Papers and Originals

The Adrenal Cortex in Internal Medicine*—Part I

C. L. COPE,† D.M., F.R.C.P.

Brit. med. J., 1966. 2, 847–853

Fellows of Lumley's time were probably aware of the existence of the adrenal glands, which had been described in 1563 by Bartholomaeus Eustachius in Rome. Subsequent theories regarding their functions covered the occupation of space to support the stomach, the cleansing of the blood, and the maintenance of potency (Shumacker, 1936), and 117 years ago Thomas Addison (1849), addressing the South London Medical Society, first suggested "that a diseased condition of the adrenal glands, functional or structural, may interfere with the proper elaboration of the body generally."

In the late 1930s the structure of a few of the adrenal steroids was partly clarified, and Sayers and Sayers (1949) reviewed their work on the sensitivity of the adrenal cortex to noxious stimuli. This was the main stimulus to my own interest in these glands.

Technical Advances

The subsequent rapid advances in our knowledge have been intimately dependent on a series of major technical advances. The introduction of paper chromatography (Bush, 1951; Burton *et al.*, 1951) offered for the first time the possibility of separating out the very large number of steroidal components of plasma and urine, and of isolating the more important ones in relatively pure form, even when the quantities were infinitesimal. This calls for no high degree of technical skill, and it can be applied quickly and simply to the diagnostic problems of individual patients. The technique enabled Bush and Sandberg (1953) to prove that the adrenal cortical hormone in plasma is mainly cortisol with a small addition of corticosterone, the amount of cortisone being negligible. Both cortisol and cortisone were recognized in normal urine; and the more important metabolites of adrenal cortical hormone degradation were defined, the main ones being the tetrahydro-derivatives, which, before excretion by the kidney, are rendered freely water-soluble by conjugation with glucuronic acid (Cope and Hurlock, 1953). With these techniques, aldosterone was discovered by Tait *et al.* (1952).

Several years earlier Venning (1945), using the very laborious technique of bioassay on mice, showed that the human adrenal was stimulated into activity by trauma and other noxious stimuli or stresses, and the urinary content of adrenal hormone was thereby increased. That the human adrenal responded as sensitively as did the adrenal of experimental animals was widely confirmed a few years later by indirect evidence based on the behaviour of circulating eosinophil cells. This sensitive response to the varying stresses and strains of daily life, both mental and physical, provided valuable evidence of the reality of the psychosomatic response: the behaviour of the adrenals of the crews, coxswains, and coaches in the Harvard-Yale boat race was an intriguing example (Renold *et al.*, 1951).

Porter and Silber (1950) developed their sensitive chemical side-chain reaction for determination of adrenal steroids, and Nelson and Samuels (1952) applied this to the plasma levels. Because the reaction was by no means specific, purification stages had to be introduced, and optical difficulties owing to the very small volumes led to failure in many laboratories. In spite of this, human adrenal cortical behaviour—in both health and disease—was elucidated by this method combined with skill and enthusiasm. Clinically the method was little accepted even when subsequent modifications made it more reliable (Petersen *et al.*, 1957).

For some years the adrenal type of steroids had been known to fluoresce powerfully when dissolved in sulphuric acid and exposed to a suitable wavelength of incident light. Two properties of the molecule were shown to be essential for this reaction—a hydroxyl group on C11 and a double bond of unsaturation between C3 and C4. The first of these is unique to steroids of adrenal cortical origin in the body, and the second is essential to their hormonal activity. Because it required both features in the molecule, the reaction had a very high degree of specificity: it was given mainly by cortisol and corticosterone—the chief adrenal cortical hormones—and not in significant degree by the breakdown products. In this, and its great sensitivity, it differs sharply from the Porter and Silber reaction.

The high specificity of this fluorescence reaction is very important to the clinician, because De Moor *et al.* (1960) in Belgium and Mattingly (1962) at Hammersmith Hospital developed a rapid and simple method for estimating the plasma adrenal cortical hormones. When this method is used they are referred to as the 11-hydroxycorticosteroids to distinguish them from the older 17-hydroxycorticosteroids, based on the Porter and Silber reaction. The reaction is so sensitive that a fraction of a microgram of cortisol in 5 ml. of plasma can be estimated with acceptable accuracy. Mattingly's (1962) normal range of 11-hydroxycorticosteroids is from 6.5 to 26 μg./100 ml., with a mean of 14 μg., and he regularly performed unaided six complete analyses in one and a half hours. The method required meticulous attention to cleanliness and detail. If delegated to poorly supervised technicians because of its apparent simplicity it will speedily sink into disrepute. However, the method has already revolutionized clinical endocrine practice because it can produce, within a matter of hours, the answers to many clinical problems.

One further technical advance, which is complementary to the preceding, has been of major clinical significance. The synthesis of adrenal steroid hormones labelled firmly in the molecule with either ^{14}C or tritium has made possible the direct estimation of the actual rate of production of these hormones in man, and the relatively high degree of accuracy and reliability are both well maintained at low levels of adrenal activity (Cope and Black, 1958a). This can be done with cortisol because its specific metabolism makes it possible to apply the fundamental principle of isotope dilution to the problem. It is unreliable

* Lumleian Lecture (slightly abbreviated) delivered at the Royal College of Physicians of London on 7 March 1966.

† Physician, Postgraduate Medical School of London.

with some of the other adrenal hormones because their metabolic pathways are more complex. Relatively simple techniques are adequate for measuring cortisol secretion, but a number of conditions (which need not be enumerated here) are essential for valid estimates. Thus with negligible inconvenience to the patient, and with exposure to isotopic radiation no greater than arises from a few hours' stay in Aberdeen (Cope and Black, 1959b), the actual secretion rate of cortisol can usually be measured with an error of probably less than 10%. Unlike plasma-cortisol assays, these secretion-rate studies require isotope measurement and are less rapidly completed. They are unlikely in the near future to become routinely available, but their great value clinically is in providing reliable objective information in the difficult case, and also a reliable standard of reference for judging the validity of other more easily applicable tests. This is important because before the development of secretion-rate techniques the only criterion of validity of an adrenal-function test was that it agreed well with the clinical impression ; it could not therefore give a reliable lead when clinical doubt and judgement faltered.

