Altered Pathways of Urinary Corticosteroid Excretion in Liver Disease Studied by a Clinical Laboratory Method

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A method of sequential extraction of \( \beta \)-glucuronidase-hydrolyzed urine, which permits the assessment of alternative pathways of corticosteroid excretion, was employed to study urine samples from normal subjects and from patients with hepatic cirrhosis. In patients with hepatic cirrhosis there is a decrease in the excretion of methylene dichloride-soluble 17-hydroxycorticosteroids; this decrease is due to significantly lower levels of tetrahydrocortisone, with moderately low levels of tetrahydrocortisol. A significant increase was observed in the levels of 17-OH,20,21-glycols, confined mainly to cortols in the urine of patients with cirrhosis; a less marked increase in this fraction was also observed in patients with fatty metamorphosis of the liver. Urinary 17-ketosteroids were decreased in patients with hepatic cirrhosis. The intravenous infusion of adrenocorticotropin to normal subjects was followed by increased 17-hydroxycorticosteroids in all solvent fractions of the urine. In patients with hepatic cirrhosis similarly treated, there was no significant rise in the level of 17-hydroxycorticosteroids extracted with methylene dichloride, but significant increases in polar 17-hydroxycorticosteroids were found.

The relative magnitudes of the alternate pathways of corticosteroid excretion were determined by calculation of the ratios of (1) total ketols to total glycols, (2) polar ketols to total glycols, and (3) less polar ketols to polar ketols; it was shown that in hepatic cirrhosis there are significant alterations in the excretory pathways of cortisol metabolites, with relative decreases in the excretion as tetrahydrocortisone and increases in the excretion as cortols. Based on these data it is clear that values of routine 17-hydroxycorticosterone and 17-ketosteroid excretion should be evaluated carefully as to whether they represent abnormal adrenal function or alterations in the peripheral metabolism of cortisol. The method is sufficiently simple to be employed in the clinical laboratory.

The standard methods for determination of urinary 17-hydroxycorticosteroids: 17-OHCS, Porter-Silber chromogens (1, 2), and 17-

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Presented at the 52nd Annual Meeting of the Federation of American Societies for Experimental Biology, Apr. 15-20, 1968, Atlantic City, N. J.

The author acknowledges the kindness of Dr. Eugene A. Clark for referring several of patients for these studies.

Received for publication May 31, 1968; accepted for publication July 23, 1968.
ketosteroids: 17-KS (3, 4) are extensively employed for assessment of adrenocortical activity. Such measurements are also employed to determine the reactivity of the pituitary adrenocortical axis after the use of metyrapone (SU 4885) or of the adrenal cortex after injection of adrenocorticotropin, with generally good accuracy. There are conditions however, in which the levels of these urinary metabolites are influenced by nonadrenal factors. This paper reports data on urinary corticosteroid metabolites in normal subjects and in patients with hepatocellular disease. It is shown that in patients with hepatic cirrhosis there are alterations in the major pathways of corticosteroid excretion—with decreased levels of 17-ketosteroids and 17-hydroxy corticosteroids, and increased levels of more polar compounds with the glycol side chain. Relatively simple methods are described which aid in identifying the altered pathways.

Materials and Methods

Terminology

The following abbreviations are used in this paper:

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\[ \text{Cortisol} = \Delta^1\text{pregnen,11\beta,17\alpha,21-triol,3,20-dione} \]
- 

\[ \text{Cortisone} = \Delta^1\text{pregnen,17\alpha,21-diol-3,11,20-trione} \]
- 

\[ \text{THF} = \text{tetrahydrocortisol} = 5\beta\text{-pregnan-3a,11\beta,17\alpha,21-tetrol,20-one} \]
- 

\[ \text{THE} = \text{tetrahydrocortisone} = 5\beta\text{-pregnan3a,17\alpha,21-triol,11,20-dione} \]
- 

\[ S = 11\text{-deoxy cortisol} = \Delta^1\text{pregnan-17\alpha,21-triol,3,20-dione} \]
- 

