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Research Article

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Cortisol Metabolism in Cirrhosis *

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Abstract. **The production and peripheral metabolism of cortisol have been studied in 10 cirrhotics and 11 controls after i.v. tracers of cortisol-^{14}C. The findings were as follows: (a) Total urinary excretion of radioactivity was normal (81% of the dose) but a decreased fraction was present as glucosiduronates: 18–47% of the dose (average 34%) compared to a normal average of 54%. (b) There was a distinctively abnormal pattern of cortisol metabolites, not previously observed in other illnesses: tetrahydrocortisone was decreased to 14% of the enzyme hydrolysate (normal 26%); cortolones were increased to 34% (normal 19%), owing entirely to an increase in cortolone (20α) formation, since β-cortolone (20β) was not significantly increased; Reichstein's substances U and epi-U were increased, averaging 2.6% for the former and 4.7% for the latter; tetrahydrocortisol, allotetrahydrocortisol and cortols were normal. This pattern was independent of the degree of decreased glucosiduronate formation and also independent of the presence or absence of a portacaval shunt. (c) Cortisol production, determined by isotope dilution, was normal in each of six cirrhotic patients. From these data, taken in conjunction with our previously reported findings concerning the influence of norethandrolone on cortisol metabolism, the following conclusions were drawn: (a) Cirrhotic patients have decreased A-ring reduction of cortisone to tetrahydrocortisone and correspondingly increased 20-ketone reduction of cortisone to Reichstein's substances U and epi-U and then to the cortolones. (b) Intrahepatic cholestasis, a regular pathophysiological feature of cirrhosis, may be responsible for the observed abnormal cortisol metabolite pattern in this disease. (c) The slowed metabolic turnover rate of cortisol in cirrhosis may be due to decreased tranpsort and/or binding of cortisol to its intracellular metabolic sites rather than to abnormalities of any specific metabolizing enzymes.**

Introduction

Disturbances of cortisol metabolism in liver disease have been described by many investigators. It has been reported that formation of glucuronic acid conjugates of cortisol metabolites is decreased (2), that the removal rate of an infused cortisol load and the turnover rate of radioactive cortisol tracers are slowed (3), that cortisol production is abnormally low (3), and that the excretion of 17-hydroxycorticoids is decreased (3–5). In contrast, the removal rate of infused cortisone is normal (3). In the present study the formation of individual metabolites [1] from tracer amounts of cortisol-^{14}C has been measured in patients with cirrhosis and their cortisol production has been determined. An abnormality of the metabolite pattern, not previously observed in other illnesses, has

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TABLE I

Clinical data concerning the cirrhotic subjects

Patient	Age	Sex	Degree of illness [scale 0 → 5]*	Portacaval shunt
ET	64	M	1	No
WL	56	M	4	No
HN	44	F	4	No
AS	55	M	2	Yes
CY	41	M	3	No
HS	39	M	4	Yes
LE	60	F	3	Yes
MS	54	M	5	Yes
IN	49	M	1	No
BO	50	M	3	No

* 0, good health, 5, critically ill.

been found which appears to clarify some prior findings but raises further questions concerning cortisol metabolism in both cirrhotic patients and normal subjects. Contrary to a prior report (3), cortisol production was found to be normal in the cirrhotic patients.

Methods

Patients and procedures. 11 normal control subjects (9 men and 2 women, aged 21–35 [2]), 10 patients with Laennec's cirrhosis, and 3 noncirrhotic patients with miscellaneous illnesses were studied. All the cirrhotic subjects had typical clinical and laboratory findings as well as a long history of alcoholism. Four had an end-to-side portacaval shunt (Table 1). The quantitative pattern of urinary excretion and conjugation and the distribution of metabolites in the neutral extract were determined for all the cirrhotic subjects and normal controls. In addition, the cortisol production rate was determined for six of the cirrhotic patients and compared to that for the normal controls. The cortolone fractions from the extracts of eight of the cirrhotic patients' urines were separated into the α- and β-epimers and the amounts of these epimers were compared with the values for three of the normal controls' urines and the three noncirrhotic ill subjects' urines. The cortol fractions of five cirrhotic patients were similarly compared to those of five controls.