Thus during the past 10 years methods of clinical study have been developed which can give precise answers for the individual patient to almost any question from the clinician relating to cortisol, corticosterone, or aldosterone metabolism. Our diagnoses and actions need no longer be based on vague speculation about these hormones. But the very precision of the techniques available necessitates finer judgement on the clinician's part in posing his questions.

Cushing's Syndrome

Cushing's syndrome illustrates this well, not because of its frequency, but because it represents a relatively pure disturbance of cortisol metabolism (overproduction), which is amenable to precise quantitative expression. It follows that demonstration of a normal cortisol secretion rate must exclude the diagnosis, except possibly in those very rare cases with paroxysmal hypercorticism. The diagnostic validity of the secretion-rate test in this respect is very nearly 100%, and I know of no reported exception. The reverse, however, is less true: a raised cortisol secretion rate can have several causes besides Cushing's syndrome. The normal range is from 7 to 26 mg. daily (Cope and Black, 1958b) ; but 26 recent cases of Cushing's syndrome studied in this way, with daily cortisol secretion rates above 35 mg. and below 120 mg., have all ultimately come to operation for proper clinical indications (Fig. 1). The upper limit needs to be imposed for cases requiring active therapy because higher secretion rates are likely to result, either from inoperable carcinoma of the adrenal cortex, or from hypercorticism caused by bronchial oat-cell carcinoma, or other non-endocrine tumour not amenable to operation.

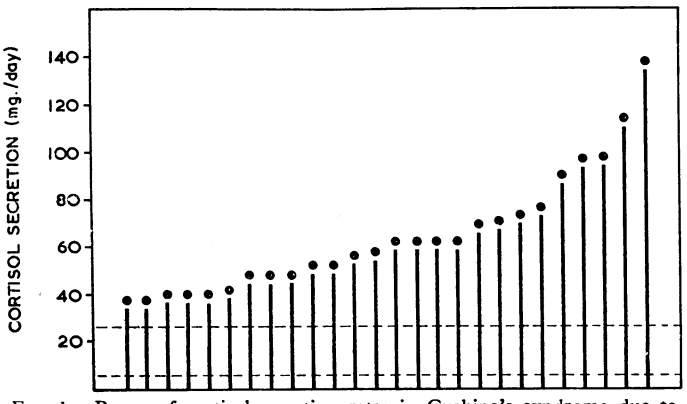

FIG. 1.—Range of cortisol secretion rates in Cushing's syndrome due to hyperplasia. Normal range between horizontal lines (7–26 mg.). (From Cope and Pearson, 1965.)

One might reasonably expect a rough correlation between the severity of the clinical picture and the height of the secretion rate, but this does not emerge, and traditional clinical criteria are unreliable in estimating the intensity of adrenal deviation. Thus it would be quite wrong to use the cortisol secretion rate as an indication for active therapeutic intervention. Medical hypophysectomy has recently benefited two subjects with secretion rates between 25 and 35 mg. daily and severe clinical symptoms (excessive osteoporosis with continuing calcium loss, and hypertension and resistant diabetes). But interference in adults with lower cortisol secretion rates—that is, within the normal range—is not advisable, since the symptoms are unlikely to improve. During the past seven years the secretion rate has been a valuable guide, especially in borderline cases, because it precisely measures the adrenocortical activity ; but the severity of the illness and the need for treatment are not determined by that factor alone, and clinical judgement is still needed.

For most clinical situations this test will not be available for many years to come, however, and the now traditional 17-oxogenic steroid assay will still be used.

Comparison of this analysis with the actual cortisol secretion rate measured at the same time shows that when adrenal cortical activity is high the 17-oxogenic steroid excretion averages about half the actual cortisol production, though with considerable scatter (Fig. 2) ; as activity falls towards the normal correlation becomes more blurred ; and at levels where the clinician needs most guidance the 17-oxogenic steroid assay is least reliable. Thus in a series of 100 the likelihood of a 17-oxogenic steroid excretion of less than 15 mg. daily being associated with a cortisol secretion above the upper limit of normal (26 mg.) was 14 in 60, or 23.3%. The 10 to 17.5 mg. range can be particularly misleading: among 56 subjects with 17-oxogenic steroid excretions in this range 13 had cortisol secretion rates between 25 and 35 mg., and a further nine—nearly all with florid hypercorticism requiring active treatment—had secretion rates between 35 and 60 mg. daily.

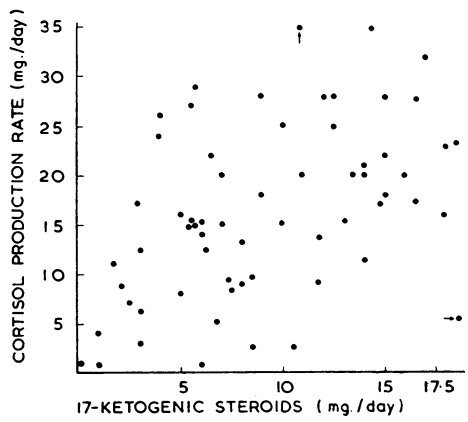

FIG. 2.—Relation of 17-ketogenic steroid excretion to cortisol secretion rate in near normal range. (From Cope and Pearson, 1965.)

The ambiguities in the interpretation of the 17-oxogenic steroid assay are such that we must turn to the plasma 11-hydroxycorticosteroid assay, which is likely to became widely available in the near future. It has been extensively explored by Mattingly (1962, 1963) and Mattingly and Tyler (1965).

