17-HYDROXYCORTICOSTEROID METABOLISM IN LIVER DISEASE [1]

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Considerable evidence has been accumulated to indicate that the liver is concerned in the metabolism of steroids (1). The development of methods for the measurement of 17-hydroxycorticosteroids in the plasma (2) and in the urine (3) has enabled us to study the role of the liver in the handling of 17-hydroxycorticosterone (hydrocortisone) and related compounds. This communication will consider the plasma levels and the urinary excretion of the 17-hydroxycorticoids in patients with liver disease as compared with a group of normal subjects.

MATERIALS AND METHODS

Twelve patients with liver disease and eleven normal subjects were studied. Of the twelve patients with liver disease, there were nine with cirrhosis, two with hepatitis and one with metastatic carcinoma. Serial studies were carried out in the patients with hepatitis to note the effects of improvement in liver function. The "normal subjects" were hospital personnel or convalescing hospital patients who were free of liver disease, fever, and endocrine abnormalities.

These subjects were given intravenously a solution containing 0.02 per cent hydrocortisone (free alcohol), 5 per cent dextrose and 1 per cent alcohol. A sufficient quantity of this commercially-prepared solution [2] was administered to give the subject 1 mg. of hydrocortisone for each kilogram of body weight.

Control bloods were drawn shortly after 8 A.M., following which the infusion was administered over a 30-minute period. Blood samples for the estimation of the 17-hydroxycorticosteroid level of the plasma were drawn at 1, 2, 4, and 6 hours after the beginning of the infusion. After the infusion, the subjects were allowed to have breakfast and to be up and about, if they were ambulatory.

The plasma was separated within two hours and stored in a frozen state until the analysis could be carried out. The estimation of the plasma 17-hydroxycorticosteroid level was performed in accordance with the modified technique of Nelson, Samuels, and Eik-Nes (2, 4).

Urines were collected for 24-hour periods and stored in the refrigerator without a preservative. In several studies, the urine samples on the day of the hydrocortisone were divided into 2-hour collections for the first six hours and then into 6-hour periods. After the collection period, an aliquot was stored in the frozen state until subsequent analysis for creatinine (5) and for free and conjugated 17-hydroxycorticoids.

The 17-hydroxycorticoid level in the urine was measured by a modification (6) of the technique of Reddy, Jenkins, and Thorn (3). Chloroform prepared as described by Eik-Nes, Nelson, and Samuels (4) was used for the extraction of the unconjugated compounds from urine. The Porter-Silber reaction was carried out on this residue as described by Reddy, Jenkins, and Thorn (3) and the results were reported in terms of a cortisone standard.

The total urinary 17-hydroxycorticoids were determined on a butanol extract of urine as described by Reddy, Jenkins, and Thorn (3) except for the following modification.

Because of the high optical density values obtained in the reagent blanks involving butanol, the n-butyl alcohol was treated in the following manner: 700 ml. of n-butyl alcohol (Merck) was put in a 1-liter distilling flask and the pH was adjusted to 1–2 with 50 per cent H_2SO_4 (v./v.). About 50 to 60 mg. of phenylhydrazine, which had been twice recrystallized from absolute alcohol, was added and the mixture was allowed to react at 60° C. for thirty minutes and then distilled. The first 50 ml. of distillate was discarded and distillation of the solution was continued until only about 100 ml. of a dark brown residue remained in the distilling flask. The redistilled n-butyl alcohol was stored in a dark brown bottle in the refrigerator and was stable for at least two to three weeks. This procedure removes nearly all of the interfering chromogenic material from the butanol, as can be seen in Table I. The values for conjugated 17-hydroxycorticoid in urine were estimated by subtraction of the value for the free from the total 17-hydroxycorticoid value.

Liver function was evaluated in each subject by the usual liver function tests, but particular attention was paid to the sulfobromphthalein (BSP) retention at 45 minutes, using 5 mg. per Kg. of body weight (7).

In eight subjects with cirrhosis of the liver, 25 In-

[1] Part of this study was presented at the meeting of the American Society for Clinical Investigation at Atlantic City, N. J., May 4, 1954.

[2] Generously supplied by Merck and Company.