Tracers. Cortisol-4-^{14}C was obtained from the New England Nuclear Corp., Boston, Mass. The purity was checked by chromatography in system A, and the product was at least 97% radiochemically homogeneous. The hormone (1–5 μc) was dissolved in a small volume of alcohol, added to approximately 100 ml of 5% glucose solution, and a weighed amount of this solution was administered intravenously over 15–20 min, as previously described (8).

Urine collections and hydrolysis. Complete urine collections, judged from the constancy of the creatinine content, were made for 2 days after the intravenous administration. In the earliest studies samples were counted at or corrected to infinite thinness using a Tracerlab SC-50 automatic sample changer. In subsequent work, samples were counted in a Packard 3000 scintillation counter using the diotol scintillant described by Herberg (9). Quenching corrections were made from internal standards. The excretion of radioactivity is summarized in Table II. The 2 day urine collections were combined and enzymic hydrolysis of the steroid conjugates carried out as previously described (10). Briefly, the urine, buffered with acetate to pH 5.0, was incubated with 300 U of β-glucuronidase (Ketodase, Warner-Chilcott Laboratories, Morris Plains, N. J.) per ml of urine for 5 days at 38°C followed by continuous ether extraction for 48 hr. The extract was washed with aqueous alkali, with 9% sodium chloride solution, and with a small amount of water. The ether extract was dried and the solvent was removed to give the enzyme hydrolysate, which was assayed for radioactivity. The per cent hydrolysis is summarized in Table II.

Tracer analysis. Paper chromatographic analysis was carried out by the method previously described from this laboratory (8). Portions of the enzyme hydrolysate were chromatographed in system A for 24 hr. The papers

[1] The following trivial names have been used for steroid hormone metabolites.

6β-hydroxycortisol	=6β,11β,17,21-tetrahydroxy-Δ^4-pregnene-3,20-dione
dihydrocortisol (DHF)	=11β,17,21-trihydroxypregnane-3,20-dione
dihydrocortisone (DHE)	=17,21-dihydroxypregnane-3,11,20-trione
tetrahydrocortisol (THF)	=3α,11β,17,21-tetrahydroxypregnan-20-one
tetrahydrocortisone (THE)	=3β,17,21-trihydroxypregnane-11,20-dione
allotetrahydrocortisol (ATHF)	=3α,11β,17,21-tetrahydroxy-5α-pregnan-20-one
cortols	=cortol+β-cortol
cortol	=pregnane-3α,11β,17,20α,21-pentol
β-cortol	=pregnane-3α,11β,17,20β,21-pentol
cortolones	=cortolone+β-cortolone
cortolone	=3α,17,20α,21-tetrahydroxypregnan-11-one
β-cortolone	=3α,17,20β,21-tetrahydroxypregnan-11-one
20-reduced cortisols	=Reichstein's substance E+Reichstein's substance epi-E
Reichstein's substance E (R-E)	=11β,17,20β,21-tetrahydroxy-Δ^4-pregnen-3-one
Reichstein's substance epi-E (R-epi-E)	=11β,17,20α,21-tetrahydroxy-Δ^4-pregnen-3-one
20-reduced cortisones	=Reichstein's substance U+Reichstein's substance epi-U
Reichstein's substance U (R-U)	=17,20β,21-trihydroxy-Δ^4-pregnene-3,11-dione
Reichstein's substance epi-U (R-epi-U)	=17,20α,21-trihydroxy-Δ^4-pregnene-3-11-dione
11β-hydroxyetiocholanolone	=3α,11β-dihydroxyetiocholan-17-one
11-ketoetiocholanolone	=3α-hydroxyetiocholane-11,17-dione

[2] Studies by Romanoff and coworkers (6, 7) have shown that cortisol metabolism is independent of age and sex.