The 11-O.H.C.S. values on plasma drawn at 9 a.m. show, even in clinically evident Cushing's syndrome, a considerable overlap with the normal range, the proportion being 29 out of a total of 106 readings—that is, 27.4% (Fig. 3). This is not surprising when one recalls the many transient causes for a raised figure, the adrenal cortex being very labile in health, though such lability is apt to be lost in disease. The healthy adrenal exhibits a very pronounced diurnal rhythm, in which the plasma 11-O.H.C.S. falls to low levels in the evening and night: the mean midnight value in 10 normal subjects (Mattingly, 1963) was only 3.3 μg.—a fifth

of the morning value. This diurnal rhythm persists with mild stress, though it may be overcome in a severely ill patient, but the overacting gland in Cushing's syndrome tends to maintain this overaction throughout the 24 hours: the diurnal rhythm is either absent or very greatly reduced in nearly all cases, whether due to hyperplasia, adenoma, or carcinoma. A blood sample drawn at midnight greatly enhances the diagnostic significance of the plasma 11-O.H.C.S. assay, especially if the patient has slept. Of 27 assays so performed in subsequently proved hypercorticism all exceeded the upper limit of the normal range for that time. Raised cortisol secretion rates may be encountered with normal *morning* plasma 11-O.H.C.S., but no patient has yet shown normal plasma levels during the late night hours.

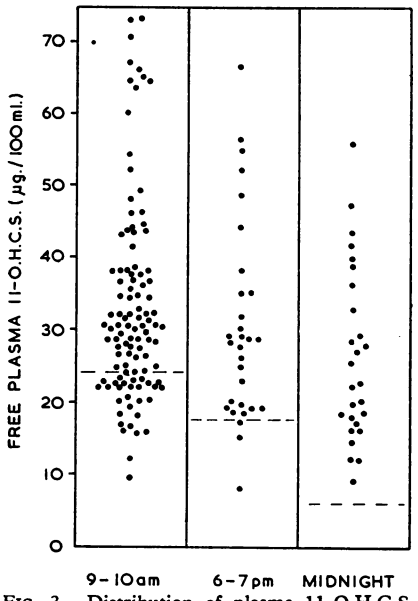

FIG. 3.—Distribution of plasma 11-O.H.C.S. levels in proved Cushing's syndrome. Interrupted lines indicate upper limit of normal range. (Data of Mattingly.)

One further form of estimation is of clinical value in adrenal hypercorticism. Since 1953 cortisol has been known to be present in the urine. In 1959 it was reported (Cope and Black, 1959a) that, with increasing adrenal activity, the urinary cortisol rose faster than any other factor. In 12 cases of hypercorticism the mean rise was eight times the normal, compared with only four times for the next most sensitive index—the 17-oxogenic steroids. This sensitivity seems to result because when plasma cortisol rises the proportion remaining free and unbound to protein increases greatly, and this determines the urinary content (Beisel *et al.*, 1964). The urinary free cortisol, which has proved for us a valuable diagnostic tool for about 13 years, has not achieved the popularity it deserves, probably because few have the time or ability for the analyses. Its use is now being explored in the United States. We have found that, with a cortisol excretion below 100 µg. daily, Cushing's syndrome is very improbable (Cope and Black, 1959a) ; see also Rosner *et al.*, 1963 ; Harris and Crane, 1964).

The outstanding value of the fluorescent method for plasma cortisol in the form of 11-O.H.C.S. clearly made it desirable to apply the same technique to urinary cortisol. But urine is very impure, and the prospect of success with a simple technique was low. The relatively good correlation between the simply determined urinary 11-O.H.C.S. and the actual secretion rate of cortisol was a purely empirical and unexpected discovery. It gives a better correlation with actual cortisol secretion than does the 17-oxogenic steroid excretion (Fig. 4). We therefore proposed this assay as a screening test for adrenal overactivity (Mattingly *et al.*, 1964), believing it to be the first adrenal test offered which has been checked against the cortisol secretion rate as reference standard. In numerical terms we can say that daily excretion of 220 µg. or less gives a 95% chance of a normal or low cortisol secretion, and an excretion of 500 µg. a 66% chance of a raised cortisol secretion. This simple method does not estimate urinary cortisol alone, but also corticosterone, 20 α-hydroxycortisol, and possibly other steroids. Espiner (1965), who measured the true cortisol content in my laboratory by internal isotope checks, found a variable composition of 11-O.H.C.S., the mean cortisol content being only about 30% of the total, though occasionally it was as high as 80%.

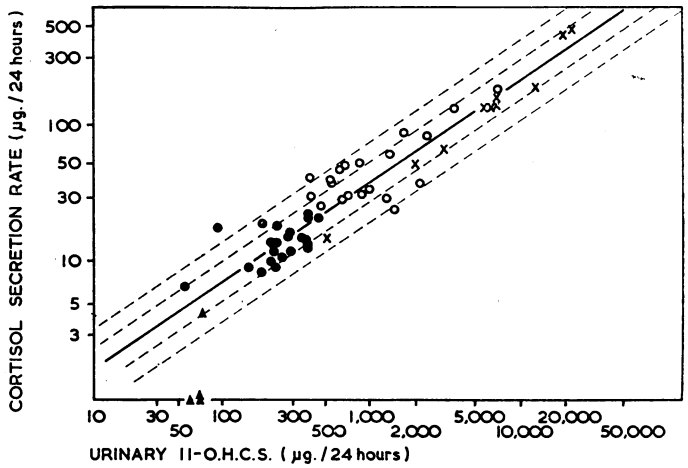

FIG. 4.—Rapid screening test. Relation of urinary 11-O.H.C.S. to cortisol secretion rate. Log-log scale. (From Mattingly *et al.*, 1964.)