TABLE I

Effect of treatment of N-butyl alcohol on the optical density of reagent blanks *

	Tube "A"	Tube "B"	C.O.D. "A" − "B"	C.O.D. of reagent blank Range of 12 specimens
Untreated	.100	.040	.060	.055–.090
N-butyl alcohol	.115	.035	.080	
Treated	.015	.010	.005	.004–.010
N-butyl alcohol	.030	.020	.010	

* Tube "B" contained n-butyl alcohol plus H_2SO_4 (620 ml. concentrated H_2SO_4 diluted to one liter with distilled water). Tube "A" contained n-butyl alcohol plus the phenylhydrazine-H_2SO_4 mixture. The optical densities were read at 410λ on a Coleman Junior spectrophotometer. The optical density reading of Tube "A" minus the reading of Tube "B" gave a corrected optical density (C.O.D.) value, which was due to the phenylhydrazine and any reaction of the phenylhydrazine with products in the n-butyl alcohol which might cause the formation of chromogens. The results in two instances are tabulated. In the last column, the range is given for twelve specimens.

ternational Units (I.U.) of adrenocorticotrophic hormone were administered intravenously over a 6-hour period and the plasma levels of 17-hydroxycorticosteroids were measured at 2, 4, 6, 8, and 10 hours after the beginning of the infusion (8).

Two normal subjects and two patients with severe cirrhosis of the liver were given an infusion of 1 mg. per Kg. of tetrahydrocortisone. This material was first dissolved in a small volume of absolute alcohol (50 mg. per ml.), the solution being then added to 250 ml. of normal saline. This was administered intravenously over a 30-minute period and the bloods were drawn at 30, 45, 60, 90 and 120 minutes after the beginning of the infusion for the determination of the plasma 17-hydroxycorticosteroid level.

RESULTS

Plasma 17-hydroxycorticosteroid levels after the infusion of hydrocortisone

The plasma levels of 17-hydroxycorticosteroids after the infusion of hydrocortisone are shown in Table II. It is to be noted that the plasma levels of the 17-hydroxycorticosteroids were not significantly different in the patients with liver disease from those of the normal subjects prior to the infusion. However, in the patients with liver disease, the levels were significantly higher after the standard infusion. In Figure 1, the plasma level expressed logarithmically is plotted against time. In both groups of cases, the rate of removal was proportional to the concentration at any given time, but the 17-hydroxycorticosteroids disappeared more slowly in the liver disease group.

At one hour after the beginning of the infusion, less than 5 per cent of the infused steroid could be accounted for in the plasma by multiplying an estimated plasma volume by the 17-hydroxycorticosteroid plasma level. This was noted even when the infusion lasted 59 minutes.

Two patients with homologous serum hepatitis were given hydrocortisone infusions on two sepa-

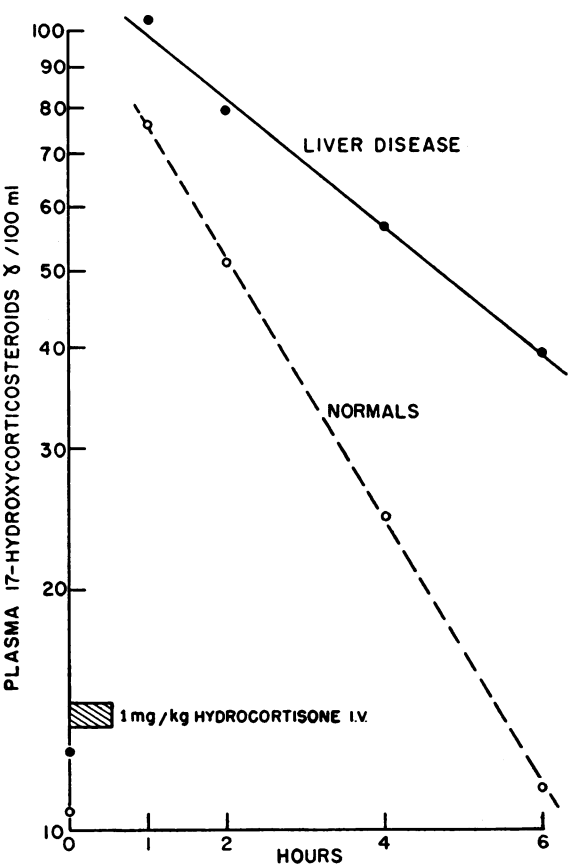

FIG. 1. THE MEAN 17-HYDROXYCORTICOSTEROID LEVELS IN THE PLASMA AFTER A STANDARD INTRAVENOUS INFUSION OF HYDROCORTISONE 1 MG. PER KG. ADMINISTERED OVER 30 MINUTES

The data are given in Table II.

