TECHNIQUES FOR THE EVALUATION OF ADRENAL CORTICAL FUNCTION BY THE USE OF ADRENOCORTICOTROPHIN : A REVIEW

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The number of conditions in which abnormal adrenal cortical function can occur is an ever-widening field, including certain well recognized endocrine abnormalities in addition to extremes of environment, trauma, "non-specific" disease processes, and surgical procedures which may excite the so-called "stress reaction" (Selye, 1946). In addition, alteration in adrenal cortical activity seems to occur in some psychotic states. A short classification of conditions in which adrenal cortical function varies from normal is as follows:

Increased Adrenal Cortical Activity

Cushing's syndrome
Adrenogenital syndrome
Oestrogen-producing tumours of the adrenal cortex
Active stages of acromegaly
Pharmacological response to adrenocorticotrophin therapy
"Resistance phase" of stress reaction

Decreased Adrenal Cortical Activity

Acute Insufficiency
Waterhouse-Friderichsen syndrome
Following adrenal operations
Chronic Insufficiency
Addison's disease
Panhypopituitarism
Myxoedema
"Exhaustion stage" of stress reactions
Schizophrenia
(Decreased adrenal cortical "functional reserve." Pincus, Hoagland, Freeman, and Elmedjian, 1949).

It is becoming of considerable importance to possess rapid and easy criteria for detecting variations of adrenal cortical activity. Before considering relevant laboratory, as opposed to clinical procedures, for detecting abnormalities of function, the present concept of the physiological relations of the adrenal cortex in the organism must be outlined.

Physiological Relations of the Adrenal Cortex

That the adrenal cortex is immediately under the control of the anterior pituitary, which secretes adrenocorticotrophin (ACTH) is indicated in Fig. 1. Since the early

FIG. 1.—Physiological relations of the adrenal cortex.

rapid advances have been made in the study of this substance. The activity of ACTH is mediated through the secretion of cortical steroid substances which are probably synthesized from the cholesterol contained in the gland (Long, 1947). The exact nature of the steroids secreted by the cortex is not known with certainty. Metabolic and clinical data show that activity which is known to be obtained with 11-oxygenated and 11-desoxy C_{21} steroids is manifest on stimulation of the adrenal cortex (Fig. 2). There is also evidence from the increased excretion of 17-ketosteroids to show that the C_{19} steroids are produced as a result of this stimulation, and previously urinary 17-ketosteroids were known to be derived from the adrenal cortex (Callow, 1938; Callow, Callow, and Emmens, 1938). The isolation of steroids from the adrenal suggests that 17-hydroxy-11-dehydrocorticosterone ("cortisone"; "Compound E" of Kendall) and 17-hydroxy-corticosterone ("Compound F" of Kendall) are synthesized by the gland (Reichstein and Shoppee, 1945; Haines, Johnson, Brunner, Pabst, and Kuizenga, 1949). These compounds are representative of 11-oxygenated C_{21} steroids, and they may also appear in the urine; for instance, large amounts of "Compound F" have been isolated from the urine of a patient with Cushing's syndrome by Mason and Sprague (1948). On the other hand very small quantities of desoxycorticosterone and C_{19} steroids only have been isolated from adrenals, and doubts exist that they may be artefacts of the process; it is therefore uncertain that the adrenal in fact synthesizes these compounds. Studies of the chemistry of 17-ketosteroids isolated from the urine suggest an origin of some at least of these excretory products as C_{21} adrenal steroids, which may be metabolized in the body at some site remote from the adrenal cortex, possibly the liver (Lieberman, Dobriner, Hill, Fieser, and Rhoads, 1948).