Pal and Smith (1965) have modified the simple method, claiming as a result that only cortisol is measured, but their evidence scarcely justifies this conclusion. Though quite rapid, the simple urine test is of positive value in diagnosing hypercorticism, and it is probably more useful than the usual 17-oxogenic steroids, because correlation with the cortisol secretion rate is much better. The test is still on trial, however, and much more experience is needed before its true value can be reliably assessed.

Suppression Tests

Various forms of suppression test are used to elucidate the mechanisms of hypercorticism. Apparent autonomy, or lack of corticotrophin dependence, as implied by complete failure of dexamethasone suppression, suggests an adenoma or carcinoma, and treatment by direct inspection of the adrenals with subtotal adrenalectomy is indicated ; moreover, medical or surgical hypophysectomy is strongly contraindicated. Rapid plasma 11-O.H.C.S. assays are admirable for observing such responses, but they are even better adapted to the incomplete suppression tests which commonly utilize a 2-mg. dose of dexamethasone, as originally advocated to differentiate hyperplastic forms of hypercorticism from the normal (Liddle, 1960). In practice it is rarely possible to make such a sharp distinction between normal and abnormal degrees of function, and this test not infrequently fails (see Braverman *et al.*, 1965).

The data on dexamethasone suppression, studied in our clinic by 11-O.H.C.S. assay in confirmed cases of bilateral hyperplasia, have been analysed by Mattingly. Even with a full 8-mg. dose of dexamethasone the sensitivity to inhibition ranges from a nearly normal ease of suppression to a high degree of resistance to suppression which is only slightly less than that found in carcinoma or adenoma (Fig. 5). At either end of this continuous spectrum of responses ambiguity of interpretation easily arises ; these are the cases which tend to be reported. Easy suppression with a 2-mg. dose should not counter a diagnosis of Cushing's syndrome if there is cogent evidence of sustained hypercorticism ; similarly doubtful or slight suppression with 8 mg. does not completely exclude hyperplasia, but it does call for inspection of both adrenals at open operation. The sup-

pression test thus greatly influences both diagnosis and therapeutic policy. With the plasma 11-O.H.C.S. assay results can be obtained in little more than 24 hours ; and with an intravenous infusion of dexamethasone the test can be completed in three to four hours (James *et al.*, 1965).

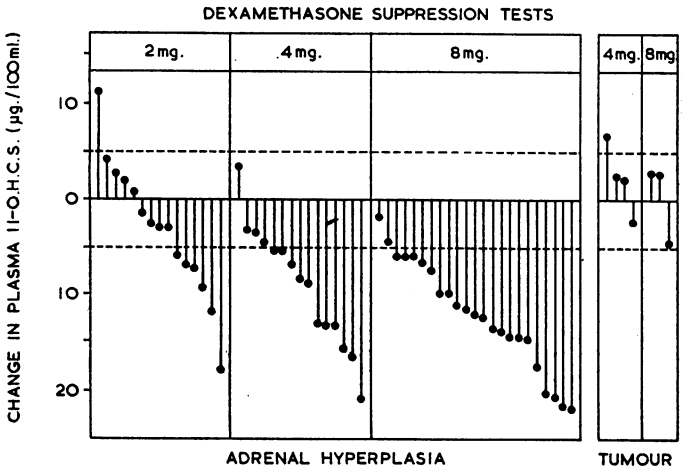

FIG. 5.—Range of responses to suppression by three doses of dexamethasone in cases of Cushing's syndrome due to hyperplasia and tumour. (Data of Mattingly.)

Hypoadrenalism

In theory the same tests can be used to diagnose hypoadrenalism, but in fact this is not so. Absence of cortisol from the urine is not abnormal and does not necessarily indicate underfunction of the adrenal. The urinary 11-O.H.C.S. is too insensitive, like the 17-oxogenic steroids, to distinguish clearly between absent adrenal function and the low degree of function encountered in some wasting disorders, yet this distinction is important.

If cases with total surgical adrenalectomy are excluded the lowest degrees of adrenal cortical activity are found in clinical panhypopituitarism. The cortisol-secretion-rate technique gives adequately accurate results even at these low levels ; 14 cases of panhypopituitarism showed daily values of 0.6 to 2.1 mg., with a mean of 1.4 mg. (Cope and Pearson, 1965). Measurements as low as this provide valuable criteria of the effectiveness of medical hypophysectomy by ^{90}Yt or other implant. Although therapeutic benefit correlates poorly with suppression of cortisol secretion, nevertheless persistent cortisol secretion, by indicating incomplete pituitary damage, may explain a disappointing therapeutic result and justify the insertion of further implants. At low levels other measures of adrenal activity are probably not sufficiently sensitive to make such a delicate distinction, and even plasma 11-O.H.C.S. concentrations are likely to prove less sensitive for this purpose.

Addison's disease, in which the cortisol secretion may be anywhere between normal and nearly zero, must be distinguished from hypoadrenalism. First, the presenting symptoms are due more commonly to aldosterone lack than to cortisol deficiency. Secondly, the adrenal cortex possesses appreciable powers of recuperation when an actively destructive tuberculous process has been controlled. The result may be an adequate but fixed, not necessarily reduced, output of cortisol, which is characteristic of Addison's disease. Haydar *et al.* (1958) have collected seven such cases ; and I have a patient who had been in Addisonian crisis in 1935 but had recovered sufficiently by 1939 to serve in a Guards Regiment in France without hormonal replacement, having a fixed output of 19 mg. daily.

When testing a suspect with corticotrophin stimulation one need not withhold maintenance steroids if the response is to be judged by the plasma 11-O.H.C.S. rise, because the analogues are not included in this estimate. But to distinguish clearly between true lack of response and sluggish stimulation induced by maintenance therapy, it is desirable to continue the test for three to four days, and to estimate plasma 11-O.H.C.S. daily before reaching a conclusion.

