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THE EXCRETION PATTERN OF 17-KETOSTEROIDS AND CORTICOSTEROIDS IN SURGICAL STRESS

By

G. Birke, C. Franksson and L.-O. Plantin

Surgical interventions undoubtedly involve stress-producing factors. Clinical and experimental studies have shown that such procedures entail increased adrenal activity (Selye, 1936, Reed, 1938, Selye et al., 1940, Forbes et al., 1947, Roche et al., 1950, Kinnunen, 1951, Franksson & Gemzell, 1953, and Franksson, Gemzell & v. Euler, 1954). Although these investigations demonstrated that the secretion of adrenal hormones is markedly intensified in association with operations, they gave no information of possible qualitative changes in adrenal function in such conditions of acute stress. We therefore considered it warrantable to investigate if, in addition to heightened adrenal activity, acute stress also brings about qualitative changes in the adrenal secretion or disturbances of steroid metabolism. Since satisfactory hydrolytic techniques have recently been evolved for the study of the urinary corticoids, we also thought it justifiable to investigate if the estimation of these steroids provides a better measure of adrenal activity in stress than do previously reported methods, if changes in the excretion of the various 17-ketosteroids and the corticoids are in any way correlated, and how long disturbances in the steroid pattern persist after operation. Further considerations were the changes in the steroid pattern in relation to the extent of the operation and the age of the patient.

PRESENT INVESTIGATION

METHODS

Determination of the different 17-ketosteroids (17-KS)

The urine was hydrolyzed with sulphuric acid at pH 0.4 and boiled for 25 minutes. A continuous separate extraction was then performed with ethyl-ether for 16 hours.
The crude extract was separated with Girard's reagent T. Colorimetric determination of the 17-KS was then performed, according to Callow's modification (1938) of Zimmermann's method (1935). Chromatography of the extracts was carried out according to the micromethod of Zygmuntowicz et al. (1951). To allow for identification of the different steroids with infrared spectrography, however, a modified method has been developed. This modification was discussed in detail in a previous paper (Plantin & Birke, 1954).

**Determination of reducing corticoids**

Sterile urine was obtained by boiling and the urine was subsequently adjusted to pH 5.0 with buffer solution. It was then incubated with β-glucuronidase from calf spleen for 42 hours at 37° C. (Cohen, 1951). Extraction was done with chloroform according to Sprechler (1950). Thereafter colorimetric determination of reducing corticoids was carried out with phosphomolybdic acid reagent on the ketonic fraction after separation with Girard's reagent T.

**Clinical Material**

18 patients were studied, 12 men whose ages ranged from 32 to 61 years and 6 women aged from 24 to 72 years. Ten of the patients underwent partial gastrectomy for duodenal or gastric ulcers; cholecystectomy was performed in four others; three underwent segmental bowel resection for cancer and one nephrolithotomy. In all cases the postoperative course was almost entirely uneventful. Shock, significant haemorrhage or embolism did not occur. The excretion of the various 17-KS was measured for three days prior to operation and postoperatively until the initial values were regained. In 10 cases the excretion of corticoids was studied in the same way.

**Results**

In 12 of the patients the preoperative total excretion of 17-KS was normal for the age and sex, according to the requirements stated by Hamburger (1948). The pathologically low values in the other patients were probably attributable to the fact that these patients had chronic, serious diseases, mainly cancer or long-standing severe ulceration, with markedly impaired nutrition.