work of Evans (1923-4) and Smith (1930) showing that the anterior pituitary is required for adrenal cortical maintenance in animals, great strides have been made in the separation and preparation of a pure protein possessing the activity of ACTH (Collip, Anderson, and Thomson, 1933; Sayers, White, and Long, 1943; Li, Evans, and Simpson, 1943). This hormone has now been shown to be obtainable as an active peptide (Li, 1950; Morris and Morris, 1950). In man the purified protein ACTH has been found to stimulate all the known metabolic activities of the adrenal cortex (Forsham, Thorn, Prunty, and Hills, 1948; Mason, Power, Ryncarson, Ciaramelli, Li, and Evans, 1948), and since that time

It will be seen that the precise nature of the steroids secreted by the gland is not at present clear, nor is the endogenous metabolism of these substances nearly fully elucidated. Fig. 1 illustrates the possibility of stimulating the anterior pituitary

FIG. 2.—Formulae of some steroid compounds. Adrenosterone and dehydro-iso-androsterone are 17-ketosteroids; testosterone, for comparison, is a C₁₉ steroid but not a 17-ketosteroid.

to secrete ACTH by means of adrenaline. The experiments of Vogt (1945) with prolonged adrenaline injections in normal and hypophysectomized animals, and of Long (1947) using short-term experiments, would seem to indicate that adrenaline stimulates the anterior pituitary to secrete ACTH. There is an admitted difficulty in that an earlier experiment of Vogt (1944) showed that adrenaline could directly stimulate the cortex in a decapitated dog. Recent work by Spiers and Meyer (1949) using eosinophil counts in mice confirms that the main action of adrenaline is on the anterior pituitary, but there may be some direct action on the cortex. This matter is further complicated by the report of Hume (1949) showing that damage to certain parts of the hypothalamus results in failure of the pituitary-adrenal

system to respond to adrenaline, and suggesting that the latter acts directly on the former structure. Nevertheless the presence of an intact pituitary is necessary for the normal reaction of the organism to stress stimuli (Long, 1947). It seems then that it may be taken as a working hypothesis that adrenaline is capable of causing ACTH production by the anterior pituitary which results in adrenal cortical stimulation. Adrenal steroids are effective in acting as a break on ACTH secretion by the pituitary (Sayers and Sayers, 1949).

Indications of Disturbance in Adrenal Cortical Function

Before the use of ACTH for evaluating adrenal cortical function, reliance had to be placed on the use of procedures summarized in Table I. Included in Table I

TABLE I
METHODS OF ASSESSING ADRENAL CORTICAL FUNCTION

	Hypofunction (Addison's Disease)	Hyperfunction (Cushing's Syndrome)
A. C₂₁ STEROIDAL ACTIVITY		
1. 11-oxygenated steroids :		
(i) Carbohydrate metabolism		
Fasting blood sugar	Low	High
Intravenous glucose curve	Hypoglycaemic phase	Diabetic-like
Insulin sensitivity	Great	Small
(ii) Tissue response		
Lymphoid (circulating lymphocytes)	High	Low
Eosinophils	May be high	"
(iii) Steroid excretion		
Neutral reducing steroids ..	Low	High
2. Salt-retaining steroids :		
Electrolyte metabolism		
Haematocrit	High	—
Serum sodium	Low	High
Serum chloride	Low	Low
Serum potassium	High	"
Serum carbon dioxide	Low	High
Cutler Low NaCl, high K regime:		
Urine Na	High	—
Cl	"	—
Administration of desoxycortico-		
sterone acetate	Chloride retention	Chloride loss
3. Kepler diuresis test	Impaired	—
B. C₁₉ STEROIDS		
17-ketosteroid excretion	Low	Normal or high
3 β -hydroxy 17-ketosteroids	—	Sometimes high in tumours

is the use of eosinophil counts, the value of which did not, however, become apparent until later. The tests in this table are classified under the headings of the types of steroid, the function of which they may be considered to be an index. In many respects the table is self-explanatory, but a few comments may be needed. That important changes occur in the blood counts of patients with adrenal cortical abnormality was pointed out by de la Balze, Reifstein, and Albright (1946) as a result of experiments in animals at that time. Patients with Addison's disease have exces-

sive amounts of lymphoid tissue accompanied by high blood lymphocyte counts, and the reverse is true in Cushing's syndrome. In 1948 the importance of the eosinophil in relation to the adrenal cortex became apparent (Hills, Forsham, and Finch; Forsham *et al.*), and in Cushing's syndrome eosinopenia may be profound (Thorn and Forsham, 1949). In Addison's disease there may be a high eosinophil count, but statistically this is not significant and suggests that the control of the basal level of circulating eosinophils is governed by factors other than the adrenal cortex.