Pseudohypoadrenalism

True hypoadrenalism is relatively uncommon, though it is quite rightly often suspected: pigmentation, low blood-pressure, hyponatraemia, and low 17-oxosteroid and 17-oxogenic steroid excretions are common to a number of wasting disorders, such as advanced tuberculosis, widespread neoplasia, and malabsorption.

Shuster (1957), using the diagnostic tests then available, concluded that the overall picture in advanced tuberculosis suggested a selective hypopituitarism affecting secretion of corticotrophin. This illustrates the inadequacy of these earlier tests, for in the great majority of such cases adrenal function is in fact hyperactive. For more than 10 years we have shown this by demonstration of free cortisol in the urine of such patients, a simple way of establishing the essential adequacy of adrenal function: urinary excretions of more than 50 µg. daily are usual. Today the best way of disproving hypoadrenalism in wasting conditions is by plasma 11-O.H.C.S. assay: the answer comes the same day, the value being above rather than below the normal range. Shuster (1960) subsequently found a mean plasma 17-O.H.C.S. of 25.9 µg., compared with a normal of 16 µg., in 29 such subjects, of whom 13 had levels above the normal range.

Another form of "pseudohypoadrenalism" is found among the varied types of collapse or shock after surgical operations. Such collapse in patients who have previously undergone long-term steroid therapy brings the adrenal at once under suspicion ; and if the clinical condition is improved by intravenous cortisol the diagnosis is often regarded as practically proved. Yet this is very false logic. The number of such collapses with real proof of hypoadrenalism is surprisingly small ; the first proved case in this country was reported by Sampson *et al.* (1961). True adrenal failure as a cause of shock in these circumstances must be rare. Mattingly and Tyler (1965), at Hammersmith, measured the plasma 11-O.H.C.S. in 14 post-operative and 23 acute medical patients in circulatory collapse, some of whom had had previous steroid therapy: in all of them the plasma 11-O.H.C.S. was 20 µg. or above, a figure near the upper limit of normal (Fig. 6), though many had shown apparent clinical benefit from intravenous cortisol.

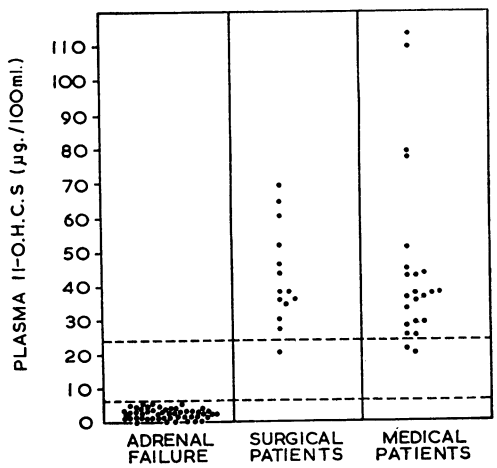

FIG. 6.—Plasma 11-O.H.C.S. values in true adrenal failure and in medical and post-operative shock. (From Mattingly and Tyler, 1965.)

Yet another form of pseudohypoadrenalism has been described by Kyle *et al.* (1961) in chronic leukaemia treated with busulphan ; but since the urinary 17-oxosteroids and 17-O.H.C.S. and the corticotrophin response were normal, no confusion in diagnosis need ever arise.

Weaning from Steroids

Suspicion of hypoadrenalism is high during the process of weaning patients from steroids, especially when therapy has been long-continued. In spite of much work on adrenal recovery after steroid therapy, almost all clinicians have had to, and still do, conduct such weanings with a blind faith. But the effects of tailing-off the steroids, or of giving stimulating doses of corticotrophin, are ideally suited to control by rapid plasma 11-O.H.C.S. assay, not only because of the speedy results but also because the validity is in no way impaired by simultaneous administration of a corticosteroid analogue, which it is quite unnecessary to withhold. It is relatively simple to watch the revival of the patient's own adrenal activity independently of the exogenous steroid. During steroid weaning the physician needs reassurance on two main points: first, that the patient's adrenal—atrophied by lengthy steroid therapy—can be aroused to sufficient activity either by his own or by administered corticotrophin ; and, secondly, that this activity can be maintained by the patient's own corticotrophin production. The plasma 11-O.H.C.S. gives quick answers to both these points. Adrenal activity can be very readily restored by corticotrophin administration, even after several years of continuous steroid therapy (Fig. 7)—indeed, the rate of revival seems to be little

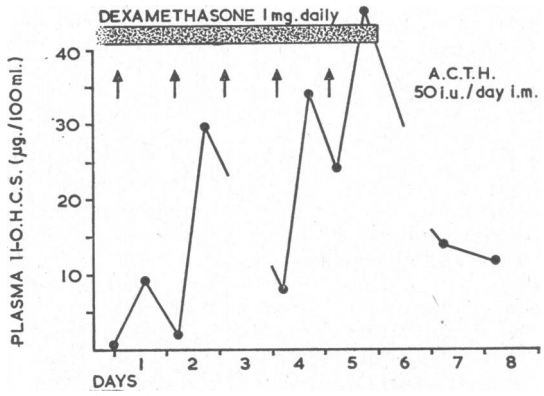

FIG. 7.—Weaning from steroids after seven years of therapy. Control of stages by plasma 11-O.H.C.S. assay.

less than after a brief course of steroids. The observed rise in plasma 11-O.H.C.S. after each injection rapidly falls as its effect wears off. But this alone gives no guidance on the efficiency of the patient's own corticotrophin-releasing mechanism. When no corticotrophin has been given, the patient's own endogenous sources being utilized, near normal plasma levels are usually restored in 48 hours, provided the steroid therapy is first stopped. We found (Robinson *et al.*, 1962) that this was true in all save one of 13 cases. Roe *et al.* (1966) also found that plasma 17-O.H.C.S. levels had risen to normal or