The patients with preoperatively normal 17-KS excretion showed a clear, although not particularly substantial rise in the total excretion after operation (Table 1). The mean increase was 45 per cent, with a range of 9 to 170 per cent. In all but one (O. F.) of the patients with pathologically low 17-KS excretion prior to operation the postoperative increase was obviously either less or absent. The individual 17-KS in these latter patients showed insignificant changes, with no definite increase in any one steroid or any other change in the pattern. In the 14 patients with preoperatively normal and postoperatively raised 17-KS excretion (Table 1) there was a conspicuous rise in dehydroepiandrosterone excretion, but no comparable increase in androsterone and etiocholanolone. De-
Table 1.
The excretion of the main 17-ketosteroids and reducing corticoids before and after operation.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>17-KS mg./24 h.</td>
<td>17-KS mg./24 h.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Dehydroepiandrosterone</td>
</tr>
<tr>
<td>G. S.</td>
<td>M</td>
<td>32</td>
<td>8.7</td>
<td>0.8</td>
</tr>
<tr>
<td>D. T.</td>
<td>M</td>
<td>52</td>
<td>4.6</td>
<td>0.2</td>
</tr>
<tr>
<td>J. R.</td>
<td>F</td>
<td>58</td>
<td>3.0</td>
<td>0.4</td>
</tr>
<tr>
<td>M. T.</td>
<td>M</td>
<td>58</td>
<td>4.0</td>
<td>0.3</td>
</tr>
<tr>
<td>D. S.</td>
<td>M</td>
<td>50</td>
<td>5.0</td>
<td>0.6</td>
</tr>
<tr>
<td>F. W.</td>
<td>M</td>
<td>57</td>
<td>6.4</td>
<td>0.9</td>
</tr>
<tr>
<td>C. Z.</td>
<td>M</td>
<td>45</td>
<td>10.9</td>
<td>1.4</td>
</tr>
<tr>
<td>J. C.</td>
<td>M</td>
<td>45</td>
<td>10.8</td>
<td>0.8</td>
</tr>
<tr>
<td>S. U.</td>
<td>M</td>
<td>61</td>
<td>3.4</td>
<td>0.1</td>
</tr>
<tr>
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<td>M</td>
<td>34</td>
<td>10.9</td>
<td>0.7</td>
</tr>
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<td>F</td>
<td>32</td>
<td>8.9</td>
<td>1.8</td>
</tr>
<tr>
<td>M. A.</td>
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<td>5.8</td>
<td>0.5</td>
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<tr>
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<td>40</td>
<td>3.4</td>
<td>0.4</td>
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<td>F. V.</td>
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<td>47</td>
<td>1.7</td>
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</tr>
<tr>
<td>I. R.</td>
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<td>24</td>
<td>2.0</td>
<td>0.1</td>
</tr>
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<td>26</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>O. U.</td>
<td>M</td>
<td>55</td>
<td>4.0</td>
<td>0.3</td>
</tr>
<tr>
<td>V. A.</td>
<td>F</td>
<td>72</td>
<td>1.3</td>
<td>0.2</td>
</tr>
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</table>
Fig. 1.

Fig. 2.

Fig. 3.
hydroepiandrosterone increased in association with operation by 60 to 700 per cent, with a mean rise of 210 per cent. The corresponding figures for androsterone and etiocholanolone were only 0 to 190 per cent and 20 per cent. It was remarkable that the 11-oxy-metabolites showed no statistically verifiable increase immediately after surgery. Five patients, on the other hand, showed decreased excretion of 17-keto-11-oxy-steroid metabolites. Only one of the patients (C. Z.) with a pronounced rise in total 17-KS excretion even showed an insignificant increase in 17-keto-11-oxy-steroid metabolites.

Study of the steroid pattern after operation showed that the excretion of androsterone and etiocholanolone very rapidly regained preoperative levels, in all cases within three days. Figs. 1–3 represent typical examples. The conspicuous increase in dehydroepiandrosterone, which was most marked during the first few postoperative days, subsequently showed a rapid, successive decline, reaching preoperative values 7 to 9 days after the intervention. The excretion of 17-keto-11-oxy-metabolites, on the other hand, which in the first two postoperative days showed no rise but in some cases an insignificant decrease, later reached levels which in all cases were moderately but definitely higher than the preoperative values. These initial values were regained 9 to 11 days after operation.

The reducing corticoids, which were studied in 10 patients, showed varying degrees of increase. The preoperative excretion ranged from 3.4 to 16.3 mg. in 24 hours and rose by 53 to 390 per cent, with a mean rise of 130 per cent. Thus the response of the corticoids was greater than that of the total 17-KS excretion. But dehydroepiandrosterone increased more than the corticoids. It is necessary, however, to consider the limitations of the type of corticosteroids that are determined by the method chosen, i.e. only reducing ketonic corticosteroids are measured. Table 1 shows that the excretion of corticoids rose after operation even in the patients with no increased 17-KS. No definite correlation was observed between the rise in corticoid and in 17-KS excretion. On the contrary, the level of the former rose considerably in some cases in which there was only an insignificant increase in 17-KS. The reverse was also noted. As a rule the corticoids reached their preoperative values by the third to the fifth postoperative day.