\[ \text{THS} = \text{tetrahydrodesoxycortisol} = 5\beta\text{-pregnan-3a,17\alpha,21-triol,20-one} \]
- 

\[ 6\beta\text{-hydroxycortisol} = \Delta^1\text{pregnen6\beta,11\beta,17\alpha,21-tetrol,20-one} \]
- 

\[ \text{Cortol} = 5\beta\text{-pregnan-3a,11\beta,17\alpha,20\alpha,21-pentol} \]
- 

\[ \text{Cortolone} = 5\beta\text{-pregnan-3a,17\alpha,20\alpha,21-tetrol,11-one} \]
- 

\[ \text{Metyrapone (SU 4885)} = 2\text{-methyl-1,2-di-3-pyridyl-1-propanone} \]
- 

\[ \text{PSC} = \text{Porter-Silber chromagens} = 17\text{-OH,20-21-ketols} \]
- 

\[ 17\text{-KGS} = 17\text{-ketogenic steroids} \]
- 

\[ 17\text{-OH,21-DOCS} = 17\text{-OH,21-deoxycorticosteroids} \]

Subjects

Twenty-four-hour urine samples were obtained from 20 normal subjects under care in private practice and in the hospital in-patient and clinic services. Urine specimens also were obtained from 7 patients with fatty metamorphosis of the liver, and from 10 patients with hepatic cirrhosis. All patients diagnosed as having fatty metamorphosis had the following significant clinical characteristics: (1) increased alcohol intake of several months’ duration, (2) clinically palpable enlarged liver, (3) increased Bromsulphalein (BSP) retention, and a liver scan consistent with fatty metamorphosis. In 3 of these patients the above parameters returned to normal after dietary management and avoidance of alcohol. The patients with hepatic cirrhosis were diagnosed by standard clinical methods, including increased BSP retention, de-
creased prothrombin activity, decreased serum albumin and α-globulin, and increased γ-globulin. Four patients of this group also had roentgenologic evidence of esophageal varices without any active bleeding during this study. In 3 patients needle biopsy of the liver revealed portal cirrhosis. Eight patients of this group showed moderate elevation in serum bilirubin levels.

The response to intravenous (I.V.) adrenocorticotropin (25 units aqueous adrenocorticotropin in 1000 ml saline I.V. over an 8-hr. period) was studied in 5 normal subjects and in 3 with cirrhosis. The response to I.V. metyrapone (SU4885, 30 mg/kg given I.V. in 500–1000 ml saline over a 4-hr. period) was studied in 3 normal subjects and in 3 with cirrhosis.

**Chemical Methods: Extraction**

The urinary corticosteroids were quantitated by the method of sequential extraction of β-glucuronidase-hydrolyzed urine (5). In the present study the adjustment of the enzyme-hydrolyzed urine to pH 1.0 was omitted. In the sequential extraction method, carbon tetrachloride, CCl₄ (Fraction A) extracts the relatively weakly polar corticosteroids, including THS and S (5–7) and 17-OH,21-DOCS (5–7), for the purposes of this study. Methylene dichloride (Fraction B) extracts cortisol, cortisone, THE, and THF from the residual urine (5); treatment of the residual urine (employing the salting out procedure, 20% of sodium sulfate added to the urine) with ethyl acetate (Fraction C) extracts the more polar corticosteroids including 6β-hydroxycortisol, 6β-hydroxycortisone, cortols, and cortolones (5).

**Analytic Methods**

The steroids in the various solvent fractions were quantitated by the method for 17-ketogenic steroids—borohydride reduction followed by bismuthate oxidation (4, 7, 8)—and by the method for PSC’s (1, 2, 5). The PSC’s in the CCl₄ extract are a measure of THS (and of small amounts of S) if present (9). The difference between the 17-KGS’s and PSC’s in Fraction A represents the levels of 17-OH,21-DOCS. The PSC’s in Fraction B are a measure of THF, THE, and of cortisol and cortisone, if present; the 17-KGS levels in this fraction had been found (5) to be identical to the PSC level and therefore were not determined in this study. The PSC’s in Fraction C represent the polar 17-OH,20,21-ketols, including 6β-hydroxycortisol and 6β-hydroxycortisone. The difference between the 17-KGS’s in Fraction C and the PSC’s represents the levels of 17-OH,20,21-glycols, as in cortols and cortolones.

