THE DIAGNOSIS OF ADRENAL CORTICAL DYSFUNCTION

BY

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Although the history and clinical findings are important in the diagnosis of endocrine disorders, confirmatory biochemical tests are necessary in many cases. If possible, these biochemical tests should give direct evidence of the gland's activity by measuring the hormone levels in the blood or, where applicable, excretion in the urine. When this cannot be done it is necessary to rely on indirect evidence, usually metabolic changes resulting from altered hormone production.

In the case of the adrenal cortex, assessment is complicated by the multiplicity of hormones produced. Three require particular consideration: hydrocortisone, aldosterone, and the adrenal androgens. Of these the most important is hydrocortisone. Although sometimes referred to as the glucocorticoid hormone because it increases gluconeogenesis, it has other more important actions affecting the blood pressure, renal function, and homeostasis of the extracellular fluid (Mendelsohn and Pearson, 1955).

It is unfortunate that the only direct measurement of adrenal cortical hormones commonly undertaken is the urinary excretion of 17-ketosteroids. These are for the most part metabolites of adrenal androgens, which are among the less important of the adrenal cortical hormones. Furthermore, 17-ketosteroid excretion may be reduced in any chronic serious illness without impairment of the essential activities of the adrenal cortex. Measurement of aldosterone excretion in blood and urine is possible, but only as a complex research procedure, and there is little likelihood of its becoming practicable for routine study of patients. A number of methods for estimation of hydrocortisone and its metabolites in the blood or urine have been described during the last 10 years. The blood methods and the earlier urinary ones are complex and undertaken in only a few centres.

In recent years two comparatively simple methods for measuring urinary glucocorticoid excretion have been described. The first is the Reddy-Jenkins-Thorn (1952) procedure in which the steroids are extracted with butanol and after purification measured colorimetrically by the Porter-Silber reaction. The second is the Norymberski method (Norymberski *et al.*, 1953), in which the steroids to be measured are converted to 17-ketosteroids by oxidation with sodium bismuthate. The 17-ketosteroids already present and those formed by this oxidation are extracted, purified, and measured by the Zimmerman colour reaction that is routinely used for this purpose. The increase of 17-ketosteroids following oxidation—the urinary 17-ketogenic steroids—are an index of glucocorticoid excretion. A comparison of these two methods has been reported elsewhere (Moxham and Nabarro, 1956). Norymberski (Appleby *et al.*, 1955) subsequently described a modification of his original procedure, in which the pre-existing 17-ketosteroids in the urine are first destroyed so that all 17-ketosteroids present after oxidation are derived from hydrocortisone and its metabolites. They are referred to as the total 17-hydroxycorticosteroids. Studies have been made of the value of urinary total 17-hydroxycorticosteroid estimations in patients suspected of having dysfunction of the adrenal cortex. The method used is a modification of the original, and is very similar to that already used in hospital laboratories for estimations of the urinary 17-ketosteroids (Medical Research Council, 1951). The method is described in the Appendix to this paper.

Results

The urinary total 17-hydroxycorticosteroids (total 17-OHCS) have been estimated (by this method) in a number of adults with normal adrenal cortical function, and the results were:

	No.	Mg. 17-OHCS per 24 hours	
		Mean	Range
Men	41	14·9	7·3–23·6 (S.D. 4·4)
Women	57	11·0	4·6–17·8 (S.D. 3·5)

Total 17-OHCS estimations have been made on 100 urine collections from 22 adrenalectomized patients receiving oral cortisone acetate up to 150 mg. a day, or 9α-fluorohydrocortisone 0.5 mg. daily. The results are shown in Fig. 1. The correlation coefficient between steroid dose and steroid excretion is 0.94. The relationship between them is given by the equation:

Cortisone acetate dose = 2.88 × total 17-OHCS − 3.0.

Steroid excretion is approximately 36% of the daily dose of cortisone acetate.