The rationale of the test described by Robinson, Power, and Kepler (1941), in which diuresis after the injection of water is impaired in Addison's disease, is more difficult to interpret. It is not restored to normal by the control of the patient with desoxycorticosterone, and there is some evidence that this alteration of adrenal function may be dependent upon deficiency of 11-oxygenated steroids (Gaunt, 1946). Delayed diuresis may occur in diseases other than Addison's disease where there is deficiency of water absorption, abnormal hydration, or renal insufficiency (Levy, Power, and Kepler, 1946). The specificity of the test is considerably enhanced by applying the second part described by the authors in which additional account is taken of the frequently associated decrease in urea clearance and increase of clearance of chloride. If this is done about 12% of cases give false negatives, and in practice it proves to be a most useful screening procedure.

Alterations in blood electrolyte composition cannot be regarded as very sensitive indices of changes of activity dependent upon salt-retaining steroids. In Addison's disease, even in instances where plasma volume is decreased, the normal concentration of electrolyte tends to be maintained until late, in spite of a total body deficiency of sodium. By the application of the test devised by Cutler, Power, and Wilder (1938) this system may be put under stress. The test represents a more carefully controlled procedure employing the older methods of applying stress to the adrenal by salt withdrawal. The patient is given a standard low salt diet with increased potassium intake for two days, and the excretion of sodium and chloride is estimated in the urine. In adrenal cortical failure the amounts of these remain high compared with the falling values in patients who do not have Addison's disease. In the absence of renal disease the test has a high degree of specificity (Willson, Robinson, Power, and Wilder, 1942), but suffers from the grave disadvantage that the patient with Addison's disease is subjected to considerable danger from the risk of an adrenal crisis, and for this reason it is becoming superseded by more recent techniques.

Excess secretion of salt-retaining steroids in Cushing's syndrome might be supposed to occur. The response of such patients to administration of desoxycorticosterone acetate has in fact been observed to be the reverse of that found in Addison's disease in 10 of 15 patients with increased adrenal cortical function (Soffer, Gabrilove, and Jacobs, 1949). The response to these steroids must therefore depend upon the existing salt balance in the body and on the magnitude of the activity of the adrenal cortex at the time of the observation (Prunty, 1949a).

"Neutral Reducing Steroids" and "Glyco-corticoids"

The estimation of the urinary excretion of "reducing steroids" has come to be of considerable value in assessing adrenal cortical function, in spite of the lengthiness of

FIG. 3.—Principles of the estimations of reducing steroids and glyco-corticoids.

the procedures. The principles of this estimation are shown schematically in Fig. 3, where it will be seen that a variety of methods are at present available. It is not yet known which of these techniques is the most valuable as an indication of cortical steroid excretion, but there is in disease a general correlation between them and the biological technique of Venning, Kazmin, and Bell (1946), based on the ability of the urine extracts to cause deposition of glycogen in the mouse liver. The test measures the glyco-corticoids which may be assumed to be a biological index of excretion of steroids of the 11-oxygenated class. This estimation of the glyco-corticoids is a somewhat laborious technique, not suited to general use. The application of the chemical methods is, therefore, more widely favoured, and technically probably the easiest is the molybdate method of Heard and Sobel (1946) for reducing

lipids. The use of the benzene: water partition step in the fractionation of the crude extracts and Girard separation favours the concentration in the extract of ketones with hydroxyl groups at the 11,17 positions, for example “Compound F.” It is nevertheless questionable whether these steps are entirely desirable in a test

