THE RESPONSE OF URINARY 17-HYDROXYCORTICOID TO CORTICOTROPHIN ZINC AS A TEST OF ADRENAL CORTICAL FUNCTION

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An increased interest in the study of the adrenal cortex, together with the ready availability of corticotrophin (A.C.T.H.), have, in recent years, led to the development of improved methods for the assessment of adrenal cortical function. The first of such methods was devised by Thorn, Forsham, Prunty, and Hills (1948) and depended upon the fall in blood eosinophils after injection of a single dose of corticotrophin. It was subsequently realized, however, that this test was by no means specific, since a fall in eosinophils could be produced by the injection of adrenaline, for example, without any effect on the adrenal steroids (Nelson, Sandberg, Palmer, and Glenn, 1952). Moreover, there is a considerable spontaneous variation in the eosinophil count and results may be difficult to interpret when the initial count is low.

The urinary 17-ketosteroid excretion after corticotrophin provides a more valuable indication of adrenal cortical function and many of the current tests rely on this estimation. In males, however, the 17-ketosteroids are only partly derived from the adrenal cortex, and sometimes a clearly defined rise after corticotrophin is not obtained even in normal individuals. Jenkins, Forsham, Laidlaw, Reddy, and Thorn (1955) find that 14% of normal controls showed a rise of less than 2 mg. in 24 hours.

Recently, relatively simple methods have been devised for the estimation of urinary 17-hydroxycorticoids, and it seems that these methods provide a more direct measure of adrenal cortical function since the 17-hydroxycorticoids represent that fraction of the adrenal steroids known as the glucocorticoids, of which hydrocortisone and its urinary metabolites are the most important. It is now recognized that for an adequate assessment of its function a prolonged stimulation of the adrenal cortex is necessary. Jenkins et al. (1955) describe the use of an eight-hour intravenous infusion of corticotrophin, but this is inconvenient, and occasional anaphylactic reactions have been reported. The same authors find that corticotrophin gel injected intramuscularly is equally effective in producing a rise in 17-hydroxycorticoids. Recently, a very effective aqueous suspension of corticotrophin and zinc phosphate has been introduced, which is more easily injected than the gel preparation. Greene and Vaughan-Morgan (1954), den Oudsten, van Leeuwen, and Coers (1954), and Ferriman, Anderson, and Turner (1954) have shown that corticotrophin-zinc phosphate has a prolonged and powerful stimulatory effect on the adrenal cortex, as reflected by the eosinophil count and 17-ketosteroid excretion. The purpose of the present report is to describe a test of adrenal cortical function based upon the estimation of urinary 17-hydroxycorticoids before and after the administration of corticotrophin zinc.

Methods

Twenty-four-hour urine collections were started at 9 a.m. The first day was used as a control, and on the second day 40 units of corticotrophin zinc was injected intramuscularly at 9 a.m. and 9 p.m. In most cases urine collection was continued for the third day.

Urinary 17-hydroxycorticoids were estimated by the method of Reddy, Jenkins, and Thorn (1952) as modified by Reddy (1954) and involved the extraction of urine with butanol followed by the colour reaction of Porter and Silber (1950). This method has the merit of being relatively simple to perform and rapid enough for estimations to be completed within a few hours. Two alterations have been made in the procedure described by Reddy (1954). The first employs the suggestion of Smith, Mellinger, and Patti (1954), who found that the use of 56% instead of 62% sulphuric acid, allowing a longer incubation time, reduced blank values and increased colour development. The second modification involves a simplified method...
for the purification of the commercial butanol. One
litre of butanol ("analar") was acidified to pH 1
with 50% sulphuric acid. One hundred milligrams of
recrystallized phenylhydrazine was then added and
the mixture heated to 60° C. for 30 minutes. At the
end of this time the temperature was raised to boiling
point and the butanol distilled at 117° C., discarding
the first 50 ml. Urinary 17-hydroxycorticoids esti-
mated by this method, using cortisone as a standard,
showed in 50 individuals a normal range of 3 to
10.2 mg. per 24 hours (mean 6.3 mg.).
Urinary 17-ketosteroids were estimated by the stan-
dard method proposed by the Medical Research
Council Committee on Clinical Endocrinology (1951).