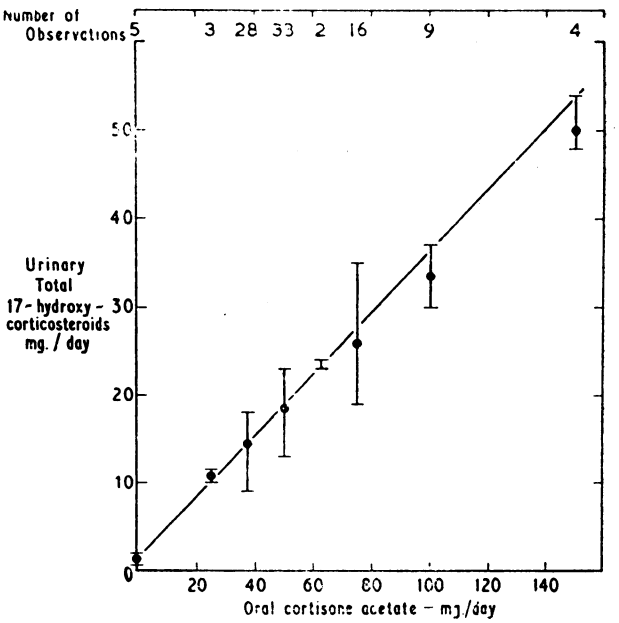

FIG. 1.—17-OHCS excretion of adrenalectomized patients receiving oral cortisone acetate. At each dose level the range and mean excretions are shown, and also the calculated regression line.

Diagnosis of Adrenal Cortical Dysfunction

The main syndromes of adrenal cortical dysfunction are:

1. Chronic adrenal cortical insufficiency: (*a*) Primary—Addison's disease; (*b*) secondary—in panhypopituitarism.

2. Adrenal cortical overactivity due to tumour, hyperplasia, or simple hyperfunction: (*a*) Cushing's syndrome—

secretion of excess glucocorticoids ; (*b*) syndromes of excess androgen secretion ; (*c*) hyperaldosteronaemia ; (*d*) iatrogenic hyperfunction from injected corticotrophin.

3. Dysfunction due to failure of normal hormone synthesis—congenital adrenal cortical hyperplasia.

Measurement of urinary glucocorticoid excretion may be of considerable value in the diagnosis of a number of these syndromes.

Diagnosis of Chronic Adrenal Insufficiency

Cases of Addison's disease vary in severity. In some the adrenal cortex is almost entirely destroyed or atrophic, whereas in others there is a significant remnant, able, if continuously stimulated by endogenous corticotrophin, to maintain an adequate steroid output. There is, however, no adrenal cortical reserve, and no response to injected corticotrophin can be demonstrated.

In the severe cases the diagnosis can usually be made on clinical grounds, and may be confirmed by biochemical tests. The plasma sodium will be low, the plasma potassium and blood urea raised, and despite the hyponatraemia a random urine specimen will contain 20 mEq./l. or more of sodium. If these abnormalities are found it is dangerous to delay treatment for further investigation, although later a confirmatory corticotrophin test may be made to differentiate a salt-losing renal lesion from Addison's disease.

In the mild or incomplete case the diagnosis is more difficult. The plasma electrolytes and blood urea are unaltered, the urinary excretion of 17-ketosteroids is low, but this may be a non-specific effect, and the urinary glucocorticoid excretion is usually in the lower part of the normal range. A number of tests have been suggested based on the secondary metabolic changes resulting from deficiency of adrenal cortical hormones. The simplest is the water-load test, with and without cortisone (Soffer and Gabrilove, 1952). Delayed water excretion has been noted in many conditions, but it is seldom improved by cortisone except when caused by primary or secondary adrenal cortical insufficiency. There may, however, be some improvement in cases of steatorrhoea and hepatic fibrosis, and patients with mild Addison's disease are occasionally able to excrete a water load normally (Fig. 2). The Cutler salt-deprivation test, although safe in a mild case, is dangerous in more severe adrenal cortical insufficiency, and may give false positive results in chronic renal disease. The Robinson-Power-Kepler test is known to give false positive results in many conditions, and its use can no longer be recommended. These indirect tests have been superseded by the corticotrophin tests of adrenal cortical reserve. In a severe case they may be applied after cortisone therapy has started, and steroid administration can be continued during the test. In the incomplete case absence of any reserve capable of responding to additional corticotrophin is the essential feature of the condition.