TABLE II

Recovery of radioactivity after cortisol-^{14}C

Cirrhotic patients	Per cent of administered radioactivity		Per cent hydrolysis of urinary conjugates	Per cent of enzyme hydrolysate						
	Urine	Enzyme hydrolysate		THF	ATHF	THE	Cortols	Cortolones	R-U	R-epi-U
ET	80	47	59	20	1	21	6	30	*	*
WL	84	25	30	10	13	16	3	38	*	*
HN	87	29	33	24	0	12	3	29	*	*
AS	67	34	51	13	4	20	4	38	*	*
CY	73	31	43	13	2	6	5	33	*	*
HS	86	24	28	19	7	9	11	29	2.7	4.0
LE	82	41	50	14	4	15	8	41	1.1	4.4
MS‡	73	18	25	7	2	7	5	37	7.3	11.0
IN	89	49	55	15	6	22	7	31	0.3	0.9
BO	89	43	49	16	8	16	9	35	1.6	3.3
Avg of 10 cirrhotic patients	81	34	42	15	5	14	6	34	—	—
Avg of 4 with portacaval shunts	77	29	38	13	4	13	8	36	—	—
Avg of 5 "poorer" glucosiduronate formers	81	25	31	15	5	10	5	33	—	—
Avg of 5 "better" glucosiduronate formers	81	43	53	15	5	18	7	35	—	—
Avg of 11 normal controls	78	54	68	17	8	26	6	19	§	§
(Range)	(70–95)	(43–66)	(61–88)	(14–20)	(5–15)	(18–36)	(3–11)	(13–23)		

* The earlier patients studied were not critically evaluated for these compounds.
‡ Enzyme hydrolysate also contained 6% of 20-reduced cortisols.
§ Not detectable in normal subjects.

were then scanned in a Vanguard 880 chromatogram scanner. The radioactive areas were identified by comparison with standards run simultaneously on side legs. In the subjects noted in Patients and Procedures the areas containing the cortols and those containing the cortolones were eluted and separately rechromatographed in system B. In this system, β-cortolone moves faster than cortolone and β-cortol moves faster than cortol (11). The epimers were eluted and counted. The chromatographic peaks corresponding to tetrahydrocortisol (THF), allotetrahydrocortisol (ATHF), tetrahydrocortisone (THE), cortols, and cortolones were further characterized by conversion to the corresponding 17-ketosteroids. The tetrahydro compounds were reduced with sodium borohydride and treated with periodic acid as described by Bush (12). Cortols and cortolones were oxidized with periodic acid. The products were chromatographed in system C. Only a single peak corresponding to the anticipated compound was seen, except with subject MS who was studied as follows.

Isolation of the epimers of 20-reduced cortisone from subject MS. The enzyme hydrolysate from this patient contained a large amount of radioactivity with the mobility in system A of allotetrahydrocortisol or a 20-reduced cortisone. This area, designated X, was eluted and further examined. A portion was acetylated and chromatographed on a thin layer of Silica Gel G, with benzene : ethyl acetate, 1 : 1. The plate was then scanned on a Vanguard 885 chromatoplate scanner. Two separated areas of radioactivity were found, one with the mobility of a 20-reduced cortisone diacetate and a second, less prominent, with the mobility of tetrahydrocortisol diacetate. Another portion of the eluate from area X was oxidized with sodium bismuthate as described by Bush (12), and the extract was chromatographed in paper system B. Two radioactive areas were identified by comparison with the mobility of standards. One was a large area with the same mobility as Δ^4-androstene-3,11,17-trione (derived from 20-reduced cortisones) and the other was smaller, with the mobility of 11-β-hydroxyetiocholanolone (derived from THF). Additional portions of the eluate from area X were separately added to 30.9 mg of unlabeled Reichstein's substance epi-U and to 37.5 mg of unlabeled Reichstein's substance U, respectively. The mixtures were acetylated and recrystallized to constant specific activity.

