THE EXCRETION OF TOTAL NEUTRAL 17-KETOSTEROIDS AND OF DEHYDROISOANDROSTERONE POSTOPERATIVELY

By

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As is well known, the general adaptation syndrome, as described by Selye (1950), is also seen following major operations. After surgery it is possible by various means to record the adrenal response produced by the alarm reaction: inter alia by eosinophil counts and by determination of the postoperative variations in the excretion of 17-ketosteroids (17-KS) and corticoids in the urine. Recently, these adrenal postoperative reactions have also been evaluated by daily determinations of 17-hydroxy-11-oxy steroids in the blood. All these investigations appear to indicate a definite stimulation of the adrenal cortex immediately after operation.

Hormonal analysis of blood as well as determinations of corticoids in urine are, however, fairly complicated procedures, hardly suitable for the daily clinical routine or particularly when it is desirable to follow the relation between the postoperative course and the deviations of the adrenal functions.

However, determinations of 17-KS in the urine is quite straightforward, particularly if Vestergård's method (1951) is used.

17-KS excreted in the urine mainly consist of androsterone, etiocholanolone and dehydroisoandrosterone (DHA). Androsterone and etiocholanolone must be assumed to be decomposition products of androgens from the adrenal glands as well as from the gonads, particularly the testes. Numerous investigations, however, seem to indicate that DHA is derived from the adrenal cortex only.

Chemically DHA is \( \Delta^5,6 \)-androsten-3\( \beta \)-ol-17-one. It is characteristic in that it has, in contrast to androsterone and etiocholanolone, a) the hydroxyl group at C\( _9 \) in \( \beta \)-position and b) that it is an unsaturated compound with a double bond between C\( _9 \) and C\( _\alpha \).
Normally DHA appears to make up 10–15 per cent of the total neutral 17-KS in the urine. As for 17-KS only comparatively insignificant individual variations are observed from day to day under normal conditions. With regard to DHA, Landau et al. (1951) observed deviations of about 15 per cent. More recent investigators, applying an improved method of hydrolysis, maintain that this fraction makes up a somewhat larger proportion of the total amount of 17-KS. Thus, it has been shown that hydrolysis during heating with acid occasioned a certain, though constant, loss of DHA.

It was previously believed that DHA was a decomposition product of ketosteroids, but later investigations - i.e. by Liebermann & Teich (1953), Mason & Kepler (1947), Miller, Dorfman & Miller (1950), Munson, Labhart & Forsham (1952), Samuels & West (1952), and Wolfson, Eya & Robinson (1952) indicate that it is either a preliminary or intermediary stage between some of the active cortical hormones. In support of this is the fact that unsaturated 3-ß compounds have never been excreted as such. They are invariably reduced by the organism to saturated 3-α compounds.

It has never been possible to demonstrate the presence of DHA in extracts of the adrenal cortex, and it has only slight biological activity. Stimulation of the adrenal cortex by corticotrophin will increase the excretion of 17-KS and DHA, but with comparatively higher quantities of DHA than the total 17-KS.

At any rate there appears to be a close correlation between the glandular activity and the quantity of DHA excreted in urine; and Liebermann & Teich (1953), as well as Ronzoni (1952), maintain that the determination of DHA is equally specific as the determination of corticoids in evaluating the adrenocortical activity.

Previously, DHA in the urine was determined by extraction of all neutral 17-KS, followed by precipitation with digitonin. This method is, however, somewhat troublesome. In 1950, Allen, Hayward & Pintu described a colorimetric method of analysis, based on the so-called Pettenkofer reaction in which steroid compounds with a hydroxy group in the β-position at C₃ and a double bond at C₃, C₄ or C₅ gives a blue colour when sulphuric acid is added. The method is well suited for use on a great number of samples. It may be carried out simultaneously with determinations of total 17-KS.

Since the determination of DHA in the urine appears to yield a fairly specific expression of the adrenocortical activity, it is of particular interest to ascertain the postoperative excretion of this fraction of the total 17-KS: following surgery one might expect a more pronounced reaction on the part of this hormonal fraction than in the total 17-KS level.

Following operations there are frequently definite increases in the excretion of 17-KS compared with preoperative levels. Since the methods of analysis, as well as the collection of urine samples, have certain degrees of error, it is
likely that only increases of 50 per cent and more (compared with preoperative figures) are significant.

Own investigations

MATERIAL AND METHODS

17-KS determinations are undertaken by the method described by Vestergård (1951), which is a modification of the micro-method of Hamburger (1948). The advantage of this method is that it allows of the extraction and 17-KS determination of a number of urine samples simultaneously; thus, it is better suited to extensive serial examinations.