TABLE II

Effect of hydrocortisone infusion on plasma 17-hydroxycorticosteroid levels

	Patient	Control	Hours 1	Hours 2	Hours 4	Hours 6	Bromsulphalein retention at 45 min.	Diagnosis
			γ/100 ml.				%	
Liver disease	Cor*†	14	81	61	38	21	26	Infectious hepatitis
	Mee‡	8	79	65	44	31	7	Cirrhosis
	Pur†	12	124	105	85	70	70	Homologous serum hepatitis
	Wad*	18	151	117	93	59	36	Cirrhosis
	Phi	11	103	99	68	56	48	Cirrhosis
	Jep*	15	127	91	86	60	32	Cirrhosis
	Sie	5	66	45	25	7	16	Cirrhosis
	Tur‡	12	110	82	53	42	16	Cirrhosis
	War§	17	81	46	41	37		Metastatic cancer of liver
	Mee‡	10	84	63	42	25	7	Cirrhosis
	Wad*	19	129	107	68	41	27	Cirrhosis
	Pur†	16	112	82	56	30	21	Homologous serum he patitis
	Qui	8	87	63	42	27	9	
	Phi	11	113	81	53	41	35	
	Mean	12.6	103.4	79.1	56.7	39.1		
	S.D.±	4.0	23.5	22.0	19.8	16.8		
	$S.D._m$±	1.1	6.5	6.1	5.5	4.7		
Normals	Sin‡	7	56	43	16	6	2	Normal
	Sim‡	9	64	45	23	11	3	Normal
	And‡	14	71	55	20	11	3	Normal
	Sha	15	56	48	23	12	5	Normal
	Car	8	100	50	28	16	3	Normal
	Neb	8	68	34	25	13	5	Normal
	Dun	14	75	57	25	9	3	Normal
	Mau	9	85	56	28	8	1	Normal
	Mar	10	81	65	28	11	3	Normal
	Hug	11	99	71	33	17	3	Normal
	Cor†	12	78	37	23	10	2	Convalescent hepatitis
	Mean	10.6	75.7	51.0	24.7	11.3		
	S.D.±‖	2.6	28.7	10.7	4.3	3.1		
	$S.D._m$±	.8	9.1	3.4	1.4	1.0		

* Infusion lasted almost 60 minutes.
†Patients with hepatitis studied on two occasions.
‡ Fifty mg. infusion.
§ Icterus index >100.
‖ S.D. Standard Deviation.
$S.D._m$ Standard Deviation of Mean.

rate occasions as their hepatitis improved. It can be seen that the rate of removal increased as the liver disease became less severe (Figure 2).

It should be noted that, in patient War (Table II), who had metastatic carcinoma of the liver, the rate of steroid removal was only moderately reduced, although the icterus index was greater than 100.

Several patients with advanced cirrhosis of the liver, as evidenced by clinical findings and biopsy, showed impairment of the mechanism for removing the 17-hydroxycorticosteroids from the plasma even though the BSP retention at 45 minutes was only slightly elevated. In the other cases, however, there was good correlation between the BSP retention at 45 minutes and the impaired rate of 17-hydroxycorticosteroid removal. This is illustrated in Figure 3 where the plasma level of the 17-hydroxycorticosteroids at four hours is plotted against the BSP retention at 45 minutes. The four-hour level was chosen because there was a maximum spread of values at this time. The coefficient of correlation is $+0.89 \pm .04$ ($p < .01$).

The plasma 17-hydroxycorticosteroid level after ACTH stimulation

The average plasma 17-hydroxycorticosteroid levels of eight patients with cirrhosis of the liver during a 6-hour intravenous infusion of 25 mg. of ACTH are compared in Table III with the

TABLE III

Effect of ACTH on plasma 17-hydroxycorticosteroid levels

		γ 17-hydroxycorticosteroid/100 ml. plasma						
	Control	2 hrs.	4 hrs.	6 hrs.	8 hrs.	10 hrs.	12 hrs.	24 hrs.
Cirrhosis	14.3 ± 2	32.4 ± 3	41.9 ± 4.4	45.0 ± 3.8	39.3	28.5	18.0	13.6
Normal (8)	10 ± .5	26 ± 1	36 ± 2	42 ± 2	—	—	—	—

TABLE IV

The urinary excretion of 17-hydroxycorticoids

	No. of subjects	No. of days	Free mg./day	Conjugated mg./day	% Conjugated
Normals	8	11	.16 (.08–.30)	5.3 (2.0–12.2)	97.2 (91.5–98.5)
Liver disease	9	15	.15 (.03–.51)	3.3 (.39–5.4)	95.7 (79.6–99.3)

levels in a group of normal subjects who received similar infusions.