Urinary 17-ketosteroids were determined by the method of Sobel
### Table 1. Corticosteroids in Solvent Fractions of Urine from Normal Subjects and Patients with Liver Disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of pt., &amp; sex</th>
<th>17-KGS</th>
<th>PSC</th>
<th>17-OH, #1-DOCS*</th>
<th>Ethyl acetate extract</th>
<th>Total urinary 17-KS</th>
<th>Total urinary estrogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (20)</td>
<td>15 ♀, 5 ♂</td>
<td>1.80 ± 0.25</td>
<td>0.08 ± 0.025</td>
<td>1.69 ± 0.22</td>
<td>4.27 ± 0.45</td>
<td>4.27 ± 0.44</td>
<td>2.06 ± 0.23</td>
</tr>
<tr>
<td>Cirrhosis (10)</td>
<td>4 ♀, 6 ♂</td>
<td>2.29 ± 0.34</td>
<td>0.06 ± 0.03</td>
<td>2.23 ± 0.31</td>
<td>0.83 ± 0.17</td>
<td>6.01 ± 0.78</td>
<td>1.41 ± 0.17</td>
</tr>
<tr>
<td>Fatty meta-morphosis (7)</td>
<td>2 ♀, 5 ♂</td>
<td>3.72 ± 0.57</td>
<td>0.13 ± 0.048</td>
<td>3.60 ± 0.56</td>
<td>2.63 ± 0.46</td>
<td>6.55 ± 0.79</td>
<td>2.67 ± 0.41</td>
</tr>
</tbody>
</table>

p values‡

<table>
<thead>
<tr>
<th>Normal vs.</th>
<th>Fatty meta-morphosis</th>
<th>&gt;0.05</th>
<th>&gt;0.05</th>
<th>&gt;0.05</th>
<th>&gt;0.05</th>
<th>&lt;0.05</th>
<th>&gt;0.05</th>
<th>&lt;0.01</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal vs. Cirrhosis</td>
<td></td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

All results are given in milligrams per 24 hr. except for the total urinary estrogens which are in micrograms per 24 hr.

Mean values are followed by standard errors; S.E. = \( \sqrt{\frac{1}{n(n-1)} \sum (x - \bar{x})^2} \)

Urine (22 ml.) was adjusted to pH 4.6 with acetate buffer and, after addition of β-glucuronidase (333 units/ml urine), was incubated at 38° for 16 hr. One aliquot was used for determination of total estrogens (10, 11). The remaining urine was extracted sequentially with carbon tetrachloride, methylene dichloride, and ethyl acetate (8). 17-KGS's by borohydrate reduction and bismuthate oxidation (8), and PSC's (1, 2), were determined on aliquots.

* 17-OH, 21-DOCS = (17 KGS - PSC) in CCl4 fraction (5, 6).
† Polar 20-OHCS or 17-OH, 20, 21-glycols = (17 KGS - PSC) in ethyl acetate fraction; significant amounts of these compounds are not found in CCl4 or in ethylene dichloride extracts.
‡ p values taken from table of t after t was calculated as follows: \( t = \bar{x}_1 - \bar{x}_2 \sqrt{\frac{n_1(n_1 + n_2 - 2)}{(n_1 + n_2) \sum x^2}} \) based on Snedecor (15).
et al. after hot acid hydrolysis (4). Urinary total estrogens were determined by the method of Eechaute and Demeester (10), as modified (11) by employing Sephadex G-15, and quantitating the estrogens fluorometrically.

In some studies individual compounds were identified after paper chromatography, sulfuric acid chromogen spectrums, and selective oxidation and reduction studies (5, 7, 12).