TABLE II
CORTICAL STEROID EXCRETION BY DIFFERENT TECHNIQUES

	Sex	Range	Average
Venning and Browne (1949)	Females	25–65 units	41 units
	Males	40–85 units	60 units
Heard, Sobel, and Venning (1946) ..	Females	1.0–2.0 mg./day	1.3 mg./day
	Males	1.1–2.1 mg./day	1.5 mg./day
Talbot, Albright, Saltzman, Zygmuntowicz, and Wixom (1947)		0.1–0.44 mg./day	0.22 mg./day
Daughady <i>et al.</i> (1948b)		1.0–1.6 mg./day	—

designed to give a general indication of cortical steroid excretion. The normal adult values by these methods are summarized in Table II.

There is general agreement among the devisers of these methods that in Cushing's syndrome, in active stages of acromegaly, and in conditions of stress, a marked rise above the normal may be expected, whereas in Addison's disease and hypopituitarism low values are to be expected, particularly in the latter. The fact that the levels rarely reach zero in these conditions means that some of these substances are of extra-adrenal origin.

Urinary Excretion of 17-ketosteroids

The general processes involved in this estimation are shown schematically in Fig. 4. For routine use estimation of the total 17-ketosteroid in the neutral fraction by the Callow *et al.* (1938) method is most applicable, but in applying the test in patients with low values correction for non-ketosteroid chromogens by the colour method of Fraser, Forbes, Albright, Sulkowitch, and Reifenstein (1941) is necessary to avoid falsely high results. Decreases of 17-ketosteroid excretion may be observed in a wide range of debilitating diseases and occasionally under conditions of stress—e.g., post-operatively (Venning and Browne, 1949). Values approaching zero are to be expected in patients with widespread damage to the anterior pituitary, whilst levels below normal are obligatory in the diagnosis of Addison's disease; in 37 male patients with the latter condition the range of 17-ketosteroid excretion was 1.0–8.0 mg. per day with a mean of 4.2, mean deviation ± 2.7 , and 33 female patients 0.1–8.6 with a mean of 3.0 and mean deviation ± 2.0 (Thorn, Forsham, Prunty, Bergner, and Hills, 1949). In Cushing's syndrome related to adrenal cortical tumour the 17-ketosteroid excretion is frequently increased (Kepler, Sprague,

FIG. 4.—Principles of the extraction and fractionation of 17-ketosteroids.

Mason, and Power, 1948), and may be helpful in differentiating this from the syndrome associated with adrenal hyperplasia in which 17-ketosteroid levels are not greatly raised. High values for 17-ketosteroid excretion are frequently found in patients with the adrenogenital syndrome, and a value above 100 mg. per day is likely to be indicative of the presence of tumour rather than adrenal hyperplasia (Warren, 1945 ; Callow and Crooke, 1944), both of which conditions may occur in this syndrome. On the basis of the isolation of large amounts of dehydro-iso-androsterone, a 3 β -hydroxy-17-ketosteroid, in cases of tumour (Crooke and Callow, 1939 ; Mason, 1948) it has been suggested that this estimation is of value in the diagnosis of adrenal cortical tumour, values of not less than 50% of the total 17-ketosteroid being diagnostic of this lesion (Talbot, Butler, and MacLachlan, 1940a ; Callow and Crooke, 1944). The estimation of steroids of this type (Fig. 2) may conveniently be performed in two ways, by the precipitation of these compounds with digitonin and estimation of the 3 β -steroids in solution by the Callow method (Talbot *et al.*, 1940b), or by direct estimation in the ketonic fraction (Fig. 4) with the use of the furfural reagent (Munson, Jones, McCall, and Gallagher, 1948). That good agreement can be obtained by these methods is shown by the results in Table III (Prunty, unpublished).