Identification of 20-reduced cortisols in subject MS. A portion of the eluate from the cortolones area (in system A) was oxidized with sodium bismuthate (12). The product was chromatographed in paper system B. Two spots with mobilities corresponding to 11-ketoetiocholanolone (derived from the cortolones) and 11β-hydroxy-Δ^4-androstene-3,17-dione (derived from 20-reduced cortisols) was obtained. These were eluted and the identity of each 17-ketosteroid was confirmed by addition of the appropriate carrier to portions of the eluate, followed

by recrystallization to constant specific activity. No attempt was made to fractionate the epimers of 20-reduced cortisol.

Isolation of the epimers of 20-reduced cortisone from subjects HS, LE, BO, and IN. In view of the findings in subject MS, the enzyme hydrolysates from subjects HS, LE, BO, and IN were reexamined to determine whether they contained 20-reduced cortisones. Since these compounds have somewhat variable chromatographic mobility which may correspond to that of ATHF or THF, the eluates from both these areas were examined. Portions of the eluates (from system A) were acetylated overnight with pyridine and acetic anhydride. The solvent was removed in vacuo, and the residue was chromatographed in system D for 8 hr. The strips were scanned in the usual manner. The ATHF eluates in all four subjects showed only a single peak which was ATHF. However, the THF eluates showed an additional radioactive peak, corresponding to the epimeric 20-reduced cortisone diacetates. All the radioactive areas were eluted with ethanol and assayed for radioactivity. THF content was determined by the Mader-Buck reaction (13). The 20-reduced cortisone diacetates were chromatographed on thin layers of Silica Gel G with ethyl acetate: cyclohexane, 9:1. The plates were developed twice, with brief drying in between, and scanned in the usual manner to locate the radioactive areas. The areas corresponding in mobility to Reichstein's substance epi-U and Reichstein's substance U were scraped off and eluted with acetone. The identifications were confirmed by dilution of each of the eluates with the corresponding carrier and recrystallization to constant specific activity. The expected compound constituted over 90% of the eluted radioactivity in all cases.

Paper chromatography. All samples were examined on 118 × 18 cm strips of Whatman No. 1 paper cut to give a center strip, 2–4 cm wide, and two side strips. The extract was placed on the center strip, with reference steroids and dye marker on the side strips, and was chromatographed until the dye marker was at the bottom of the paper strip. System A was benzene: methanol: ethyl acetate: water, 1:1:0.1:1, and the dye marker was isatin. System B was isooctane: methanol: toluene: 0.1 M borate buffer (pH 9.2), 7:6:35:20. Paper strips were dipped in buffer and allowed to dry before the samples were applied. System C was isooctane: methanol: toluene: water, 175:160:125:40.

Cortisol production rate. Cortisol production rate was determined by isotope dilution, using the method of Laumas, Tait, and Tait (14). Specific activities of both THE and THF were measured and agreed satisfactorily (Table III). The values are expressed as milligram per gram of creatinine. The urinary excretion of creatinine has been accepted as a good indirect measure of the mass of metabolically active tissue. Expressing cortisol production in this "normalized" manner greatly reduces the variation between individuals and eliminates the influences of age, body size, and sex.

TABLE III

Cortisol production in cirrhotic subjects

Subject	Cortisol production: Based on THE	Based on THF	Avg	Creatinine excretion
	mg/g creatinine			*g/day*
CY	15	15	15	1.03
IN	12	12	12	2.12
AS*	20	16	18	1.16
HS*	19	21	20	1.45
LE*	14	14	14	0.97
BO	16	16	16	0.85
Avg of all cirrhotics			16	
Avg of 3 with portacaval shunts			17	
Normal avg (Range)			15 (10–20)	

* Portacaval shunt present.

Results

Recovery of radioactivity

It is evident from Table II that the recovery of radioactivity in the urine was quite similar for cirrhotic patients and control subjects. On the other hand, the cirrhotic patients showed varying degrees of subnormal hydrolysis of the urinary steroid conjugates by β-glucuronidase. The extent of this disturbance was unrelated to the presence or absence of a portacaval shunt.