Results

Corticosteroids in Solvent Fractions of Urine from Normal Subjects and Patients with Liver Disease

The data presented in Table 1 reveal that the levels of 11-deoxy,17-OHCS (PSC's in Fraction A) in all urines studied were negligible. The 17-OH,21-DOCS levels are similar to normal values reported earlier (5) and are comparable for the three groups. The levels of PSC's in the methylene dichloride extract ("11 oxy",17-OHCS) are significantly lower in patients with hepatic cirrhosis. The levels of polar 17-OH,20,21-ketols (PSC's in the ethyl acetate extract) are somewhat lower than normal in the patients with cirrhosis. The levels of 17-OH,20,21-glycols are shown to be significantly elevated in the urine of patients with cirrhosis. Urinary 17-KS's are decreased in patients with cirrhosis. Total estrogens are somewhat higher than normal in patients with cirrhosis.

The data in Table 2 present the relative magnitudes of the excretory pathways. The total ketols represent the PSC levels in Fraction B and C; the total glycols represent the 17-OH,20,21-glycols (in Fraction C). It is noted that in patients with fatty metamorphosis there is a normal total ketol to total glycol ratio. In cirrhosis this is significantly lower than normal, approximately one-fourth the normal ratio. The polar ketols (i.e., PSC's in Fraction C) normally equal the total glycols. This ratio is altered in fatty metamorphosis, and is especially decreased in cirrhosis. The ratio of the less-polar 17-OHCS's (i.e., PSC's in Fraction B, representing THF and THE) to the polar 17-OHCS's is significantly and markedly decreased in the urine of patients with hepatic cirrhosis, and moderately decreased in patients with fatty metamorphosis of the liver. Three patients of the fatty metamorphosis group were studied several months after dietary management, at which time there was clinical improvement. The urinary corticosteroid patterns were normal at that time.

Identification studies revealed that the major decrease in PSC in the methylene dichloride extract involved THE, which was present only in trace amounts in the urine of cirrhotic patients. There was a smaller
Table 2. Relative Magnitudes of Excretory Pathways

<table>
<thead>
<tr>
<th>Diagnosis &amp; No. of pt.</th>
<th>Total ketols: Total glycols</th>
<th>Polar ketols: Total glycols</th>
<th>Less-polar ketols: Polar ketols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (20)</td>
<td>1.95 ± 0.024</td>
<td>1.25 ± 0.190</td>
<td>2.33 ± 0.300</td>
</tr>
<tr>
<td>Cirrhosis (10)</td>
<td>0.52 ± 0.061</td>
<td>0.35 ± 0.040</td>
<td>0.49 ± 0.095</td>
</tr>
<tr>
<td>Fatty metamorphosis (7)</td>
<td>1.71 ± 0.156</td>
<td>0.76 ± 0.056</td>
<td>0.99 ± 0.220</td>
</tr>
</tbody>
</table>

Statistical analysis

<table>
<thead>
<tr>
<th>Normal vs. Fatty metamorphosis</th>
<th>p &lt; 0.05</th>
<th>p &lt; 0.05</th>
<th>p &lt; 0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal vs. Cirrhosis</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Fatty metamorphosis vs. Cirrhosis</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &gt; 0.05</td>
</tr>
</tbody>
</table>

Ratios calculated from individual values on the various urine specimens. The figures following the ratios are the standard errors.

* Total ketols = PSC's in methylene dichloride extract plus PSC in ethyl acetate extract.
Total glycols = 17-OH, 20, 21-glycols = (17 KGS - PSC) in ethyl acetate fraction. Less-polar ketols = PSC's in methylene dichloride extract. Polar ketols = PSC's in ethyl acetate extract.

decrease in the excretion of THF. In the ethyl acetate fraction of normal urine there was an almost equal distribution of cortisol and cortolone; in this fraction in cirrhotic patients, the major component was cortisol, with only small amounts of cortolone.

It is clear from these data that in the presence of hepatic cirrhosis there are profound alterations in the pathway of corticosteroid excretion.