It is apparent that there was no significant difference in the levels in the two groups during the 6-hour test. The ACTH infusions caused a mean rise in the plasma levels in the patients with cirrhosis of 30.7 μg, as compared with 32 μg in the normal group.

The plasma 17-hydroxycorticosteroid levels after the infusion of tetrahydrocortisone

The 17-hydroxycorticosteroid levels of the plasma after the intravenous infusion of tetrahydrocortisone, 1 mg. per Kg., are plotted in Figure 4. It is evident that this material was cleared much more rapidly than hydrocortisone, both in the normals and in the patients with liver disease. In the four patients studied, two with cirrhosis of the liver and two normal subjects, the levels had returned to the normal range in about 90 minutes. In one subject with biliary cirrhosis, who was being treated by means of biliary drainage, the rate of 17-hydroxycorticosteroid removal was more rapid than in the two normal subjects.

The urinary excretion of free and conjugated 17-hydroxycorticoids

The results of the daily urinary excretions of 17-hydroxycorticoids during the control period are summarized in Table IV. Although the urinary excretion of the free compound was the same in the two groups, the patients with liver disease excreted less 17-hydroxycorticoids in the conjugated form than did the normal subjects.

The increased excretion of total 17-hydroxycorticoids per 24 hours, occasioned by the infusion of hydrocortisone, in the normal subjects averaged 19.6 per cent (11.0 to 26.0 per cent) of the amount infused and 18.2 per cent (15.2 to 25.2 per cent) in the subjects with liver disease (as

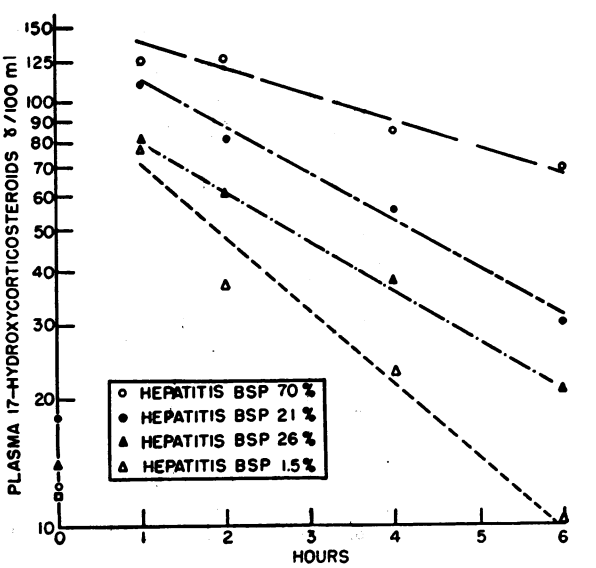

FIG. 2. THE PLASMA 17-HYDROXYCORTICOSTEROID LEVELS OF TWO PATIENTS WITH HEPATITIS AFTER THE STANDARD HYDROCORTISONE INFUSION

Two infusions were carried out on each patient as his hepatitis improved. The circles refer to patient Pur and the triangles to patient Cor (Table II).

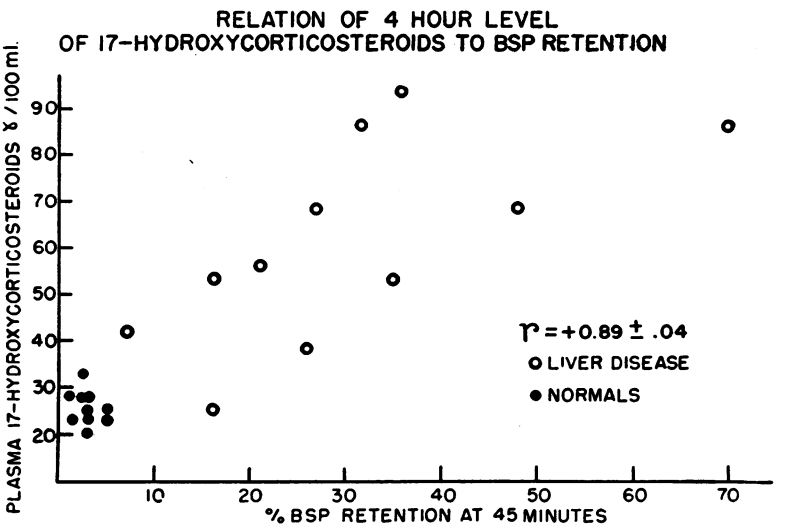

FIG. 3. THE RELATION OF BSP RETENTION AND THE FOUR-HOUR PLASMA 17-HYDROXYCORTICOSTEROID LEVEL AFTER THE STANDARD HYDROCORTISONE INFUSION

