanol and one-fifth volumes were assayed for steroid content by the Porter-Silber reaction (33). A nonchromatographed standard of 4.82 μg. THE was similarly estimated. The recovery ranged from 93 to 98 per cent, with a mean of 95 per cent.

The efficiencies of extraction Procedures I and II were determined. For Procedure I, six samples of 37 ml. of unhydrolyzed urine and six samples of 37 ml. of unhydrolyzed urine plus a mixture of 50 μg. each of THF, THE, F and E were extracted. Six controls contained the steroids only and were not extracted. For Procedure

[4] A sample of crystalline allo-THF was graciously contributed by Prof. T. Reichstein of the University of Basle. Dr. H. Rosenkrantz of the Worcester Foundation for Experimental Biology, Shrewsbury, Mass., kindly performed the infrared analysis.

[5] Obtained from Dajac Laboratories, Division of Monomer-Polymer, Leominster, Mass.

II, eight samples of 5 ml. of hydrolyzed urine and eight samples of 5 ml. of hydrolyzed urine plus a mixture of 20 μg. each of THF, THE and F were extracted. Four control samples contained the steroids only and were not extracted. All the samples were chromatographed and quantitatively evaluated with BT. For Procedure I, the per cent recoveries plus or minus one standard error were as follows: THF, 61 ± 2; THE, 87 ± 2; F, 88 ± 3; and E, 97 ± 2. Using Procedure II, the per cent recoveries were: THF, 86 ± 3; THE, 98 ± 2; and F, 100 ± 1. The more exhaustive extraction of Procedure II was employed in all later experiments.

Where indicated, quantitative values are corrected to 100 per cent recovery on the basis of recovery experiments. Quantitative standards of 20 μg. each of THF, THE and F were included on each sheet prepared for chromatography. Values for allo-THF in the BT estimation were calculated on the assumption that the ab-

TABLE I

The effect of surgical procedures on the relative proportions of urinary THF, allo-THF and THE

Patient	Age	Sex	Operation		Preoperation days 3	2	1	Op.	Postoperation days 1	2	3	4	5	6	7
A. G.	46	F	Abdominal hysterectomy	THF*		3.0		22.2	7.5	6.8	6.0	6.8	6.1	2.0	3.1
				THE		5.6		8.8	4.5	6.0	4.0	10.1	9.6	4.0	6.0
				Sum†		8.6		31.0	12.0	12.8	10.0	16.9	15.7	6.0	9.1
				Ratio‡		0.54		2.5	1.7	1.1	1.5	0.67	0.64	0.50	0.52
S. H.	45	F	Right ovariectomy	THF			2.3			3.9§	3.1	1.7	2.9	1.6	3.8
				allo-THF			1.0			0.4	0.4	0.4	0.5	0.5	1.1
				THE			2.6			1.0	1.4	1.2	1.4	1.9	3.2
				Sum			5.9			5.3	4.9	3.3	4.8	4.0	8.1
				Ratio			0.88			3.9	2.2	1.4	2.1	0.84	1.2
M. K.	46	F	Abdominal hysterectomy	THF*	1.8	1.7	1.7	2.4		2.8	2.2		2.0	2.3	2.1
				THE	2.3	2.2	2.1	1.6		2.4	2.1		3.4	2.9	3.6
				Sum	4.1	3.9	3.8	4.0		5.2	4.3		5.4	5.2	5.7
				Ratio	0.78	0.77	0.81	1.5		1.2	1.0		0.59	0.79	0.58
E. P.	43	F	Abdominal hysterectomy	THF*		2.1	1.5	15.1	8.8	8.2	4.8	2.4	3.5	2.3	1.7
				THE		3.4	2.3	6.3	5.5	5.1	4.1	2.4	3.6	2.0	1.9
				Sum		5.5	3.8	21.4	14.3	13.3	8.9	4.8	7.1	4.3	3.6
				Ratio		0.62	0.65	2.4	1.6	1.6	1.2	1.0	0.97	1.2	0.90
M. W.	44	F	Total hysterectomy	THF	0.96	1.04	0.92	5.1	2.9	1.9	1.8	1.5	1.3	0.92	1.7
				allo-THF	0.14	0.11	0.24	1.0	0.40	0.41	0.52	0.17	0.19	0.27	0.52
				THE	0.97	1.0	0.71	2.0	1.4	0.85	1.4	1.1	1.2	1.2	1.5
				Sum	2.1	2.1	1.9	8.1	4.7	3.2	3.7	2.8	2.7	2.4	3.7
				Ratio	0.99	1.0	1.3	2.6	2.1	2.2	1.3	1.4	1.1	0.77	1.1
M. H.	54	F	Bilateral ovariectomy	THF		3.0	3.3	25.0	3.9	4.6	2.5	2.0	1.5	3.1	3.3
				allo-THF		1.8	1.9	8.0	1.6	2.2	1.4	0.4	0.2	0.7	0.7
				THE		4.6	4.8	8.0	1.6	3.8	4.4	3.0	1.6	3.3	3.5
				Sum		9.4	10.0	41.0	7.1	10.6	8.3	5.4	3.3	7.1	7.5
				Ratio		0.65	0.69	3.1	2.4	1.2	0.57	0.67	0.94	0.94	0.94
P. L.	29	M	Repair knee ligament	THF				7.4	2.4						
				allo-THF				0.5	0.1						
				THE				2.5	1.8						
				Sum				10.4	4.3						
				Ratio				3.0	1.3						
J. L.	45	M	Sub-total gastrectomy	THF	2.7	2.6	3.4	14.6	7.6	5.2	5.4				3.3
				allo-THF	2.1	1.4	1.6	7.6	4.8	2.8	2.1				1.2
				THE	4.1	3.0	3.7	7.3	4.4	3.1	3.5				3.0
				Sum	8.9	7.0	8.7	29.5	16.8	11.1	11.0				7.5
				Ratio	0.66	0.87	0.92	2.0	1.7	1.7	1.5				1.1
L. L.	59	M	Fractured humerus set	THF				17.8	8.4	8.1	4.0	4.2	3.9		
				allo-THF				6.3	3.1	3.6	2.1	2.2	2.0		
				THE				8.1	4.2	6.4	4.7	5.6	5.9		
				Sum				32.2	15.7	18.1	10.8	12.0	11.8		
				Ratio				2.2	2.0	1.3	0.85	0.75	0.66		