Individual metabolites (Table II)

Individual metabolites are expressed as a percentage of the enzyme hydrolysate. Implicit in this way of expressing the data is the assumption that the conversion to various metabolites is independent of subsequent conjugation, so that the percentage of each metabolite in the enzyme hydrolysate reflects the over-all bodily conversion to that metabolite. Strongly supporting this assumption was the finding that the abnormal metabolite pattern observed in cirrhotic patients was quite unrelated to the degree of disturbance in glucosiduronate formation. Although the "better" five cirrhotic patients formed nearly twice as much glucosiduronate as the "poorer" five (43% of the dose vs. 25%), there were no significant differences in the percentage of any metabolite in the enzyme hydrolysates of the two subgroups. The degree of abnormality of the pattern was likewise unrelated to the presence or absence of a portacaval shunt.

TABLE IV

Recovery of urinary cortolone and β-cortolone after cortisol-^{14}C

Subject	Per cent of enzyme hydrolysate			Cortolone / β-Cortolone
	Cortolone	β-Cortolone	Total cortolones	
Cirrhotic				
IN	20	11	31	2.0
BO	20	15	35	1.3
AS	26	13	39	2.0
MS	21	16	37	1.3
LE	30	11	41	2.9
HS	17	12	29	1.5
HN	17	12	29	1.4
ET	24	6	30	3.8
Avg	22	12	34	2.0
Noncirrhotic				
FA (normal)	11	9	20	1.2
SG (normal)	10	9	20	1.1
CI (normal)	12	10	22	1.2
MN (multiple sclerosis)	8	6		1.3
GG (amyotrophic lateral sclerosis)	9	7	16	1.3
LR (alopecia totalis)	10	10	20	1.0
Avg	10	9	39	1.2

THE. There was a decrease in formation of this metabolite in the cirrhotic patients, to a value about half that of the control subjects.

Cortolones. The cirrhotic patients showed an increase in cortolones to a value nearly double that of the control subjects. This increase in cortolones was almost precisely equal to the fall in THE. Thus, the sum of THE and cortolones constituted 47% of the extracts in cirrhotic patients and 44% in control subjects. In the six cirrhotic subjects studied appropriately it appeared that the increase in cortolones was almost entirely accounted for by increased cortolone; there was virtually no change in β-cortolone when compared with the control subjects (Table IV). Thus the ratio cortolone: β-cortolone was elevated in the cirrhotic subjects from a normal value of 1.2 to an average value of 2.0 (range 1.3–3.8).

THF, ATHF, and cortols. THF was present in essentially the same amounts in cirrhotic patients and controls. The average value for ATHF was decreased in the cirrhotic patients but the difference is difficult to interpret because of the small amounts of radioactivity present as this metabolite. Cortols were present in the same amounts in cirrhotic patients and controls; in five cirrhotic patients the ratio cortol: β-cortol ranged from 0.7 to 1.5, a range similar to that reported for normal subjects (8).

Reichstein's substances U and epi-U. Significant amounts of these two metabolites were found in the five cirrhotic patients who were critically evaluated (Table II) in contrast to its absence in six normal subjects and six patients with miscellaneous noncirrhotic illnesses similarly studied. In each of the five cirrhotic patients, the $\alpha:\beta$ ratio of the epimeric 20-reduced cortisones was similar to the $\alpha:\beta$ ratio of the epimeric cortolones, with a tendency for the former to be somewhat higher than the latter (Table V).

20-reduced cortisols. In subject MS these compounds, isolated as their common degradation product 11β-hydroxy-Δ^4-androstene-3,17-dione, accounted for 6% of the neutral steroid extract.

6β-hydroxycortisol. No significant amounts of this metabolite were detected.

TABLE V

Comparison of α:β-epimer ratios of 20-reduced metabolites in cirrhotic patients

Subject	Reichstein's epi-U / Reichstein's U	Cortolone / β-Cortolone
MS	1.5	1.3
LE	4.0	2.9
HS	1.5	1.5
BO	2.0	1.3
IN	2.9	2.0

Cortisol production

Cortisol production varied from low normal to high normal in the six cirrhotic subjects studied. The average value was essentially the same as that of control subjects (Table III).