**DISCUSSION**

The investigation was undertaken with a twofold purpose. The first object was to study the steroid metabolism in acute stress and the second to observe how the steroid metabolism in patients of different ages and with different basic diseases reacted to major surgical procedures. To ensure uniformity of evaluation, therefore, the clinical material was limited primarily to patients without
any serious postoperative complications. Comparison of the steroid metabolism in complicated and complication-free surgical cases must await later study.

The series comprised patients of different ages, but it is seen that in several of them the 17-KS values were pathologically low prior to operation, in all probability because these patients had malignant or severe ulcerative conditions which had given rise to gravely defective nutrition. The response of their 17-KS excretion to operation was very insignificant. Although some patients showed inappreciable increase in the total excretion, with slightly raised values of dehydroepiandrosterone, androsterone and etiocholanolone, the 17-keto-11-oxy-steroids did not increase. The fact that patients with initially low 17-KS excretion react poorly to acute stress has previously been reported (Forbes et al., 1947, Birke, 1954 a).

The impaired response of the 17-KS was not accompanied by a smaller increase in the excretion of corticoids, which could be initially high and also yet react satisfactorily. On the whole the excretion values of 17-KS and of corticoids did not appear to be correlated preoperatively in these patients. A high level of the former could be observed with low values of the latter, and vice versa. This demonstrates that the metabolism of these two groups of steroids may be different in the same subject. Whether or not this apparently defective correlation is also to be found in larger clinical series and in healthy subjects remains to be investigated. The patients with pathologically low 17-KS excretion prior to operation and insignificant or no postoperative increase showed no clinical signs of complications after surgery. Nor was there, in these cases, any change in the period required for the corticoids to regain their preoperative values.

Administration of ACTH results in an increase of all the 17-KS, as shown by Lieberman et al. (1950, 1951), Dobriner et al. (1950), Dingemanse (1950), Martii (1951) and Birke (1954 b). A moderate increase in dehydroepiandrosterone is sometimes observed, but this is by no means predominant. The 11-oxy-metabolites, on the other hand, comprise the greatest portion of the increase. These results were achieved by chromatographic separation and identification of the steroids with infrared spectrography. Ronzoni (1952), however, expressed the opinion that ACTH stimulation is followed by a marked increase in dehydroepiandrosterone; but his findings were based on determinations of chromogens reacting with Allen's blue test and this, according to Wolfson's (1953) latest communication, has proved to be an unsatisfactory method.

In acute stress ACTH is liberated and stimulates the adrenal secretion. It is therefore surprising that the metabolites of »Compound F« did not increase in our cases. In the patients with preoperatively normal 17-KS excretion the various steroids did not react uniformly to the operation. While dehydroepiandrostergone greatly increased, the rise in androsterone and etiocholanol-
The 17-keto-11-oxy-steroids rather showed a tendency to decrease during the period of acute stress. Similar changes in the 17-KS pattern are seen in febrile diseases such as rheumatic fever or pneumonia, but then the increase in dehydroepiandrosterone is even greater and persists for a considerably longer time. Since these changes are not the same as those produced by ACTH stimulation, it would seem that either the adrenal production of steroids or the steroid metabolism is altered in acute stress.

We do not as yet know the precursor of the urinary dehydroepiandrosterone, but the configuration of the steroid makes it reasonable to assume that its precursor appears relatively early in the adrenal synthesis. The steric resemblance to cholesterol lends support to this assumption, and it is probable that a $\Delta^5$-steroid is the precursor, since conversion from a $\Delta^4$ to a $\Delta^5$-steroid is unlikely. The pronounced increase in dehydroepiandrosterone, which averaged 210 per cent, may possibly be interpreted as showing that in acute stress the adrenal synthesis is disturbed by a substantial increase in steroids of the $\Delta^5$ type. The smaller rise in androsterone and etiocholanolone excretion and the lack of increase in the 11-oxy-metabolites may support this shift in the steroid production from $\Delta^4$-11-oxy-steroids to $\Delta^4$-11-desoxy-steroids and further to $\Delta^5$-steroids in acute stress. Another conceivable explanation is that the precursor of these first-named steroids increases to the same extent as the precursor of dehydroepiandrosterone, androsterone and etiocholanolone, but that during the increased needs of the body in acute stress the breakdown of the precursor of the 17-keto-11-oxy-steroids is more complete than that of the other steroids, the precursors of which may be presumed to be biologically inactive. This complete utilization and breakdown of the active steroids may thus lead to chemical substances without steroid configuration. That Franksson et al. (1954) observed an increase in the 17-hydroxy-corticoids of the blood in such conditions need not contradict this assumption, since the increase in the venous blood is not necessarily attributable to »Compounds F« or »E«, but may equally well have been caused by a $\Delta^5$-steroid with a dihydroxyacetone side chain. Investigations are at present in progress to determine which steroid increases in the venous blood during acute stress (Birke & Plantin).