measured in milligrams of cortisone). These data are tabulated in Table V.

On the day of infusion, the patients with liver disease excreted larger amounts of the 17-hydroxycorticoids in the free form than the normal subjects. In two patients (one normal subject and one patient with hepatitis), urines were collected at 6-hour intervals on the day of the hydrocortisone infusion. In this study, the normal subject excreted practically all of the free steroid during the first 6-hour period whereas the subject with liver disease excreted a significant portion of

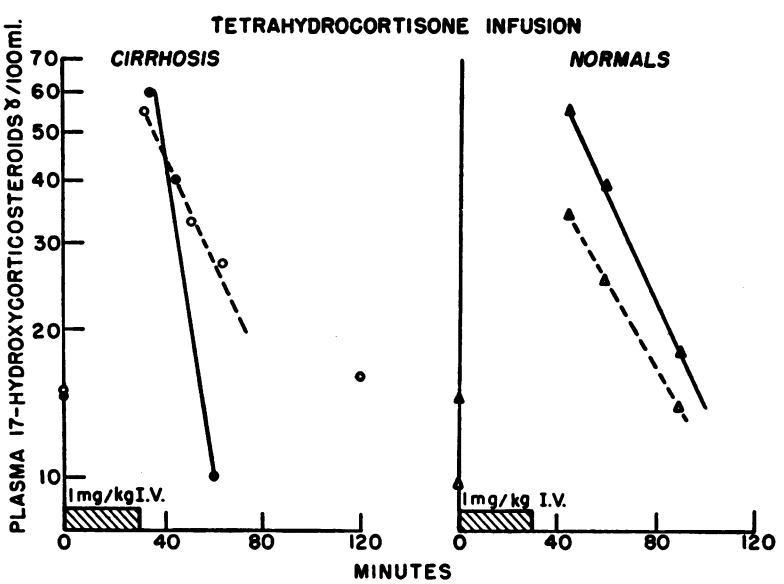

FIG. 4. THE PLASMA 17-HYDROXYCORTICOSTEROID LEVELS AFTER THE INTRAVENOUS INFUSION OF TETRAHYDROCORTISONE 1 MG. PER KG. OVER A 30-MINUTE PERIOD

The steeper curve on the left depicts a study in a female patient with biliary cirrhosis whose bile was being drained externally.

TABLE V

Urinary excretion of 17-hydroxycorticoids after hydrocortisone infusion

	Patient	Dose infused mg.	Excretion: Control day mg./day total 17-hydroxycorticoid	Excretion: Day of infusion mg./day total 17-hydroxycorticoid	Excretion: Increase mg./day total 17-hydroxycorticoid	% Dose excreted
Liver disease	Mee	50	4.4	13.8	9.4	18.8
	Tur	50	.9	13.9	13.0	26.0
	Pur	66	3.4	15.2	11.8	17.9
	Phi	59	3.0	9.5	6.5	11.0
	Jep	91	4.8	16.7	11.9	13.1
	War	81	3.3	21.5	18.2	22.5
	Pur	68	4.7	16.5	11.8	17.3
	Qui	60	1.9	11.0	9.1	15.1
	Cor	59	4.8	17.9	13.1	22.2
Mean			3.5 ± .4			18.2 ± 1.1
Normal	Neb	50	12.3	24.9	12.6	25.2
	Sha	50	9.5	19.2	9.7	19.4
	Car	89	2.8	21.5	18.7	21.0
	Mar	87	5.5	21.5	16.0	18.4
	Dun	61	3.7	15.4	11.7	19.2
	Mau	64	6.0	19.7	13.7	21.4
	Hug	51	2.1	11.1	9.0	17.6
	Cor	60	4.8	13.9	9.1	15.2
Mean			5.8 ± 1.2			19.6 ± 1.2