* THF and allo-THF are combined.
† Sum is THF plus allo-THF plus THE. Values are corrected for extraction losses.
‡ Ratio is THF:THE. Other values are in mg. per 24 hrs.
§ Two day urine pool.

TABLE II

The effect of a surgical procedure on the relative proportions of urinary THF, allo-THF and THE—Detailed analysis *

Time	Operation	THF	Allo-THF	THE	Sum	THF	Allo-THF	THE	THF:THE
			μg./hr.				*% of sum*		
Day 1		114	89	172	375	30	24	46	0.65
Day 2	Difficult intubation;								
7 A.M.–3:30 P.M.	oper. postponed	502	217	378	1,115	47	19	33	1.4
3:30– 5:30 P.M.		152	118	152	422	36	28	36	1.0
5:30– 9:00 P.M.		300	154	258	712	42	22	36	1.2
9:00–10:45 P.M.		140	48	153	341	41	15	44	0.92
10:45– 4:00 A.M.		164	94	157	415	39	22	38	1.0
4:00– 7:00 A.M.		84	42	96	222	38	18	44	0.88
Day 3									
7:00–11:00 A.M.		144	72	158	374	38	19	42	0.90
11:00–12:00 P.M.	Subtotal	96	58	132	286	34	20	46	0.72
12:00– 1:00 P.M.	gastrectomy			No urine samples available					
1:00– 3:30 P.M.		247	122	271	640	39	19	42	0.91
3:30– 9:00 P.M.		720	400	336	1,456	49	27	23	2.1
9:00–11:00 P.M.		2,400	1,100	506	4,006	60	27	13	4.8
11:00– 3:00 A.M.		758	447	500	1,705	44	26	29	1.5
3:00– 7:00 A.M.		385	202	241	827	47	24	29	1.6
Day 4		315	156	180	651	48	24	28	1.7
Day 5		218	117	128	463	47	25	28	1.7
Day 6		226	89	146	461	49	19	32	1.5
Day 9		136	48	126	310	43	15	41	1.1
Day 15		93	40	124	257	36	16	48	0.75

* This case is J.L., aged 45, of Table I.

sorption per μg. of this compound was identical with that of the standards, THF and THE. Consequently, quantitative values for allo-THF are considered to be "relative."