Discussion

The present study has disclosed an abnormality in the peripheral metabolism of cortisol-^{14}C in cirrhosis, in that there was decreased conversion to THE, and an equivalent increase in conversion to cortolone, with virtually no change in β-cortolone. Total conversion to 11-keto metabolites (i.e. THE + cortolones) was normal. Total conversion to 11-hydroxy metabolites (i.e. THF + ATHF + cortols) was also normal, as was the conversion to each of the individual 11-hydroxy metabolites. These changes were independent of the presence or absence of a portacaval shunt. They were also independent of the extent of subnormal glucosiduronate formation, which was present to a greater or lesser degree in all the cirrhotic patients, as reported by Peterson and coworkers (2).

Cortisol production was found to be within the normal range in the cirrhotic subjects. This result differs from the finding of Peterson (3), who described low cortisol production in this disease. Vermeulen and Demeulenaere (5), who reported 30% less excretion of urinary 17-hydroxycorticoids in cirrhotic patients than normal, had also concluded that these patients had decreased cortisol production. However, this did not take into account the diminished quantity of urinary Porter-Silber chromogens relative to the actual cortisol production in cirrhotic patients. This results from the decreased quantity of cortisol metabolites

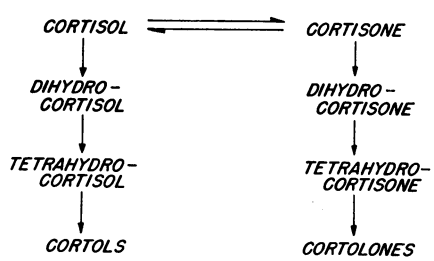

FIG. 1. CORTISOL PERIPHERAL METABOLISM (SCHEME 1). The oxidation-reduction system, cortisol ⇋ cortisone, which are converted to a sequence of metabolites by successive reductions, of the double bond, the ketone at the 3 position and the ketone at the 20 position.

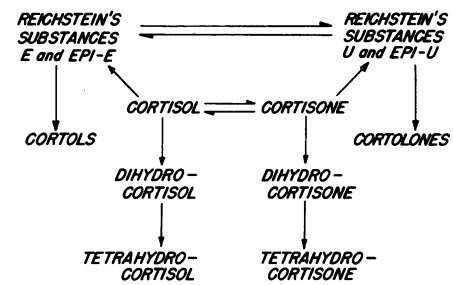

FIG. 2. CORTISOL PERIPHERAL METABOLISM (SCHEME 2). Both cortisone and cortisol can undergo alternative reductions. The double bond may be reduced first, yielding the dihydro compounds, and these are further reduced to the tetrahydro compounds. Alternatively, the 20-ketone can be reduced first. From cortisol, this yields Reichstein's substances E (20β-epimer) and epi-E (20α-epimer); from cortisone, Reichstein's substances U (20β-epimer) and epi-U (20α-epimer) are obtained. Subsequent reduction of the A-ring yields the cortols from substances E and epi-E, and the cortolones from substances U and epi-U.

recoverable after glucuronidase hydrolysis as well as the decreased amount of THF + ATHF + THE. For example, in the present study the cirrhotic subjects showed 60% less conversion of injected cortisol-^{14}C to the glucosiduronates of THF + THE + ATHF than was characteristic of normal subjects (Table II). In the patients reported by Vermeulen and Demeulenaere, transformation of exogenous cortisol and cortisone to 17-hydroxycorticoids was also decreased, by 23% and 21%, respectively. Since this was quite comparable to their decrease in endogenous corticoids, an appropriate inference would be that their endogenous production of cortisol was normal.