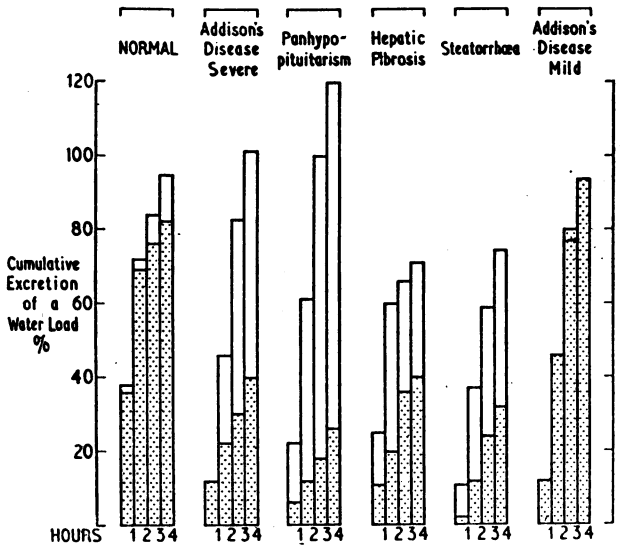

FIG. 2.—Water-load excretion tests. 20 ml. per kg. body weight of water given between 6 and 6.15 a.m.; urine collections made at 7, 8, 9, and 10 a.m. Shaded area represents excretion without cortisone; clear area, the increase on a subsequent occasion when the test was preceded by 100 mg. of cortisone acetate given by mouth at 2 a.m.

In the original corticotrophin test (Thorn *et al.*, 1948) indirect indices of adrenal response—the absolute eosinophil count and urinary uric-acid:creatinine ratio—were measured. Subsequently, Thorn (Thorn *et al.*, 1951) pointed out that it was essential to have direct evidence before making the diagnosis of Addison's disease, and suggested estimating urinary 17-ketosteroids. A diagnostic test using corticotrophin gel has been described (Nabarro, 1954). Daily intramuscular injections of 100–120 units of gel were given for up to three consecutive days. Significant reduction of the absolute eosinophil count or the urinary sodium: potassium ratio was found to be satisfactory evidence of an adrenal response. In the absence of this evidence the urinary 17-ketosteroids were estimated and Addison's disease was diagnosed only if 17-ketosteroid excretion was unchanged by stimulation. The smallest increase in a patient with normal adrenal glands was 4 mg. per 24 hours. Tests have since been carried out in 22 more patients with normal adrenal glands and the increase of urinary glucocorticoid excretion (measured as total 17-OHCS by the method described) compared with that of the 17-ketosteroids. The results are shown in Fig. 3, the maximum total 17-OHCS excretion was from 38 to 225 mg. per 24 hours, whereas that of 17-ketosteroids was 5 to 63 mg. The method for estimating the 17-OHCS is as sensitive as that for the 17-ketosteroids, and therefore the test becomes much more delicate if the urinary total 17-OHCS can be measured. The results in four patients are shown in Fig. 4; one is a typical normal response, and one that of a patient with Addison's disease. The last two patients were suspected of having Addison's disease, and it will be noted that the increase of urinary 17-ketosteroids was very small, but the increase of total 17-OHCS was unequivocal.

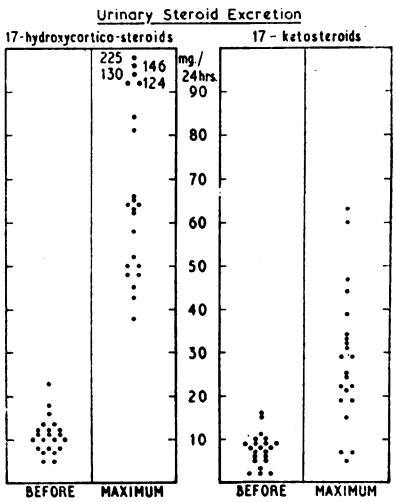

FIG. 3.—Comparison of the increase of total 17-hydroxycorticosteroids and 17-ketosteroids in 22 patients with normal adrenal glands given corticotrophin gel 100 units daily for three days.

The corticotrophin test is of limited value in the diagnosis of secondary adrenal cortical insufficiency. The control levels of 17-ketosteroids and total 17-OHCS excretion are usually below normal, but the adrenal cortex has responded to corticotrophin in the nine cases we have studied. The response may be delayed and smaller than normal, depending on the duration of the pituitary failure.