The urinary 17-keto-11-oxy-steroids (11-keto-, 11-hydroxy-etiocholanolone) represent 2 to 8 per cent of the breakdown from »Compounds E« and »F« (Birke & Plantin, 1954), while 25 to 30 per cent of these biologically active steroids are metabolized to reducing corticoids (Birke & Plantin, 1954). It therefore seems probable that, since reducing corticoids represent a greater part of the conversion, their marked increase after surgical procedures should be regarded as an expression of intensified production and conversion of »Compound F« to its metabolites. This assumption cannot be definitely proved until it is shown which corticoids increase in response to acute stress. It may very well be that a change in the corticoid pattern similar to that in the 17-KS
excretion takes place, with no increase in the metabolites of "Compound F", primarily tetra-hydro-E. This question is at present being investigated (Birke & Plantin). It would imply that in moderate stress entailed by surgical intervention the conspicuous increase in the adrenal steroids is utilized differently in the body, so that no increase occurs in the urinary breakdown products of the body's active 11-oxy-steroids.

None of the patients showed any signs of serious postoperative shock and their general reactions were on the whole similar; but the degree of increase in the various steroids excreted differed greatly in the individual patients. The reason for this is difficult to decide, but it was evident that the very ill patients with impaired general condition did not respond with the customary increase in 17-KS excretion. The prognostic implications of this are not clear since the postoperative course at least was uneventful in these cases. It was noteworthy that as long as 9 to 11 days elapsed before the steroid metabolism regained its preoperative values. This suggests that disturbances of the homeostasis which may be associated with adrenal activity can be expected to appear late in the postoperative course. The tendency of the 11-oxy-17-ketosteroids to increase about 4 to 11 days after the operation may be interpreted as implying that the adrenal secretion of biologically active steroids was still raised but, that the body's requirements of these steroids was less than before and thus Franksson et al. (1954) were able to report an increase of 17-hydroxy-corticosteroids in the blood at this time, but they did not discuss this finding.

An important question is which method may be regarded as providing the best measurement of adrenal activity. In our cases we found that the mean excretion of dehydroepiandrosterone increased by 210 per cent, while the mean 17-KS excretion rose by only 45 per cent and androsterone and etiocholanolone by 20 per cent. The mean increase in the reducing corticoids was 130 per cent. Thus the excretion of corticoids rose considerably more than the total 17-KS, while dehydroepiandrosterone showed an appreciably greater rise than the other groups of steroids.

SUMMARY

1) The most important 17-ketosteroids (17-KS) were studied by chromatography and infrared spectrographic identification before and after major surgical procedures. The total excretion of reducing corticoids after hydrolysis with β-glucuronidase was also investigated. In a series of 18 patients the following results were obtained.

2) Dehydroepiandrosterone excretion showed a mean postoperative rise of 210 per cent. Androsterone and etiocholanolone rose by 20 per cent, while the total excretion of 17-KS increased by only 45 per cent. The 17-keto-11-oxy-metabolites did not increase immediately after surgery. The reducing corticoids

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showed a mean rise of 130 per cent in response to the acute stress. Five patients with pathologically low 17-KS excretion prior to operation did not respond with any marked rise in steroid excretion.

3) Normal excretion was again recorded 9 to 11 days after operation. The corticoids regained preoperative levels by the third to the fifth postoperative day and androsterone and etiocholanolone excretion within 3 days. The level of dehydroepiandrosterone fell rapidly and was completely normal after 7 to 9 days. The 17-keto-11-oxy-steroid metabolites showed an insignificant increase on the third or fourth postoperative day and returned to their initial values 9 to 11 days after operation.

4) No definite correlation was observed between the rise in the total excretion of 17-KS and that of dehydroepiandrosterone or the corticoids.

5) The raised values of dehydroepiandrosterone provided in most cases a good indicator of the increased adrenal activity in acute stress.

REFERENCES

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