In attempting to account for the abnormal peripheral metabolism of cortisol in the cirrhotic subjects, it is helpful to try to reconcile the data with possible pathways of cortisol metabolism. Fig. 1 depicts one such scheme: the oxidation-reduction system, cortisol ⇌ cortisone, which are converted to a sequence of metabolites by successive reductions, of the double bond, the ketone at the 3 position and the ketone at the 20 position. According to this view, the present findings of decreased THE and equivalent increase of cortolone, and the reported normal removal rate of infused cortisone (3), would imply normal reduction of cortisone to THE and displacement of the reaction THE → cortolones towards cortolones. This explanation appears unsatisfactory for two reasons: (*a*) it has

recently been shown that the reaction THE → cortolones leads almost exclusively to β-cortolone,[3] and therefore, increased transformation by this pathway would not account for the observed increased formation of the α-oriented epimer, cortolone; and (*b*) it does not account for the findings by Kandrac, Keclik, and Jirasek (15) and this laboratory of increased amounts of Reichstein's substances U and epi-U in the urine of cirrhotic patients.

Another possible scheme is represented in Fig. 2. Here both cortisone and cortisol can undergo alternative reductions. The double bond may be reduced first, yielding the dihydro compounds (DHE or DHF) and these are further reduced to THE or THF. Alternatively, the 20-ketone can be reduced first. From cortisol this route yields Reichstein's substances E (20β-epimer) and epi-E (20α-epimer); from cortisone, Reichstein's substances U (20β-epimer) and epi-U (20α-epimer) are obtained. No information is available concerning the rates or extents of the reactions of oxidation-reduction between Reichstein's substances E and U, or epi-E and epi-U. The reaction Reichstein's E → Reichstein's U has been demonstrated (16), but not its reverse. Subsequent reduction of the A-ring yields the cortols from substances E and epi-E, and the cortolones from substances U and epi-U. Accordingly, the increased conversion to cortolones and depressed conversion to THE of the cirrhotic patients would represent a change in the relative prominence of the initial alternative reductive reactions of cortisone. That is, reduction to DHE is sluggish as a result of the cirrhotic process, and reduction of the 20-ketone assumes increased prominence in the disposition of the available substrate. The increased urinary excretion of Reichstein's substances U and epi-U in this view represents "spill-over" of intermediates in pathways operating at higher than normal capacity. The close agreement between the α:β ratio of the 20-reduced cortisones and that of the cortolones in 5 subjects provides support for this viewpoint. The slightly lower α:β ratio of the cortolones would be expected because of the small additional contribution of β-cortolone, but not cortolone, from THE.

In addition to providing possible explanations for the strikingly abnormal pattern of 11-keto metabolites, it is equally necessary to consider an important negative finding: the fact that conversion to the 11-hydroxy metabolites, individually and as a group, occurs in the normal proportions. This would not be remarkable if the over-all rate of disposition of cortisol were normal, but Peterson (3) has shown unequivocally that an infused load of cortisol is removed from plasma more slowly than normal by cirrhotic patients and that the turnover of injected cortisol-^{14}C is similarly slowed.[4] Six significant metabolic reactions lead from cortisol to its metabolites: (*a*) A-ring reduction in the 5α configuration; (*b*) A-ring reduction in the 5β-configuration; (*c*) 6β-hydroxylation; (*d*) oxidation of 11-hydroxy to 11-keto; (*e*) reduction of the 20-ketone to 20α-hydroxy; and (*f*) reduction of the 20-ketone to 20α-hydroxy. These steps lead respectively to the following urinary metabolites: (*a*) THF; (*b*) ATHF; (*c*) 6β-hydroxycortisol; (*d*) the 11-keto metabolites as a group (i.e. THE + cortolones); (*e*) cortol; and (*f*) β-cortol. Each of these six metabolites is present to an essentially normal proportion in cirrhosis—the small decrease in ATHF is of dubious analytic significance, and the virtual undetectability of 6β-hydroxycortisol is comparable to the finding in normal controls. If the rate of formation of one or more of these metabolites were less depressed than the others one would expect that an increased proportion of the less depressed metabolites would be formed, just as we have postulated that intact 20-ketone reduction of cortisone in the presence of decreased A-ring reduction causes an increased diversion of cortisone to the formation of cortolones. Since a comparable phenomenon is not observed among the 11-hydroxy metabolites, and since the over-all disposition of cortisol is depressed, one appears to be led inescapably to the conclusion that each of

[3] Zumoff, B., H. L. Bradlow, T. F. Gallagher, and L. Hellman. 1967. The metabolism of tetrahydrocortisone in man. Proceedings of the 49th Meeting of the Endocrine Society, June 1967, Bal Harbour, Fla. J. B. Lippincott Co., Philadelphia. 166.