Urinary Corticosteroids Following ACTH Infusion

The data of Fig. 1 confirm the increase in corticosteroids in all fractions of the normal subject (§). It is noted that there is no significant elevation of the PSC in the methylene dichloride extract (11-oxy,17-OHCS) following the infusion of ACTH to the patients with cirrhosis studied here. Chromatographic studies revealed only trace amounts of free cortisol in the urine. The elevated levels of PSC in Fraction C (polar 17-OHCS) found in the baseline urine samples of the patients with cirrhosis are further elevated following the ACTH infusion. From this study it is clear that the increased cortisol secretion induced by ACTH is not followed by increased levels of THF or THE (i.e., of PSC's in the methylene dichloride fraction); the increased excretion as polar 17-OHCS represents an alternate excretory pathway.

Urinary Corticosteroids Following Infusion of Metyrapone (SU 4885)

In this study the adrenal 11-hydroxylase inhibitor metyrapone (SU 4885) was given I.V., and two postinfusion 24-hr. urine samples were obtained from each patient. The data of Fig. 2 reveal that in the normal
subjects, as well as in the patients with cirrhosis there were significant increases in the 17-OHCS in Fraction A (i.e., 11-deoxy,17-OHCS). The slightly elevated levels of 11-oxy-17-OHCS (PSC's in Fraction B) seen in the normal subjects are not found in the patients with cirrhosis. This

Fig. 1. Corticosteroids in solvent fractions of urine after ACTH infusion. PSC's in solvent fractions of β-glucuronidase-hydrolysed urine before (urine 0) and after I.V. infusion of 25 units adrenocorticotropic in 1000 ml normal saline over 8-hr. period. Urine 1 (24-hr. specimen) was collected from start of infusion. Urine 2 was collected for 24-hr. period following Urine 1. Dots connected by solid lines refer to normal subjects. Open circles connected by broken lines refer to cirrhotic patients.

study demonstrates that infusion of the 11β-hydroxylase inhibitor meyrapone induces increased levels of 11-deoxy-17-OHCS (THS) in both the normal subjects and the cirrhotic patients. Extraction of unhydrolyzed urine confirmed that less than 10% of this fraction was in the form of 11-desoxycortisol (S) in the patients with cirrhosis.

Identification studies revealed the bulk of the 11-deoxy-17-OHCS was in the form of THS. It is clear, then, that in the patient with hepatic cirrhosis there is no significant alteration in the excretory pathway for 11-desoxycortisol.
Fig. 2. Corticosteroids in solvent fractions of urine after metyrapone infusion. PSC's in solvent fractions of β-glucuronidase-hydrolyzed urine before (urine 0) and after i.v. infusion of metyrapone (30 mg/kg body weight) in 500-1000 ml normal saline over 4 hr. Urine 1 (24-hr. specimen) collected from start of infusion. Urine 2 was collected for 24-hr. period following Urine 1. Dots connected by solid lines refer to normal subjects. Open circles connected by broken lines refer to cirrhotic patients.

Fig. 3. Alternate pathways of cortisol excretion: percent distribution. Figures in parentheses represent the mean values in milligrams per 24 hr. 17-Ketosteroids are not included in calculating these percentages.
Discussion

Several parameters employed in evaluating the activity of the adrenal cortex are shown to be altered in patients with hepatic cirrhosis. It is shown that the 17-KS levels are decreased in patients with cirrhosis. The excretion of THF and THE (PSC's in the methylene dichloride fraction) is decreased in patients with cirrhosis even after the infusion of ACTH or with metyrapone. There was a more pronounced decrease in the levels of THE in the urine of cirrhotic patients. It is shown that there is a significant excretion of compounds which possess the 17-OH,20,21-glycol side chain, with the major component being cortols. These compounds usually are not extractable into the methylene dichloride employed in the routine methods used for 17-OHCS. It is also shown that the excretion of polar compounds possessing the 17-OH,20,21-ketol side chain (not extracted with methylene dichloride) exceeds that of relatively less-polar 17-OH,20,21-ketols (THE and THF) in patients with cirrhosis.