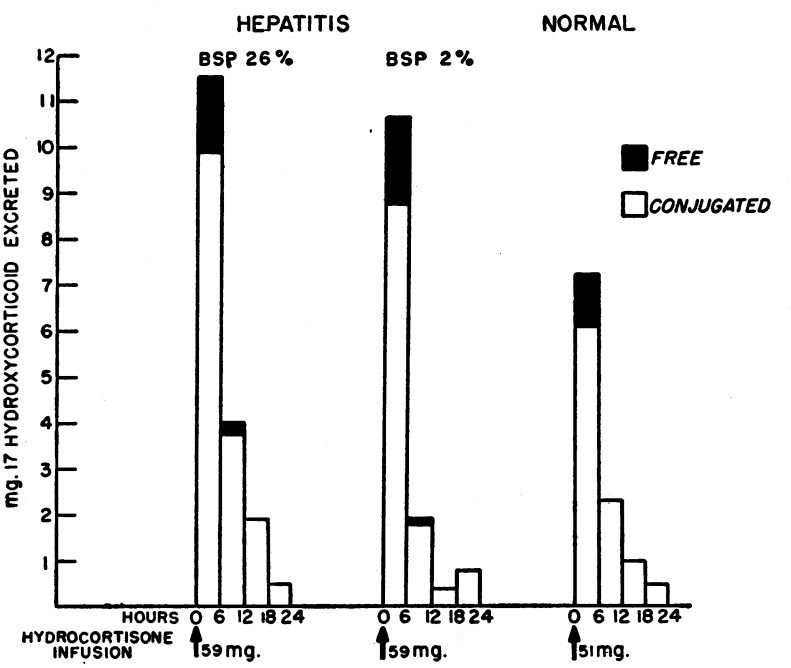

FIG. 5. THE URINARY 17-HYDROXYCORTICOID EXCRETION AFTER THE STANDARD HYDROCORTISONE INFUSION IN A PATIENT WITH HEPATITIS COMPARED WITH THAT IN A NORMAL SUBJECT

The patient with hepatitis was studied on two occasions to note the effect of improvement in liver function. As liver function improved, there was less free 17-hydroxycorticoid excreted during the second six-hour period.

free steroid during the second 6-hour period. This is illustrated in Figure 5.

In the four patients studied following the infusion of tetrahydrocortisone, 20 to 30 per cent of the infused steroid could be recovered, largely in the six hours following infusion.

DISCUSSION

The foregoing data demonstrate that there is a high degree of correlation between the rate of 17-hydroxycorticosterone (hydrocortisone) disappearance from the plasma and the clearance of sulfobromphthalein (BSP). These results suggest that the liver plays an important role in the catabolism of hydrocortisone as well as of other steroid hormones (1). Since there is evidence that 17-hydroxycorticosterone is the principal hormone secreted by the human adrenal cortex (2), it would appear that the circulating level of the adrenocortical hormones is regulated not only by the degree of stimulation of the adrenal cortex but by the functional state of the liver. In view of the fact that liver function is depressed during surgery (9) and other stresses (10–12), it is likely that the elevated plasma 17-hydroxycorticosteroid level observed under these circumstances may be ascribed in part to adrenal stimulation and in part to impaired hepatic function.

After the infusion of hydrocortisone, there was an initial rapid disappearance of the 17-hydroxycorticosteroids from the plasma. At 60 minutes after the beginning of the infusion, only about 5 per cent of the infused steroid could be accounted for by the 17-hydroxycorticosteroids in the plasma. After the initial period, in which the material was cleared very rapidly from the plasma both in the normal subjects and in the patients with liver disease, there was a period lasting many hours during which the 17-hydroxycorticosteroids disappeared at a slower rate. This, as we have pointed out, was proportional to the plasma concentration and related to the functional capacity of the liver.

It would seem, then, that the metabolism of exogenous hydrocortisone must involve at least three stages. First, there is a very rapid phase which removes more than 90 per cent of the material from the plasma in a matter of minutes; then, there is a phase in which the corticosteroids disappear in a logarithmic fashion. This phase is influenced by the function of the liver. Lastly, there must be another reaction which results in the material being excreted as a conjugate of tetrahydrocortisone.