[4] It should be noted that the simultaneous existence of normal cortisol production and slowed cortisol removal requires the conclusion that *mean* plasma cortisol concentration must be elevated. Tucci and Martin (17) have reported that in cirrhosis plasma cortisol concentration is normal in the morning but fails to undergo the normal diurnal decline in the evening. Thus, the evening concentration and the mean concentration are elevated.

the six metabolic steps leading from cortisol is depressed to the same extent.

It is difficult to visualize an explanation for this surprising conclusion. Probably, the most reasonable hypothesis is that there is a common step which precedes all these reactions. The process of transport and/or binding of cortisol to its intracellular metabolic site could certainly fulfill the role of such a "premetabolic" step, and it is suggested that this process is the step which is depressed in cirrhosis. This possibility is supported by the finding that the hepatic accumulation of thyroxine from plasma is depressed in cirrhosis (18).

This focuses attention on the anatomical changes in cirrhosis and their possible correlation with the steroid metabolic changes. The gross pathological findings in the liver, of necrosis, regeneration, and scarring are accompanied by finer structural changes involving: (*a*) decreased total blood flow with a higher than normal proportionate contribution from the hepatic artery and substantial intrahepatic portasystemic shunting; (*b*) abnormalities of the bile canalicular microvilli, the Golgi apparatus, the lysosomes, and portions of the smooth reticulum (collectively referred to as the bile secretory apparatus), constituting the syndrome of "intrahepatic cholestasis"; and (*c*) abnormalities of the remaining cellular organelles, i.e., the major part of the endoplasmic reticulum, the microsomes and the mitochondria. In view of the fact that there were no significant differences in cortisol production or metabolism between cirrhotic patients with and without surgical portacaval shunts, it seems that alterations of blood supply probably do not play a role in the observed changes. On the other hand, since administration of norethandrolone can produce the anatomical changes of intrahepatic cholestasis without the other intracellular changes of cirrhosis (19) and is also capable of reproducing the cortisol metabolic abnormalities of cirrhosis when given to patients with initially normal liver function and cortisol metabolism (1), it appears likely that the steroid abnormalities of cirrhosis are associated with its component of intrahepatic cholestasis.

The finding that the abnormal cortisol metabolite pattern in cirrhosis was the same regardless of the extent of disturbance in glucosiduronate conjugation of the metabolites implies that the cause of the former is independent of the cause of the latter. This conclusion is confirmed by the observation that the norethandrolone-treated subjects referred to above developed the abnormal metabolite pattern but not the abnormal glucosiduronate formation. Since the latter occurred to the same extent in cirrhotic patients with or without surgical portacaval shunts, it appears by exclusion that it may be related to the noncholestatic intracellular abnormalities of cirrhosis, and indeed it has been reported that glucosiduronate formation is localized to the smooth reticulum (20).

The present studies reemphasize the influence of liver disease on steroid metabolism. Although this study has not confirmed previous reports of low cortisol production in cirrhosis, specific changes in the peripheral metabolism of cortisol have been demonstrated with increased formation of certain metabolites and decreased formation of others. In light of evidence that steroid hormone metabolites can be biologically active, the finding of a shift in the relative amounts of the various cortisol metabolites in cirrhotic patients raises the possibility that the total biological activity of cortisol may be altered as a result. Other puzzling findings, such as the beneficial effect of liver disease on rheumatoid arthritis and the inability of patients with liver disease to excrete water loads normally, may be ultimately illuminated by this approach. The general applicability of this viewpoint is suggested by recent findings in this laboratory that equally significant abnormalities in estrogen metabolite pattern occur in cirrhosis [5] which may be related to the feminization which occurs in this disease.

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