Diagnosis of Cushing's Syndrome

The essential features of Cushing's syndrome result from increase of circulating hydrocortisone. For confirmation of the diagnosis it is desirable to be able to demonstrate a raised plasma hydrocortisone concentration or an increase

of the urinary excretion of hydrocortisone and its metabolites. In the past many indirect tests have been performed on patients suspected of having this condition ; these included glucose and glucose-insulin tolerance tests, urinary 17-ketosteroid estimations, plasma electrolyte concentrations,

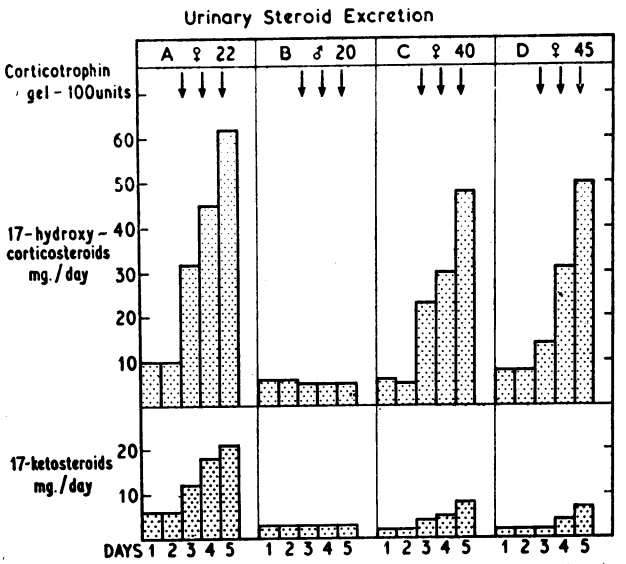

FIG. 4.—Response of steroid excretion to corticotrophin gel. A, Normal response; B, Addison's disease; C and D, Patients suspected of having Addison's disease.

and absolute eosinophil counts. In practice the results of these investigations are often equivocal, and a convenient method of urinary glucocorticoid estimation would be helpful in some of the more difficult cases.

The following results have been obtained in cases of Cushing's syndrome :

Case	Adrenal Pathology	Total 17-Hydroxy-corticosteroids mg./day	17-Ketosteroids mg./day
1 (F)	Carcinoma	73	51
2 (F)	Adenoma	29	8
3 (M)	Hyperplasia	58	40
4 (M)	,,	38	18
5 (M)	,,	38	15
6 (M)	,,	68	17
7 (F)	,,	24	15
8 (F)	,,	25	16
9 (F)	,,	25	13
10 (F)	,,	25	37
11 (F)	,,	36	16
12 (F)	,,	27	18
13 (F)	,,	60	20
14 (F)	,,	49	15
15 (F)	,,	18	12

It will be noted that the 17-ketosteroid excretion was often normal. The total 17-OHCS varied widely, but with a single exception were raised. In most cases the figure shown is the mean of several determinations ; in the last case only one urine collection was available. When the condition is due to hyperplasia there may be considerable variation due to spontaneous fluctuation in severity. If the clinical condition suggests Cushing's syndrome, but the first urine steroid estimation fails to confirm the diagnosis, further specimens should be studied. It must be pointed out that the total 17-OHCS include certain steroids not derived from hydrocortisone ; normally these are present in only small amount, but they may be greatly increased in cases of adrenal cortical carcinoma and congenital adrenal hyperplasia (Appleby and Norymberski, 1955).

Virilizing Adrenal Cortical Carcinoma

Steroid excretion has been studied in three cases of adrenal cortical carcinoma with evidence of hormone production but not of Cushing's syndrome. The steroid excretions were :

Case	Total 17-Hydroxy-corticosteroids (mg./24 hrs.)	17-Ketosteroids (mg./24 hrs.)	17-Ketogenic steroids (mg./24 hrs.)	17-Hydroxy-corticoids * (mg./24 hrs.)
A (M) ..	63	100	21	13
B (M) ..	136	122	79	50
C (F) ..	130	62	103	48

* Reddy-Jenkins-Thorn method.

In these cases it appears likely that the high level of total 17-OHCS is due to abnormal steroid compounds, but the results did not give rise to any problems of diagnosis.