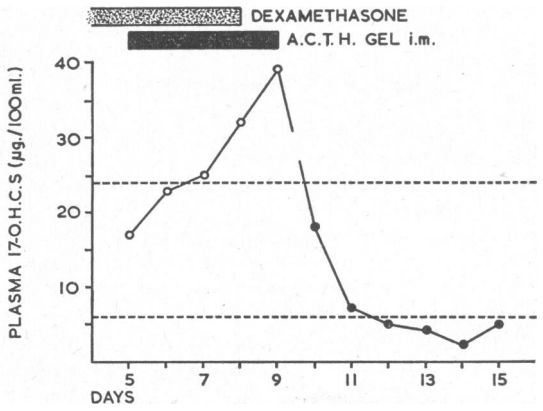

FIG. 8.—Failure of steroid-weaning. Endogenous corticotrophin unable to sustain plasma 11-O.H.C.S. (From Robinson *et al.*, 1962.)

above within 48 hours of stopping long-term steroids in all of 20 patients examined. If spontaneous revival of activity has occurred it will persist ; when corticotrophin has been given this cannot be assumed, although it is usual. A further plasma 11-O.H.C.S. should therefore be taken three days after the last dose of corticotrophin to ensure continuing activity (Fig. 7).

Thus restoration of adrenal activity after a course of steroids should present no real problem, and the very rare failures of weaning can be easily detected (Fig. 8).

Adrenal Recovery

Full responsiveness of the pituitary-adrenal axis is not regained for several months.

The response to metyrapone is reduced for as long as six weeks after the weaning (Farmer *et al.*, 1961), and there is a severe reduction shortly after stopping steroids, but only if the steroid course has lasted for more than a year (Treadwell *et al.*, 1963). As much as 300 mg. of cortisol daily for 30 days had no detectable effect on the metyrapone response in 11 normal adults (Danowski *et al.*, 1964), but after steroid therapy lasting more than a year unresponsiveness was found, though it usually persisted for less than five weeks. Tucci *et al.* (1965) followed up three patients after unilateral adrenalectomy for a functioning adrenal adenoma: compensatory hypertrophy of the surviving adrenal, as judged by urinary steroid excretion, was incomplete for four to nine months. But two similar cases (Kyle *et al.*, 1957) showed evidence of damage for up to two years. Graber *et al.* (1965) studied six patients who had had long-term steroid therapy and eight treated cases of Cushing's syndrome. After maintenance steroids were stopped both plasma A.C.T.H. and plasma 17-O.H.C.S. remained low for two months. In the next three months A.C.T.H. rose to normal levels or above, though it was still low relative to the plasma 17-O.H.C.S. Only after five to nine months did both the plasma 17-O.H.C.S. and the plasma A.C.T.H. return to their normal levels and relationships. This correlates well with the clinical impression.

To summarize the points of fairly general agreement, after short courses of steroids the pituitary-adrenal axis is little disturbed, and weaning should be trouble-free. After courses lasting a year or more spontaneous revival of adrenal cortical activity will usually occur within 48 hours, but in rare cases it may fail completely. During the weaning period corticotrophin will assist the revival, but its value is short-lived and it will not affect subsequent maintenance. The pituitary-adrenal axis continues to be subdued for up to five months after weaning, though its activity is usually adequate for ordinary maintenance purposes.

Action of Metyrapone

Metyrapone inhibits the 11-hydroxylating mechanism in the adrenal, so that it produces 11-deoxycortisol instead of cortisol. This inhibition is commonly assumed to be absolute, the stimulus to increased adrenal activity being complete lack of cortisol from the blood-stream. But this is not true: even very large doses of metyrapone to the limit of tolerance probably do not paralyse the enzyme system completely, and so long as traces of 11-hydroxylating power persist the adrenal will usually succeed in maintaining a normal plasma concentration of cortisol (Lazarus *et al.*, 1963). More recently we have simultaneously measured the secretion rates of both cortisol and 11-deoxycortisol in subjects taking metyrapone (Cope *et al.*, 1966), and so formed an estimate not only of the total adrenal activity but also of the degree of inhibition of the enzyme. Daily doses of 4.5 g. of metyrapone usually produce 95% inhibition of the enzyme system, but the frequently employed 3-g. daily dose has much fewer and much more variable effects, the range being from 66 to 95% inhibition. The actual adrenal response to the smaller dose is also very variable (Fig. 9), and if this response is measured by one of the more indirect indices—such as urinary 17-oxogenic steroid assay—still further variability will be apparent in the final response.

This is almost certainly the main cause for the widespread dissatisfaction with the metyrapone test as normally carried out. In fact the normal healthy adreno-pituitary axis responds to this drug according to the degree of inhibition of enzyme: if this is incomplete a wrong impression of a sluggish response can be created. A daily dose of only 3 g. will not ensure adequate enzyme inhibition. The magnitude of the stimulus to adrenal activity rises asymptotically with increasing efficacy of the drug until, with complete inhibition, it would become infinite (Fig. 10), though this is never achieved in practice.

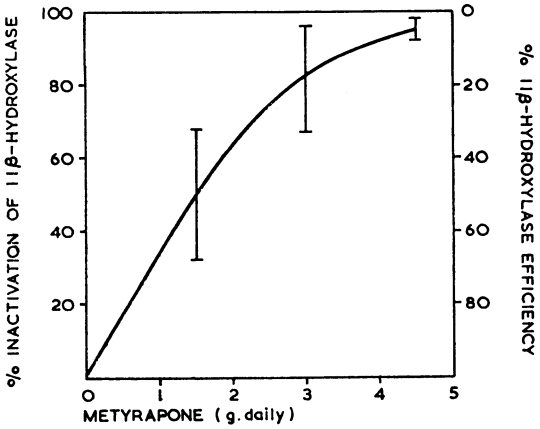

FIG. 9.—Dose-response curve for metyrapone inactivation of adrenal 11β-hydroxylase. (From Cope *et al.*, 1966.)