Experimental subjects

Effects of surgical stress were studied in six women undergoing gynecological procedures and in three men undergoing gastrointestinal surgery, bone fracture repair, and knee ligament surgery. Urines were collected at 24 hour or shorter intervals during the study, pre-, intra- and postoperatively. Catheters were used where necessary to insure urine collection.

Effects of ACTH were determined in twelve men, eight of whom were normal volunteers. Of the remaining four, three were being treated for gonadal dysfunction and one

TABLE III

The effect of adrenocorticotropin (ACTH) on the relative proportions of urinary THF, allo-THF and THE

		No ACTH					ACTH (25 I.U. 8 Hrs. I.V.)				
		mg./24 hrs.					*mg./24 hrs.*				
Volunteer		THF	Allo-THF	THE	THF:THE	Allo-THF	THF	Allo-THF	THE	THF:THE	Allo-THF
	yrs.					%					%
W. A.	25	2.4	*	7.4	0.32	*	18.8	*	21.4	0.88	*
R. P.	19	3.1	*	6.5	0.48	*	15.6	*	19.0	0.82	*
W. S.	22	2.5	*	4.1	0.61	*	12.3	*	12.6	0.98	*
J. E.	22	2.1	2.1	2.5	0.84	31† (16–45)	8.8	7.6	6.1	1.4	34† (25–42)
W. F.	21	3.4	2.0	5.7	0.61	18† (6–36)	30.2	20.4	24.9	1.2	27† (14–41)
Z.	22	1.2	1.7	2.4	0.50	31† (21–43)	7.0	9.8	7.3	0.96	41† (26–50)
W. L.	21	2.7	1.3	3.6	0.75	17† (9–26)	17.2	6.5	10.8	1.6	19† (7–29)
A. V.	63‡	0.54	*	0.61	0.81	*	5.1	*	2.6	2.0	*
R. C.	66‡	3.6	*	5.5	0.65	*	18.5	*	13.5	1.4	*
F. W.	75§	0.69	*	0.39	1.7	*	5.0	*	1.8	2.8	*
P. G.	65‡	7.0	*	6.9	1.0	*	15.7	*	11.7	1.3	*
H. M.	50	2.2	0.76	2.0	1.1	15	7.6	2.7	4.9	1.6	18

* Allo-THF was not separated from THF.

† Urine samples were collected at four hour intervals. In these instances the mean value as well as the range are presented for the "allo-THF."

‡ Treated for gonadal dysfunction.

§ Cancer present.

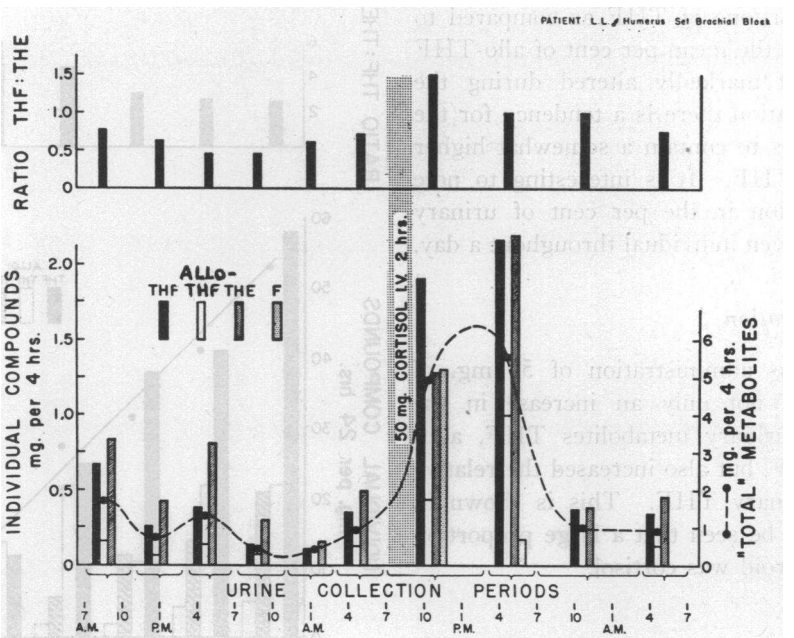

FIG. 2. THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF 50 MG. OF CORTISOL UPON THE URINARY CORTISOL METABOLITES OF PATIENT L.L.