Results

Normal Response to Corticotrophin.—The
administration of corticotrophin zinc to 24 patients
with no clinical evidence of adrenal cortical disease
resulted in an unequivocal rise in urinary 17-
hydroxycorticoids in every case, although there
were considerable differences both in the speed and
magnitude of the response. The initial control
values ranged from 3 mg. to 9.3 mg., and the
increase ranged from 6.6 mg. to 27.6 mg. (mean
16.1 mg.) during the day of corticotrophin and
from 19.4 mg. to 59 mg. (mean 36.6 mg.) during the
following day. One other normal female was not
included in this series because she had been
receiving small doses of corticotrophin twice
weekly for several months, elsewhere, on a mis-
taken diagnosis of hypopituitarism. Her initial
level was 8.1 mg. and after the corticotrophin test
there was an enormous increase of 35.4 mg. and
90.9 mg. on the successive days. This result agrees
with the finding of Forsham, di Raimondo, Island,
Rinfret, and Orr (1955) that repeated small
amounts of corticotrophin increase the sensitivity
of the adrenal to subsequent stimulation by larger
doses.

In Fig. 1 the responses of the eosinophils, the 17-keto-
steroids, and the 17-hydroxycorti-
corticoids have been com-
pared in a normal person. It
will be seen that in this
particular case a definite fall
in eosinophils was obtained,
but the rise in the 17-keto-
steroid level was much less
impressive than the rise in
17-hydroxycorticoids. Fig. 1
shows that even smaller doses
of corticotrophin zinc cause
a rise in 17-hydroxycorticoids
lasting from 24 to 48 hours.
The effect of oral cortisone,
12.5 mg. three times daily, is
included for comparison.

Addison's Disease.—The
Table and Fig. 2 show the
results obtained when the test
is carried out on patients
suffering from Addison's
disease. Three cases were
studied, of which one patient,
J.H., was untreated at the
time of investigation. This
patient showed most of the
clinical features of Addison's
disease, and the 17-hydroxy-
corticoid excretion was 2.4
mg. The administration of
corticotrophin caused no rise
in either the 17-hydroxycorti-
insufficiency secondary to hypopituitarism is shown in Fig. 2 and the Table. A male patient aged 63 suffering from hypopituitarism showed the low 17-hydroxycorticoid excretion of 1.5 mg., but this level rose to 16.5 mg. following corticotrophin. In contrast, the 17-ketosteroids rose only from 2.7 mg. to 6.6 mg. A second male patient also showed a good response, the increase in 17-hydroxycorticoids being 9.9 mg. on the day of corticotrophin and 12 mg. on the following day, although the corresponding rise in 17-ketosteroids was only 1 mg. and 1.4 mg. on these two days. A female patient with long-standing hypopituitarism following post-partum necrosis of the anterior pituitary showed a more sluggish reaction, no rise occurring on the day of corticotrophin, but a rise of 5.6 mg. was obtained on the following day. While an unequivocal increase in 17-hydroxycorticoids was obtained in all three patients using the standard test, the delayed response occurring in the third patient indicates that in cases of suspected hypopituitarism it would be wise to prolong the administration of corticotrophin for three days in order to ensure adequate stimulation.

Cushing's Syndrome.—The application of the test to two patients suffering from Cushing's syndrome due to bilateral adrenal cortical hyperplasia is also shown in Fig. 2 and the Table. In these cases the control 17-hydroxycorticoid values were higher than normal, and after corticotrophin a considerable response was obtained, indicating that the already hyperplastic adrenal cortex was capable of being further stimulated by exogenous corticotrophin.

**Discussion**

The results shown above indicate that the response of urinary 17-hydroxycorticoids after the administration of corticotrophin zinc differentiates between individuals with normal adrenal corticoid or 17-ketosteroid levels. The remaining two patients had suffered from Addison's disease for several years and were receiving treatment with oral cortisol, 12.5 mg. twice daily. This dose was sufficient to produce normal 17-hydroxycorticoid excretion under ordinary conditions, but after corticotrophin there was no significant alteration.

**Hypopituitarism**.—The use of the 17-hydroxycorticoid test in differentiating between the primary adrenal failure of Addison's disease and adrenal
count and the urinary sodium/potassium ratio as screening tests before carrying out the more time-consuming urinary 17-ketosteroid estimation. The limitations of the eosinophil count have already been mentioned and the urinary sodium/potassium ratio may be affected by variations in the diet unless this is rigidly controlled. With the development of a method for the estimation of urinary 17-hydroxycorticoids which is more rapid than the 17-ketosteroid estimation and which provides a more sensitive index of adrenal cortical response, there appears to be less need for the indirect tests.

**Summary**

An increase in urinary 17-hydroxycorticoids is a sensitive index of adrenal cortical response to corticotrophin.

Patients with Addison’s disease show no increase in 17-hydroxycorticoids whereas in hypopituitarism there is a definite response.

In Cushing’s syndrome due to adrenal cortical hyperplasia there is a good response to corticotrophin.

Corticotrophin zinc provides a sustained, intensive stimulation of the adrenal cortex in a convenient form.

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**REFERENCES**