In the present material, the DHA determination has been undertaken by the method indicated by Allen with a few modifications:

This consists of hydrolysis of 3 ml. of urine, with the addition of 0.3 ml. 40 % H₂SO₄ and boiling for 25 minutes. After cooling, extraction is undertaken with ethyl-ether, cleaning of extract with 2 x 3 ml. 2 n NaOH and 2 x 3 ml. distilled H₂O, and evaporation of the ether-extract after drying with Na₂SO₄ anhyd. The colour is developed in the evaporated extract by adding of 2 ml. of a mixture of sulphuric acid and alcohol (100 ml. H₂SO₄ conc. + 400 ml. ethylalcohol), left for 12 minutes at 55° C., cooling for 1 minute, then addition of 3 ml. ethylalcohol while cooling. The extinction is read at 560, 600 and 640 mμ in a Hilger spectrophotometer, and corrections are made for non-specific chromogens by the formula given by Allen (1950):

\[
\text{Corrected extinction} = \frac{E \cdot 600 \, m\mu}{2} - \frac{E \cdot 560 \, m\mu + E \cdot 640 \, m\mu}{2}
\]

A blank of 2 ml. H₂SO₄-ethylalcohol mixture and 3 ml. abs. ethylalcohol and a standard of 50 μg. dehydroisoandrosterone acetate are used.

The disadvantages of this method are, firstly, as already mentioned, that a certain loss of DHA occurs when boiling with acid; and secondly, that the colour of a slight content of the urine is obscured by the non-specific chromogens. The loss through boiling with acid, however, appears to be a constant percentage, according to Jacobsson's investigations (1951).

Investigations undertaken by Liebermann & Dobriner (1948) and our own investigations (not yet published) have shown that when hydrolysis is undertaken at room temperature and extraction extends over 48 hours, the DHA-yield from the urine extract is higher, and the non-specific chromogens much reduced.

As far as the present material is concerned, however, the original method embracing acid hydrolysis at 100° C. appears to have given reliable results. A determination of DHA by digitonin precipitation of the extract would not only give the loss through boiling with acid as already mentioned, but it would also show a loss through the digitonin precipitation itself; accordingly,
it would scarcely be better suited to the above mentioned investigations than Allen's method. A chromatographic determination of DHA would undoubtedly give the most exact result; but, so far, there is no chromatographic method so simple as to be deemed suitable for routine examinations, as exemplified by the material at hand. In a paper recently published, Birke, Franksson & Plantin (1955) have undertaken post-operative examinations of the urine of 18 patients by chromatographic analyses, and the results of these are consistent with those given in this article, especially with regard to the DHA-fraction.

RESULTS

The present determinations form part of a series of extensive investigations of the postoperative phase of the function of the adrenal glands; this has been undertaken in co-operation with other authors, and has been published elsewhere (Hasner et al., 1952). The material comprises 150 patients; with few exceptions, these have all undergone major surgical operations, such as gastric resections, operations on the bile-ducts, etc.; while, the few exceptions mentioned above have been subjected to minor operations, such as for hernias, haemorrhoids, etc.

Table 1.
Increase in post-operative 17-KS excretion.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Increase in post-operative 17-KS (&gt; 50 %)</th>
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<tbody>
<tr>
<td>150</td>
<td>100 (67 %)</td>
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Table 2.
Increase in post-operative DHA-excretion (and simultaneous increase in 17-KS excretion).

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Increase in postop. DHA (&gt; 50 %)</th>
<th>Increase in postop. 17-KS among these patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>64 (96 %)</td>
<td>39 (58 %)</td>
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</table>

The total 17-KS in 150 patients was determined, in each patient, 2–3 days prior to operation, and at least 6 days after operation. As appears from Tables
1 and 2, in 100 (i.e. 67 per cent) of these patients a definite increase in the 
excretion of 17-KS was found following surgery. In addition, in 67 of the 150 
patients the excretion of DHA (by Allen's method) was ascertained. In 64 
(i.e. 96 per cent) of these patients there was post-operatively a marked increase 
in the excretion of DHA. In 39 (i.e. 58 per cent) of these 67 patients there 
were, post-operatively, increase in the levels of total 17-KS.

DISCUSSION

There are individual variations in the excretion of 17-KS, depending on sex 
and age; it is highest in men, and reaches its maximum at about the age of 30 
in both sexes. In the present post-operative investigations it was established 
that there was no correlation between age and magnitude of the postoperative 
increase. This indicates that the adrenal activity brought about by the operational 
stress appears to be independent of age. The increase in excretion of total 
17-KS in men was found to be somewhat higher in the older age groups than 
in the younger males. This was not the case in the female patients; nor did it 
hold for DHA in either sex. This may possibly be ascribed to the fact that 
a higher percentage of the total 17-KS in younger men, as compared with older 
men, originates from the testes.

Moreover, there was an earlier onset in the increase of excretions of DHA 
than of the total 17-KS. This may possibly be explained by the fact that DHA, 
in contrast to the total 17-KS is not a decomposition but an intermediary pro-
duct of the adrenocortical hormones.

Comparisons between the postoperative excretion of the total neutral 17-KS 
and DHA thus seem to confirm that the excretion of DHA is a more specific 
expression of adrenal activity than the excretion of total 17-KS, and conse-
quently more valuable in assessing the adrenal reactions following surgery.

SUMMARY

The chemistry and metabolic fate of 17-ketosteroids and especially of dehydro-
isoandrosterone are described. It is emphasized that the excretion of dehydro-
isoandrosterone appears to be a more specific expression of the adrenocortical 
activity than the excretion of total 17-ketosteroids. The findings on the ex-
cretion of 17-ketosteroids and dehydroisoandrosterone before and after opera-
tion are given. The investigations appear to confirm that the excretion of de-
hydroysoandrosterone is a more valuable means of assessing the adrenocortical 
activity following surgery than is the excretion of total 17-ketosteroids.
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