The fate of the infused 17-hydroxycorticosteroid demands further study, but there are several possibilities. There is evidence that a portion is excreted into the small bowel *via* the liver and biliary tract (13). It is possible that some goes into the connective tissues (14). In addition, it should be noted that that portion in which the characteristic side chain has been modified or conjugated is not measured by our techniques. Recently, it has been shown that, in a variety of circumstances, tetrahydrocortisone is the principal 17,21-hydroxycorticoid in the urine (15). It is presumed that this metabolically inert compound is a major urinary excretion product of the adrenocortical hormone. Since we found that tetrahydrocortisone was cleared from the blood stream in both normal subjects and in patients with severe liver disease within one to two hours, it is unlikely that the elevated 17-hydroxycorticosteroid level which was present two hours after the infusion of hydrocortisone was due to tetrahydrocortisone.

The patients with liver disease excreted less 17-hydroxycorticoids (3.5 ± 0.4 mg.) per 24 hours than the normal subjects (5.5 ± 1.2 mg.). These results do not agree with those of other workers (16–20) who measured urinary corticoids by less specific methods. Although the ACTH-induced rise in the plasma 17-hydroxycorticosteroid level in the patients with liver disease was of the same magnitude as in the normal subjects, this implies a decreased adrenocortical secretion of 17-hydroxycorticosterone since its removal was impaired.

The major portion of the urinary 17-hydroxycorticoids was excreted in the conjugated fraction, probably as a glucuronide. In both normal subjects and in patients with liver disease, there was less than 5 per cent of the 17-hydroxycorticoids in the free form. However, on the day of the hydrocortisone infusion, there was an increase in the free steroid fraction excreted. This was more marked in the patients with liver disease than in the normal subjects. This increased amount probably was the result of the higher plasma levels

rather than because of saturation of the subjects' conjugating capacity.

It is well to emphasize that there are certain problems in relation to the determination of the absolute amounts of 17-hydroxycorticosteroids in the blood and urine. For the blood determinations, hydrocortisone was used as the standard of reference both for the hydrocortisone and tetrahydrocortisone infusions, and cortisone was used as the reference standard for the urine determinations since we have no information in regard to the relative proportions of cortisone, hydrocortisone and tetrahydrocortisone present under the conditions of the study. The intensity of color produced in the Porter-Silber reaction decreases with the above compounds in the order given (21).

This study again emphasizes the fine adjustment of the long-term homeostatic mechanisms (10) controlling the 17-hydroxycorticosteroid level of the plasma. Despite the markedly slower catabolism of hydrocortisone in the subjects with liver disease, the 8 A.M. levels were approximately the same in the normal subjects as in those with liver disease. Since the hormone is catabolized more slowly in the patients with liver disease, less secretion is needed to maintain the plasma level. This conclusion is supported by the observation that less hydroxycorticoid is excreted in the urine.

Finally, it should be pointed out that the metabolism of the 17-hydroxycorticosteroids at more physiological levels may not be quite the same as at the high levels induced in the present study.

SUMMARY

1. Twelve patients with liver disease and eleven normal subjects were given an infusion of 17-hydroxycorticosterone (hydrocortisone) intravenously and the plasma levels and urine excretions of the 17-hydroxycorticosteroids were measured. A similar study was made with tetrahydrocortisone.

2. The disappearance of the 17-hydroxycorticosteroids from the plasma was a first order reaction for both substances. The rate of disappearance of hydrocortisone was inversely proportional to the degree of liver damage as measured by BSP retention while tetrahydrocortisone disappeared at a much more rapid rate which was independent of liver function.

3. The urinary 17-hydroxycorticoid excretion on the day of hydrocortisone administration accounted for only about 20 per cent of the material administered in both the normal subjects and in the patients with liver disease. About 90 to 95 per cent of the urinary 17-hydroxycorticoids excreted were conjugated.

4. The rise in the plasma levels of 17-hydroxycorticosteroids in response to ACTH stimulation in patients with liver disease was normal.

5. The patients with liver disease had normal fasting levels of 17-hydroxycorticosteroids in the plasma but excreted less in the urine than did normal individuals.

6. The elevated 17-hydroxycorticosteroid level in stress must be considered as resulting both from increased adrenocortical stimulation and impaired removal by the liver.

7. Since patients with liver disease excrete less 17-hydroxycorticoids in the urine than do normal subjects despite the normal 8 A.M. plasma levels, there would seem to be an homeostatic mechanism which results in decreased adrenocortical secretion in the subjects with liver disease in whom the rate of 17-hydroxycorticosteroid removal from the plasma is impaired.

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