Iatrogenic Cushing's Syndrome

Although corticotrophin is now seldom used for the treatment of allergic, rheumatic, or haematological disorders, West (1956) has pointed out the importance of confirming from time to time that the preparations used are active and that the patients' adrenals are responding. He has shown that much more information is obtained from estimation of urinary glucocorticoid metabolites than 17-ketosteroids.

When patients are treated with cortisone or prednisone the adrenal glands are suppressed and it may be considered advisable to stimulate them periodically with corticotrophin. If this is done, evidence of an adrenal response should be sought, and an increased excretion of urinary total 17-OHCS has proved a convenient index (Fig. 5).

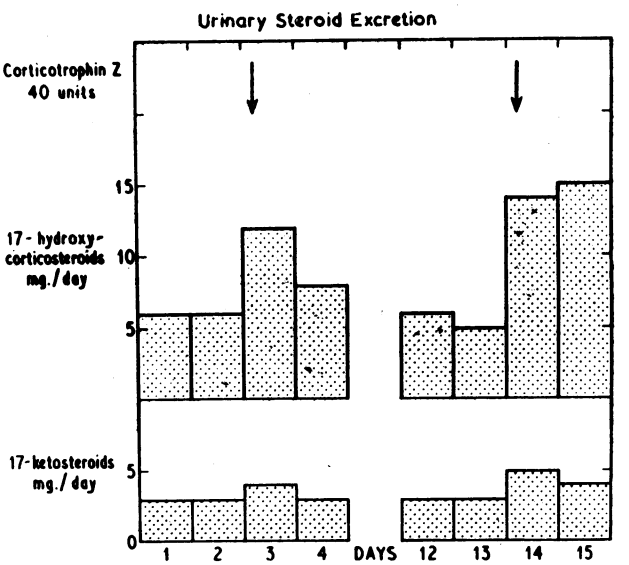

FIG. 5.—Response to corticotrophin Z. Patients with rheumatoid arthritis on steroid therapy for five months, receiving prednisone 20 mg. daily during the test.

Discussion

For many years clinicians have had to be satisfied with figures for urinary 17-ketosteroid excretion as the only direct measurement of adrenal cortical activity undertaken by hospital laboratories. The 17-ketosteroids are a poor indication of adrenal cortical activity : in men they are not derived exclusively from the adrenal cortex ; for the most part they are metabolites of the least important of the adrenal hormones, and they are reduced in any serious illness.

Simple methods for the estimation of hydrocortisone metabolites in the urine are now available. Corticotrophin tests for the diagnosis of Addison's disease are more sensitive if figures for the excretion of glucocorticoids during the period of stimulation are available. The diagnosis of Cushing's syndrome can often be made on clinical grounds. In some patients, however, it may be suspected and many investigations performed without any definite result. A simple method for estimating urinary glucocorticoids would be valuable and probably make it unnecessary to perform the numerous time-consuming investigations at present undertaken in these cases.

Increased excretion of urinary total 17-OHCS is not always the result of increased hydrocortisone secretion by the adrenal cortex. Steroids with a 17.20 diol, or 21-deoxyketol type of side chain, give rise to 17-ketosteroids in this procedure (Appleby and Norymberski, 1955). Normally they are present in only small amount, but in adrenal cortical carcinoma and congenital adrenal hyperplasia they may be greatly increased. The high urinary total 17-OHCS excretion does not then reflect an increase of hydrocortisone production, but the clinical condition is usually clear and does not suggest Cushing's syndrome.

Summary

Some of the problems in the laboratory diagnosis of chronic adrenal insufficiency and Cushing's syndrome are discussed. A major difficulty has been the lack of any convenient method for estimating hydrocortisone and its metabolites in the urine.

A method based on the Norymberski procedure for measuring total 17-hydroxycorticosteroids is described. The steroid outputs of normal subjects were 7.6 to 23.6 mg. per 24 hours in men, and 4.6 to 17.8 mg. per 24 hours in women. In 100 estimations on urines from adrenalectomized patients the steroid output showed good correlation with the dose of cortisone acetate.

The effect of injections of corticotrophin gel on total urinary 17-hydroxycorticosteroid excretion was compared with that on the 17-ketosteroids. It is concluded that corticotrophin tests for adrenal cortical insufficiency are more sensitive if total 17-hydroxycorticosteroids are estimated.