The above findings make it clear that in patients with hepatic cirrhosis there is a significant defect in the reduction of the Δ, 3-ketone group of cortisol, thereby leading to a decreased excretion of THF and THE. It is also evident, however, that the reduction and conjugation of 11-desoxycortisol are intact in patients with cirrhosis. Significant increases were noted in 17-OHCS in the CCl fraction after metyrapone infusion to normal subjects and to patients with hepatic cirrhosis.

The observations on the urinary 17-KS's might suggest that this pathway of corticosteroid excretion is also defective in cirrhotic individuals. The observations of urinary total estrogens demonstrate elevated excretion of these compounds in cirrhosis. This increase is moderate and is not comparable to the elevation found during pregnancy (14). It is known that the alterations in the corticosteroid excretion pathways during pregnancy are, at least in part, ascribed to increased levels of estrogens (15). A quantitative correlation between the moderate estrogen elevation and the markedly altered corticosteroid excretion pathway seems unlikely from our data thus far.

Presentation of the findings on the altered pathways of corticosteroid excretion in Fig. 3 and in Table 2 permits comparison with data obtained by other workers employing more elaborate methods. Ichikawa (16) made a comparison of the ratio between the 17-OH,20-21-ketols and the 17-OH,20,21-glycols in infectious and collagen diseases and in cirrhosis. While in the presence of febrile illness there was a relative increase in the 17-OH,20,21-glycols, there was no significant decrease in
the 17-OH,20,21-ketols as was found in our patients with hepatic cirrhosis. Similarly, our own unpublished observations reveal that in febrile illnesses and thyrotoxicosis there is a relative increase in the excretion of 17-OH,20,21-glycols—without significant decreases in the less-polar 17-OH,20,21-ketols. Zumoff et al. (17) studied the excretion pathways of cortisol-\textsuperscript{14}C in patients with cirrhosis. Their data showed that the ratio of the sum of THF, allo-THF, and THE (comparable to our less polar 17-OH,20,21-ketols) to the sum of the polar corticosteroids (cortols, cortolones; comparable to our total 17-OH,20,21-glycols) in the normal subject was 51/25 or 2.04; the value in patients with cirrhosis was 34/40 or 0.88. In the present study, the ratio of less polar 17-OH,20,21-ketols to total glycols in the normal subjects is 1.93, and in patients with cirrhosis 0.181. These data reveal very clearly that there are significant alterations in the excretory pathways of cortisol metabolites in cirrhosis. The findings in fatty metamorphosis represent an early derangement, which was found to be reversible in 3 patients in whom there was clinical improvement.

The mechanism of the development of alternative pathways of excretion of cortisol metabolites has been suggested to involve a decreased reduction of Ring A of the steroid nucleus due to a decrease in the production of TPNH (18), but it is not clear whether all instances of such alteration in corticosteroid degradation are due to this mechanism. It is clear from the findings that in cirrhosis there is a greater decrease in the tetrahydro end products of cortisol than in the polar 17-hydroxy-corticosteroids. It seems likely, then, that in cirrhosis the hydroxylases involved in the latter pathway are less affected than the \( \Delta^4 \), 3-ketoreductases involved in the former.

The findings presented in this paper demonstrate that this relatively simple method of fractional extraction of \( \beta \)-glucuronidase-hydrolyzed urine permits assessment of the alternate pathways of excretion. It is shown that the ratios of total ketols to total glycols, polar ketols to total glycols, and the less polar ketols to polar ketols all are altered significantly in cirrhotics. For practical purposes, the Porter-Silber reaction on the sequential Fractions B and C will give data which would suggest significant alteration in the alternate pathways of corticosteroid excretion. Based on these data, it is clear that the values obtained by means of routine 17-OHCS and 17-KS determinations should be evaluated carefully by the clinician as to their diagnostic significance. It is shown that the ACTH challenge in cirrhotic patients is not accompanied by a significant rise in the routinely determined 17-OHCS's, suggesting decreased adrenal responsiveness to ACTH. However, there is a significant rise in the polar 17-OHCS's indicating a normal adrenal
response. The urinary findings reflect altered corticosteroid excretion pathways.

References