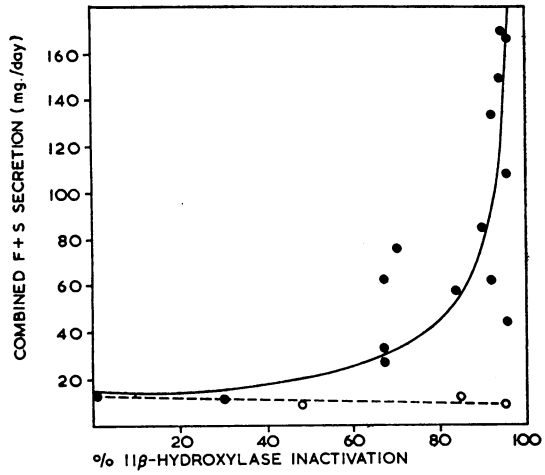

FIG. 10.—Adrenal response to progressive 11β-hydroxylase inactivation. (From Cope *et al.*, 1966.)

Reaction to Stress

We must not use conclusions about adrenal recovery to predict reaction to stress. The response to severe stress calls for greater corticotrophin release than does the restoration or maintenance of normal adrenal activity. Subjected to severe stress, a normally responsive adrenal should raise the plasma 11-O.H.C.S. or 17-O.H.C.S. to above 30 μg./100 ml., and in extreme cases to above 100 μg.—that is, two to seven times the normal. There is widespread suspicion, therefore, that the pituitary-adrenal axis, when damaged by long-continued steroid therapy, will be inadequate for the demands of stress, even though normal activity may be well within its scope. But direct evidence of this is scanty and difficult to acquire, for we know that the stress response cannot be predicted from the ordinary tests applied to the pituitary-adrenal axis.

Evidence is accumulating that this response is largely independent of the normal control of plasma cortisol by the sensitive feed-back mechanism, which can be so effectively inhibited by steroid therapy. After remaining dormant for a year or more this feed-back mechanism can revive spontaneously, though it may be sluggish for several months.

It is this feed-back mechanism which is tested by metyrapone, and which can be inhibited by steroid implantation into the hypothalamus (Smelik and Sawyer, 1962; Davidson and Feldman, 1963). It is also damaged in certain states of head injury (McCarthy *et al.*, 1964), which respond to the stress of an injected pyrogen yet not to metyrapone. Van Wyk *et al.* (1960), for therapeutic reasons in women with carcinoma, abolished the feed-back mechanism by pituitary-stalk section, and yet an adequate stress response persisted. Oppenheimer *et al.* (1961), studying central nervous system disease, found a good stress response in a subject with a temporal-lobe epilepsy, although his feed-back mechanism had been disturbed. Farmer *et al.* (1961) observed several patients who, after a year of steroid therapy, responded well to injection of a pyrogen but not to metyrapone. Arnoldsson and Helander (1963) stopped long-term steroid therapy in 11 bronchial-asthma patients and on the fourth day found a normal plasma 17-O.H.C.S. response to artificial fever.

Clearly the stress response cannot be tested by observing the rate of recovery after weaning from steroids nor by corticotrophin stimulation, dexamethasone suppression, or the response to metyrapone. That the stress response is determined by a mechanism independent of that controlling normal plasma cortisol has been elegantly demonstrated by Estep *et al.* (1963).

Using the acute and severe stress of pelvic laparotomy, they compared the adrenal responses of subjects given no extra steroid with those of subjects given large doses of dexamethasone before and during operation. Without steroids the plasma 17-O.H.C.S. rose from 17 to 36 μg., with a mean of 19 μg. With an intramuscular injection of 8 mg. of dexamethasone phosphate the mean rise was 26 μg. The rises in urinary 17-O.H.C.S. were respectively 12 and 10 mg. Thus the inhibition expected from the action of dexamethasone on the feed-back mechanism was completely overruled by the strength of the stress response. Even slow administration of dexamethasone phosphate by intravenous drip failed to inhibit the stress response, the plasma 17-O.H.C.S. rise being 21 μg. with a drip of 5 mg. an hour, and 30 μg. with 10 mg. an hour. Nor was this failure due to the use of the synthetic analogue, for the plasma corticotrophin showed the usual rise after operation, even if the plasma cortisol was as high as 200 μg./100 ml. It was even shown that adrenal activity persisted during stress when the plasma level was raised to 500 μg. by cortisol infusion. These penetrating experimental studies leave no doubt that the stress response is almost completely independent of the normol homoeostatic feed-back control, which indeed it completely overrides. The body once again proves much more complex than has been suspected. Experiences with animals have been similar. In rats corticosterone doses sufficient to raise the blood concentration to four times the maximal stress level did not diminish the rise in plasma corticotrophin provoked by stress (Hodges and Jones, 1963; Smelik, 1963; Egdahl, 1964).

The overriding independence of the stress-response mechanism in man has been clearly recognized in the past five years. We are no longer justified in making predictions about the stress response from observations of the feed-back mechanism, and we must therefore turn to the evidence of clinical observation itself.

Post-steroid-therapy Collapse

As clinical evidence, mere records of shock or collapse in vulnerable subjects are not enough. Winstone and Brooke (1961) observed fatal collapse in two patients with ulcerative colitis who came to operation without steroid cover, and suspected this was due to pituitary-adrenal failure; they encountered no such collapse in patients who had never had steroids or who had proper steroid cover for the operation. But the vast majority of such incidents seem to be associated with medical diagnostic, and not adrenal, failure: the last three that I have seen were shown at necropsy to be associated with aqueduct stenosis, acute miliary tuberculosis, and *Bacillus pyocyaneus* septicaemia. Marks *et al.* (1959) saw a serious degree of collapse develop in a patient who was actually receiving

corticotrophin, and whose plasma cortisol was well above normal and still rising. Post-operative collapse is very rarely associated with a low plasma cortisol: those few cases in which normal corticotrophin production is not restored will be diagnosed in the few days after steroid-weaning, when hypoadrenal collapse presents a medical emergency.