The total 17-hydroxycorticosteroids were measured in 15 cases of Cushing's syndrome and three of virilizing adrenal cortical carcinoma. Estimation of the total urinary 17-hydroxycorticosteroids was of diagnostic value in Cushing's syndrome and should make it unnecessary to undertake numerous time-consuming investigations in patients suspected of having this condition.

APPENDIX

Method for Estimation of Urinary Total 17-Hydroxycorticosteroids (Total 17-OHCS)

A 24-hour urine collection is made without preservative; if the total volume is less than two litres, it is made up to two litres with distilled water.

20 ml. of urine is placed in a 50-ml. ground-glass stoppered test-tube and 100 mg. sodium borohydride added. Excess frothing may be stopped by adding a few drops of ether. The tube is allowed to stand overnight at room temperature, and next morning 20 ml. glacial acetic acid (A.R.) and 4.5 g. sodium bismuthate (A.R.) are added in that order. The tube is stoppered and shaken in the dark for 30 minutes in a Kahn shaker. The stopper is removed and the tube centrifuged at 2,000 r.p.m. for five minutes; the supernatant is poured into another tube and again centrifuged. 25 ml. of the fluid is then pipetted into a 250-ml. round-bottomed flask and the following added in order: 15 drops freshly prepared 5% solution sodium metabisulphite, 25 ml. distilled water, 7.5 ml. conc. hydrochloric acid (A.R.), 25 ml. benzene (A.R.), and a few chips of porous pot. The contents of the flask are boiled under reflux for 30 minutes and then cooled. The aqueous layer is removed in a separating funnel and shaken with two further 25-ml. amounts of benzene. The three lots of benzene are pooled, washed twice with 15 ml. of normal sodium hydroxide, once with 15 ml. N/2 hydrochloric acid, and three times with 15 ml. distilled water, using a 100-ml. stoppered separating funnel shaken by hand. The washed benzene extract is transferred to a 100-ml. round-bottomed flask, evaporated to dryness under reduced pressure in a water bath, and placed in a desiccator for 12 hours. The residue is dissolved in 1 ml. ethanol (R.R. quality, Distillers Co. Ltd.), and 0.2 ml. (representing 2.5 ml. of urine) taken for the Zimmerman reaction, which is performed by the M.R.C. method (1951) using the colour correction recommended. Results are expressed as mg. dehydroepiandrosterone per 24 hours.

If the diluted urine contains more than 0.5% reducing substance, this must be removed at the beginning. 25 ml. of urine is placed in an incubator at 37° C. with about 0.75 g. baker's yeast. The concentration of reducing substance will be less than 0.5% after 6 to 24 hours; the urine is then centrifuged and an aliquot of 20 ml. taken for the estimation.

We wish to thank our colleagues at the Middlesex and other hospitals for urine specimens from cases of Cushing's syndrome. We are indebted to the Clinical Research Committee of the Middlesex Hospital for the provision of laboratory facilities and for a personal research grant to J. D. N. N. The charts were prepared by Mr. V. K. Asta.

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MATERNAL AGE AND FOETAL OXYGENATION

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In previous papers (Walker and Turnbull, 1953; Walker, 1954) it was shown that the margin of safety in the supply of oxygen to the foetus gradually diminishes in the later weeks of pregnancy, and especially when pregnancy is prolonged beyond the expected date of delivery. These findings have recently been confirmed by MacKay (1957). It was also shown (Walker and Turnbull, 1953; Turnbull and Walker, 1956) that the oxygen supply to the foetus may be reduced if pregnancy is complicated by pre-eclampsia or threatened abortion.

The purpose of the present investigation was to determine whether, in primigravidae, the margin of safety in foetal oxygenation decreases with age; this might be a factor in the well-known fact that the stillbirth rate rises with age, especially in primiparae.

Before the results of the investigation were available the staff of the Aberdeen Maternity Hospital agreed to adopt a new policy of treatment based upon the assumption that foetal oxygenation is, in fact, frequently reduced to dangerous levels in elderly primigravidae who do not go into labour before the end of the 41st week. The procedure and its results are briefly discussed.