Note the large proportion of cortisol that appears in the urine during the infusion period.

had cancer of the prostate. Urines were collected 24 hours prior to, during and 24 hours after ACTH administration. Twenty-five I.U. of ACTH was infused intravenously over eight hours.

Effects of cortisol were noted after the infusion of 50 mg. of cortisol over two hours to a man convalescing from a fractured humerus. Urine was collected periodically during the 24 hours preceding the infusion and during the 24 hour period which began with the infusion of cortisol.

Effects of severe burn trauma were observed in four patients. Three of the cases were fatal. Twenty-four hour urine collections were used. Catheters were used where necessary to insure urine collection.

Effects of prolonged stress were observed in a 50 year old man admitted with acute ulcerative colitis, who developed a pulmonary embolism, had a ligation of his inferior vena cava, developed an empyema and finally underwent a total colectomy. The effect of an ACTH infusion was observed both during the long period of illness and after successful convalescence. Urine samples were collected at 24 hour intervals.

RESULTS

Surgical "stress"

Table I expresses the daily excretion of the individual steroid metabolites, their sum, and the ratio THF: THE during the surgical procedures. In several instances where allo-THF was not separated from THF, the THF value includes allo-THF. It is apparent that the complex trauma of surgery is accompanied not only by elevations in the excretion of "total" urinary corticoids but also by increases in the relative proportions of THF: THE. In the instances in which allo-THF was evaluated no such clearly discernible pattern of variation with surgery was noted. There was, however, a consistent elevation in the rate of excretion of allo-THF during or shortly after the period of the "trauma."

A *detailed* analysis of the urinary corticoids from a patient undergoing surgery is presented in Table II. It illustrates the time relationship for the increase in the individual urinary metabolites both after the "difficult intubation" and after the gastrectomy. The increase in the proportion of THF: THE with the surgical procedures is clear, but a similar corelation is not observed between the relative per cent of allo-THF and these procedures.

Adrenocorticotropin

In all the cases presented in Table III the elevation of urinary cortisol metabolites after ACTH infusion was also accompanied by an increase in

the relative proportions of THF as compared to THE.[6] Although the mean per cent of allo-THF excreted was not markedly altered during the ACTH administration there is a tendency for the post-ACTH urines to contain a somewhat higher per cent of allo-THF. It is interesting to note the wide fluctuation in the per cent of urinary allo-THF for a given individual throughout a day.

Cortisol administration

The intravenous administration of 50 mg. of cortisol produced not only an increase in the amounts of the urinary metabolites THF, allo-THF, THE and F, but also increased the relative proportion of urinary THF. This is shown in Figure 2. It can be seen that a large proportion of the urinary steroid was cortisol.

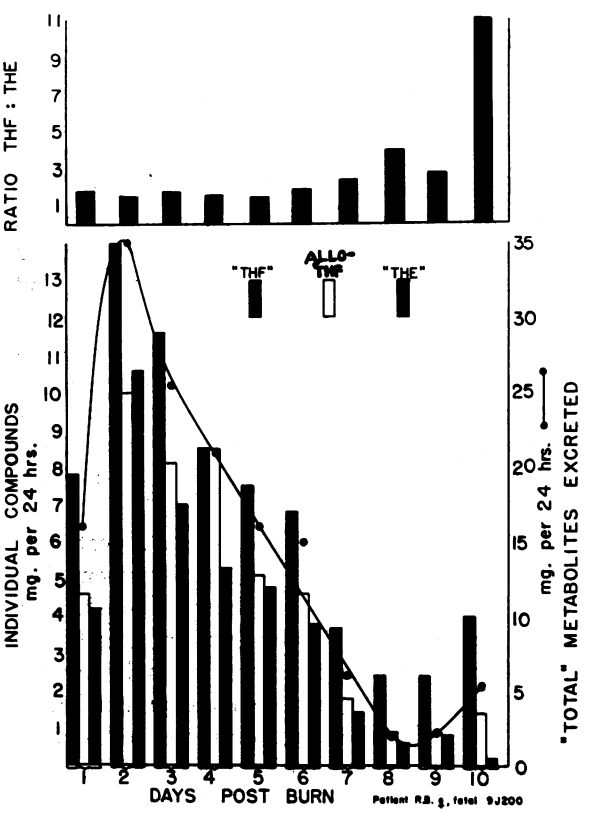

FIG. 3. URINARY CORTISOL METABOLITES IN A SEVERELY BURNED PATIENT, R.B., FEMALE, AGED 32 YEARS
The patient died on Day 11.