Evidence of later failure of the stress response is remarkably scanty, however. Although the assay of plasma corticosteroids has been possible for more than 10 years, recorded low values with post-steroid-therapy collapse are disappointingly rare: the only authenticated example was reported by Sampson *et al.* (1961). This remarkable lack of data on a widely suspected phenomenon makes one ponder in an age eager to apply every new scientific advance. It is a challenge to present-day clinicians to obtain unequivocal evidence when opportunity presents. Until this is properly done its frequence must remain a matter of speculation. But the lack of evidence after at least five years' search implies that it is a great rarity.

The fundamental requirements are the demonstration of low or normal plasma-cortisol levels during severe stress in a subject who had normal adrenal function before steroid therapy, because unsuspected cases of Addison's disease may be revealed by their inadequate reaction to stress, and in these failure of the stress response clearly cannot be imputed to steroid therapy. We still eagerly await evidence that adrenal failure of stress response is a significant risk after steroid therapy.

[*Part II, with a list of references, will appear in our next issue.*]

Immunosuppressive Therapy in Steroid-resistant Proliferative Glomerulonephritis Accompanied by the Nephrotic Syndrome

R. H. R. WHITE,* M.A., M.B., M.R.C.P., D.C.H.; J. S. CAMERON,† M.D., B.SC., M.R.C.P.
J. R. TROUNCE,‡ M.D., F.R.C.P.

[WITH SPECIAL PLATE]

Brit. med. J., 1966, **2**, 853–860

Corticosteroid therapy is well established in the management of the nephrotic syndrome due to primary renal disease. Its use has led to increased survival both in children (Riley and Scaglione, 1959; Arneil, 1961) and in adults (Hardwicke, 1965a), and to the achievement of more rapid relief from oedema, especially in children. However, with the objective sign of disappearance of proteinuria as the criterion of complete remission, about 85% of adults (Blainey *et al.*, 1960; Pearl *et al.*, 1964) and 36% of children (Arneil, 1961) ultimately fail to respond to steroids. The difference between adults and children is largely explained by the frequent histological finding in children of "minimal change." Steroid-resistant patients form a high proportion of cases referred to special clinics, and most of them are severely ill; we find that the majority of adults and children in this group have proliferative glomerulonephritis.

In this paper we describe our early experiences with immunosuppressive therapy in a group of 18 nephrotic patients suffering from proliferative glomerulonephritis. The literature on the use of immunosuppressant agents is summarized in Table I, from which it can be seen that mechlorethamine (nitrogen mustard) had been used in the pre-steroid era (Chasis *et al.*, 1949, 1950; Taylor *et al.*, 1950; Kelley and Panos, 1952). However, with the advent of prednisolone the pattern of therapeutic response improved so dramatically in children (Arneil, 1961) that the demand for more toxic drugs was no longer felt. Nevertheless, the literature contains occasional reports of the combined use of steroids and mechlorethamine in treating nephrotic children (Lestina *et al.*, 1953; Greenman *et al.*, 1955; West, 1958; Coldbeck, 1963), but the results were not convincingly better than those of steroid therapy alone.

More recently interest in the use of immunosuppressant agents has revived owing to the recognition of steroid-resistant forms of the nephrotic syndrome (Lagrue *et al.*, 1962, 1964; Goodman *et al.*, 1963; Talamo and Crawford, 1963; Payet *et al.*, 1964; Drummond *et al.*, 1964; Shearn, 1965; Milliez *et al.*, 1965; Saxina and Crawford, 1965; West *et al.*, 1965b, 1966), and the accumulation of evidence that immune mechanisms are involved in certain forms of glomerulonephritis (Peters, 1963; Michael *et al.*, 1964). At the same time less toxic orally administered drugs became available. These drugs have also been used with some success in other disorders in which immune mechanisms are believed to be involved, especially systemic lupus erythematosus (Kellum and Haserick, 1963; Demis *et al.*, 1964; Hill and Scott, 1964; Cheng Siang *et al.*, 1966).

Material and Methods

All 18 patients developed, either initially or during the course of their illness, the nephrotic syndrome, which for the purpose of this paper is defined as heavy proteinuria and hypoalbuminaemia, at some time accompanied by oedema. Fifteen had previously been treated with prednisolone in adequate dosage, and three children had also received other steroids (dexamethasone, triamcinolone, and A.C.T.H.) on account of a poor therapeutic response. All 15 had persistent proteinuria despite steroid therapy, and 13 continued to have hypoalbuminaemia with oedema. Seven children were severely cushingoid and five patients (Cases 6, 10, 11, 15, and 17) developed signs of increasing renal insufficiency while on high doses of corticosteroids. One patient (Case 4) also had steroid diabetes.

The main clinical and laboratory findings are summarized in Table II, in which the patients are arranged in order of age. There were 13 children under 15 years old and five adults, the age at onset of symptoms ranging from 5 weeks to 71 years. Six children and one adult also had anaphylactoid purpura. The antistreptolysin-O titre was considerably raised in four children and two adults. Significant hypertension occurred in 14 patients, the figures of Haggerty *et al.* (1956) being used as a reference for children. In addition to protein an excess of red blood cells, as measured by 12-hour Addis counts, was

* Senior Lecturer in Paediatrics and Child Health, University of Birmingham; lately Assistant to the Director, Department of Paediatrics, Guy's Hospital Medical School, London.
† Senior Lecturer in Medicine, Guy's Hospital Medical School, London.
‡ Professor of Clinical Pharmacology, Guy's Hospital Medical School, London.