[6] A similar observation has been reported by Romani (34) in four subjects.

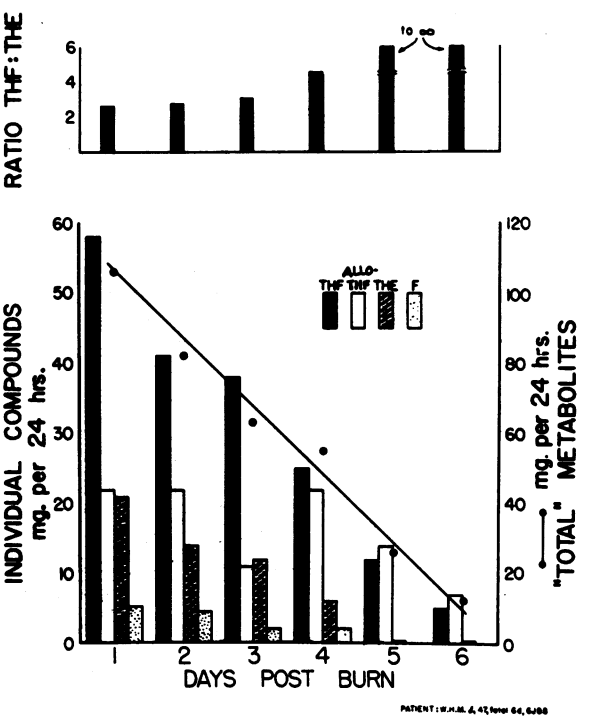

FIG. 4. URINARY CORTISOL METABOLITES IN A SEVERELY BURNED PATIENT, W.H.M., MALE, AGED 47 YEARS
The patient died on Day 7.

Severe burn trauma

The two fatal burns of Figures 3 and 4 indicate a high urinary titer of "total" metabolites at the period of initial trauma with a gradual decline to approximately normal values prior to death. All three metabolites, THF, allo-THF and THE, were excreted in greater than normal amounts. The huge excretion of "total" metabolites during Days one and two of Figure 4 suggests a very large secretion of cortisol by the adrenal.[7] This is consistent with the appearance of considerable amounts of cortisol "spilled over" into the urine on the first few days post burn. The ratio of THF : THE is elevated throughout and takes a sharp increase several days before death. The excretion of normal quantities of THF and allo-THF with markedly depressed excretions of THE suggests that the formation of THE from precursors is preferentially depressed.

In Figure 5 another fatal burn case is shown.

[7] Plasma 17-hydroxycorticoid values varied from 34 to 51 μg. F per 100 ml. plasma on the first two days post burn.

In this patient the ratio of THF: THE is considerably elevated through the seventh day post burn. By this day the excretion of THE has fallen to 1 mg. per 24 hours. On the eighth day infusions of cortisol were begun. There followed a rise in the amount of each urinary component *with the exception of THE.*

The last burn patient, shown in Figure 6, recovered. All three metabolites, THF, allo-THF (not shown in the figure) and THE, were elevated initially and gradually declined to normal values. The pattern of events was similar to that exhibited by the three fatal burns with the exceptions that the "total" metabolites excreted per 24 hours leveled off and the ratio of THF: THE returned to the normal value of 1.0 after having reached a maximum of 3.7.

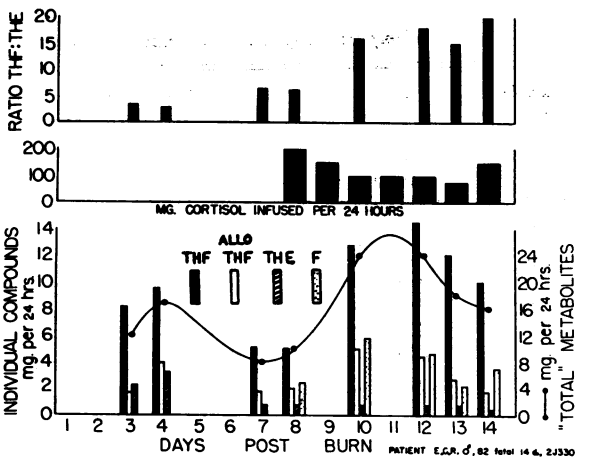

FIG. 5. URINARY CORTISOL METABOLITES IN A SEVERELY BURNED PATIENT, E.C.R., MALE, AGED 82 YEARS

The patient died on Day 15. Note that although large infusions of cortisol increased the excretion of THF, there is no increase in the quantity of THE excreted.

Prolonged stress

A quantitative study of the urinary metabolites from a patient severely ill for several months is shown in Table IV. It can be seen that: a) Allo-THF was not detectable on the chromatograms during the period of severe illness but was present in the convalescent period; b) the THF: THE ratio was elevated throughout the chronic stress period as compared to a "normal" day such as Sample 11; c) in Sample 6 during the period of surgical treatment the intravenous administration of 25 I.U. of ACTH produced a THF: THE ratio of 2.3, whereas in Sample 12, postconvalescence, the THF: THE ratio was only 1.4 after the same treatment while the "total" metabolites in each case were approximately the same; d)

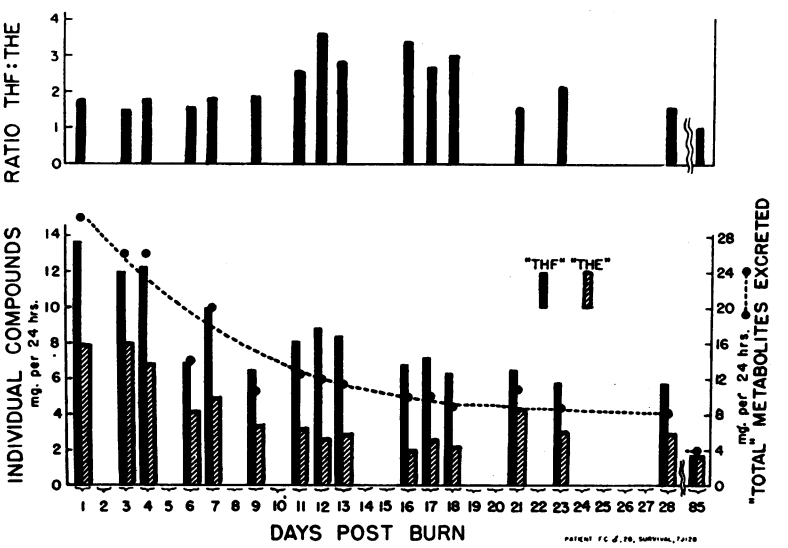

FIG. 6. URINARY CORTISOL METABOLITES IN A SEVERELY BURNED PATIENT, F.C., MALE, AGED 28 YEARS

The patient survived. Note the tendency for a further increase in the THF: THE ratio about two weeks post burn. Allo-THF, although present on the chromatogram, was omitted from the figure for purposes of clarity.

TABLE IV

Comparison of urinary metabolites during a prolonged illness with a postconvalescent period *

Sample	Date	Treatment	THF	Allo-THF†	THE	F	"Total"	THF:THE
				μg./24 hrs.			*mg./24 hrs.*	
	1956							
1.	9/10–9/11		3,660		1,890		5.6	1.9
2.	9/11–9/12		2,650		1,470		4.1	1.8
3.	9/12–9/13	Open drainage of empyema	3,670		2,260		5.9	1.6
4.	9/22–9/23		2,410		1,590		4.0	1.5
5.	9/23–9/24		3,010		2,580		5.6	1.2
6.	9/24–9/25	25 I.U. ACTH I.V.	11,600		5,100		16.7	2.3
7.	9/25–9/26		4,350		1,610		6.0	2.7
8.	9/26–9/27	Total colectomy; 200 mg. cortisol I.V.	38,300		5,600	7,180	51.1	6.8
9.	9/27–9/28	100 mg. cortisol I.V.; 25 I.U. ACTH I.V.; 40 I.U. ACTH gel I.M.	25,300		6,900	6,300	38.5	3.7
10.	10/15–10/16		3,450		1,650		5.1	2.1
Postconvalescence								
	1957							
11.	3/13–3/14		1,900	700	1,950		4.6	0.95
12.	3/14–3/15	25 I.U. ACTH I.V.	6,500	2,400	4,800		13.7	1.4
13.	3/15–3/16		2,550	1,100	2,300		6.0	1.1

* Patient H. M., male, 50 years, admitted 8/4/56 with acute ulcerative colitis, 8/8/56 developed pulmonary embolism, 8/9/56 ligation of inferior vena cava, 8/23/56 closed drainage of empyema.

† The absence of detectable quantities of allo-THF is not an artifact of the paper chromatography. Samples 8 and 12 analyzed individually and as mixed samples on the same chromatograms confirmed the "absence" of allo-THF.

during the period of prolonged stress the amount of "total" metabolites excreted in Samples 1, 2 and 4 were in the same range as in Samples 11 and 13, yet the THF: THE ratio was elevated in the stress period; e) although the excretion of "total" metabolites in Sample 8 was three times that of Sample 6 the excretion of THE was approximately the same in each sample.

DISCUSSION

It must be emphasized that the use of β-glucuronidase limits the quantitative estimation of the cortisol metabolites to the estimation of those metabolites which are excreted as glucuronides. Consequently the possibility exists that a lowered excretion of any single cortisol metabolite may be the result of: a) conjugation with a compound other than glucuronic acid, b) a decrease in the rate of filtration of the specific glucuronide by the kidney, as well as c) a depressed rate of conversion of cortisol to its metabolites.

It is clear that trauma (surgery, bone damage, burns) capable of eliciting an increase in output of urinary cortisol metabolites can also cause an increase in the per cent of urinary THF as compared to THE.[8] The greater increase in the amount of THF than THE after the administration of ACTH or cortisol suggests that this phenomenon may be directly related to the rate of cortisol secretion by the adrenal rather than to an alteration of factors concerned with the metabolism of cortisol.

However, the work of others (10–13) indicates that the ability to catabolize cortisol is reduced in surgery and that this reduction in activity involves the liver. It is conceivable then that the change in the relative quantities of THF and THE in the urine during trauma may be the result of *both* an increased adrenal secretion as well as an alteration in "metabolism" by the liver or other tissues.

In the series of burn cases it appears that there was an impairment in the conversion of cortisol to THE. Immediately post burn the THF: THE ratio was elevated above normal levels, but was in the same range as that of the surgically stressed individuals. However, in each of the four burn patients there was a further increase in this ratio after several days. In Figures 3 and 4 the largest

[8] Hemorrhage to the extent of 12 per cent of the total blood volume in 20 minutes did not elicit such a response in normal individuals.

rise in the THF: THE ratio occurred shortly before death, when the total metabolites had declined to approximately normal values. The severe depression in the relative amounts of THE excreted during the last two days prior to death of the patient of Figure 4 is strongly indicative of a preferential suppression of the reaction involving the oxidation of the hydroxyl group at position 11 to an 11-keto function. These findings tend to support the conclusions of Sandberg, Eik-Nes, Migeon, and Samuels (35) that there is an impaired metabolism of cortisol in dying patients.

That the oxidation of the 11-hydroxyl group may be inhibited is further supported by the data of Figure 5. It can be seen that by the seventh day post burn the quantity of THE excreted fell to 1 mg. per 24 hours. The infusion of large quantities of cortisol did not increase the amount of THE excreted although the amounts of THF and allo-THF rose markedly. This suggests that, although the mechanisms for the reduction and conjugation of ring A of cortisol are still active, some "physiological lesion" severely limits the conversion of cortisol to THE. The elevations of the THF: THE ratio on Day 10 of Figure 3 and the second and third weeks in the survival burn patient of Figure 6 may also represent a tendency toward the development of the same type of "physiological lesion."

An interesting illustration of altered cortisol metabolism is afforded by the patient of Table IV. This individual differed greatly from the "surgical" and "burn" patients in that his "stress," consisting of ulcerative colitis and pulmonary embolism with complications, was very prolonged, whereas the former types of stress were essentially acute. As shown in Table IV, there is an absence of detectable quantities of allo-THF during the period of extended stress and its appearance post convalescence is most striking. Although the possibility exists that allo-THF may not have been conjugated with glucuronic acid during the stress period and thus may not have been detected, such a possibility seems unlikely in view of the abundance of glucuronide conjugation occurring during the stress period.

Another indication of altered catabolism of cortisol in this same patient (Table IV) is the elevated ratio of THF: THE throughout the stress period as compared to the postconvalescence period, even though the "total" urinary metabolites in the stress period are approximately equal to those of the postconvalescent period.

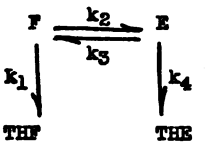

FIG. 7. SIMPLIFIED DIAGRAM OF ONE SEQUENCE IN THE CATABOLISM OF CORTISOL IN THE HUMAN (24)

Mechanisms for quantitative changes in urinary cortisol metabolites

A diagrammatic representation of several steps in the catabolism of cortisol, as shown in Figure 7, will help illustrate the experimental data. This scheme is based on the work of a number of investigators (17, 18, 36) and has recently been reviewed by Bush (24). The position of allo-THF in this scheme is not clear. It can be seen that a sudden increase in the secretion of F by the adrenal could lead to a transient increase in the relative quantity of THF formed if the resultant rates of Reactions k_2 and k_4 are slower than k_1 and k_3. In addition, it appears that either or both Reactions 2 and 4 are inhibited or that Reaction 3 is accelerated in complex trauma. Studies employing cortisol-4-C^{14} may help determine which of the kinetics of cortisol metabolism are altered.

It has been shown that there is a decreased rate of removal of cortisol from plasma in patients with cirrhosis (14, 16), in individuals undergoing surgery (10–12), and in dying patients (35). These effects have been attributed to altered liver function or to changes in hepatic flow. The role of the liver in producing the variations in urinary THF: THE ratios reported in this current investigation is not delineated.

The metabolites of cortisol which have been evaluated in this study represent only about 20 to 30 per cent of the total metabolites which appear in the urine (6, 16, 37). Another 30 per cent or more of urinary cortisol metabolites represented by the cortols and cortolones recently identified by Fukushima and co-workers (38) were not quantitatively evaluated in the current study. It may be that factors altering the kinetics of the conversion of cortisol to THF, allo-THF and THE may also involve the pathways concerned with the reduction of the C-20 keto group of cortisol.

SUMMARY

Several metabolites of cortisol were evaluated in the urine of subjects administered adrenocorticotropin and cortisol. Similar studies were made with patients undergoing such complex trauma as surgery or burns. The compounds evaluated were tetrahydrocortisol (3α,11β,17α,21-tetrahydroxypregnan-20-one), allo-tetrahydrocortisol (3α, 11β,17α,21-tetrahydroxyallopregnan-20-one), tetrahydrocortisone (3α,17α,21-trihydroxypregnan-11,20-dione), and cortisol (11β,17α,21-trihydroxypregnan-3,20-dione).

Observations showed an increase in both 1) the *rate of excretion* of each metabolite, tetrahydrocortisol, allo-tetrahydrocortisol, and tetrahydrocortisone, and 2) the *ratio of urinary tetrahydrocortisol to tetrahydrocortisone* in the following:

a) during and after *surgery,*
b) after the intravenous administration of 25 I.U. of *adrenocorticotropin* to normal subjects,
c) after the administration of 50 mg. of *cortisol,* intravenously, to one normal individual, and
d) in four patients with severe *burns.*

In three of the burn cases which were fatal the tetrahydrocortisol: tetrahydrocortisone ratio rose to extremely high values prior to death, suggesting the presence of factors which limit the conversion of cortisol to tetrahydrocortisone, *i.e.,* an inhibition of oxidation at the 11 position. There was no indication that the formation of allo-tetrahydrocortisol was grossly impaired. In one such patient the intravenous administration of large amounts of cortisol increased the daily excretion of tetrahydrocortisol and allo-tetrahydrocortisol but failed to increase the quantity of tetrahydrocortisone excreted.

The tetrahydrocortisol: tetrahydrocortisone ratio was elevated for an entire month in a chronically ill patient suffering from ulcerative colitis, pulmonary embolism, and empyema and having undergone major abdominal surgery. Allo-tetrahydrocortisol was not detected during this period. After five months' convalescence this patient showed a normal urinary pattern; allo-tetrahydrocortisol was present.

It is suggested that trauma in man can give rise to variations in the overall metabolism of cortisol.

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ADDENDUM

Gold and Moore demonstrated by *in vivo* isotope dilution procedures that THF and THE are not "interconvertible" in the normal subject (39). Details of these experiments are being prepared for publication.

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