TABLE III
ESTIMATION OF STEROIDS

Patient			Diagnosis	Total 17-ketosteroid (mg./day)	β -17-keto- steroid (% of Total)	Dehydro-iso- androsterone (% of Total 17-ketosteroid)
No.	Sex	Age				
1	F	40	Adrenal cortical carcinoma	178	34	36
2	F	21	" " "	29.8	4	—
3	M	15	Adrenal cortical hyperplasia	57.5	29	31
4	F	18	" " "	35	39	—
5	F	5	" " "	34	15	18
6	M	3	" " "	24.4	19	21
7	F	15	" " "	108	26	23
8	F	15	" " "	35.5	30	23
9	M	7	" " "	20.3	19	—
10	M	2½	Interstitial cell tumour of testes	19.6	3	13.6
11	M	5½	" " "	2.9	—	—
12	M	45	"Cushing's" syndrome	18.3	8	9
13	M	26	" " "	32.5	12	12

The estimation of the 3 β -hydroxy-17-ketosteroids might prove to be of value in the differentiation of true interstitial cell tumours of the testes from those arising from "adrenal rests," for testosterone is not metabolized to dehydro-iso-androsterone (Dorfman, 1948) and has not been isolated in the case of interstitial cell tumour described by Hoffman (1944). It is interesting to compare these results with Case 10, Table III. In our experience (Table III, Prunty, unpublished data), and of others, adrenal cortical tumour is not necessarily accompanied by very high values for 3 β -hydroxy-17-ketosteroids, whereas moderate elevations may occur in hyperplastic lesions. It would seem to be unexpected that patients with tumour excreting only moderate levels of total 17-ketosteroid, would show elevation of 3 β -17-

ketosteroid excretion (see Case 2, and Kepler and Mason, 1947). Dehydro-iso-androsterone was readily isolated with the aid of chromatography from the urine of Case 1. Table III also shows that although large amounts of 17-ketosteroid may be excreted in cases of interstitial cell tumour of the testes (Hoffman, 1944) this need not necessarily be so (compare Cases 10 and 11). In interpreting elevated levels of 17-ketosteroid excretion it should not be overlooked that trauma may be the cause of an increase, but this is not necessarily correlated with the values for neutral reducing steroids (Venning and Browne, 1949).

The Response of the Adrenal Cortex to ACTH

While for the most part the procedures in Table I test the function of the adrenal cortex in the resting state in the patient, it is now possible to stimulate the adrenal directly and so determine the functional reserve of the organ and the capacity to increase its activity. From the work of Long (1947) and of Sayers and Sayers (1949), it is apparent that the adrenal cortex reacts by the sudden release of available steroid hormones when a brief stimulus is applied, whereas with prolonged stimulation, in the absence of adrenal damage, there ensues hypertrophy of the gland with increasing steroid production. These principles may be applied in the use of ACTH in clinical work. In 1946 this material became available in the U.S.A. for clinical study in sufficient quantity to make possible extended investigations into its properties.* Two types of procedure were employed by Forsham, Thorn, Prunty, and Hills (1948): first the effects of the administration of a single dosage of ACTH, 25 mg. being that arbitrarily selected, and secondly the effects of more prolonged injection. From animal work it was apparent that ACTH must be administered in divided doses as frequently as possible, and it is now known that the life of the hormone in the body is very brief (Sayers, Burns, Tyler, Jager, Schwartz, Smith, Samuels, and Davenport, 1949; Li, 1950). It will be convenient first to consider the effects of prolonged administration of ACTH, and these effects may be classified under the headings of the types of steroid by which they are produced.

Effects of Prolonged Administration of ACTH

Stimulation of Activity Due to 11-oxygenated Steroids.—Evidence of increased activity due to the augmented secretion of these steroids may conveniently be obtained by measurement of changes in excretion of reducing steroids, alterations in carbohydrate metabolism, increased nitrogen excretion, uric acid metabolism, and changes in blood cell counts.

Increased Excretion of "Reducing Steroids."—It has been consistently found that ACTH produces an elevation in the excretion of "neutral reducing steroids." Daily doses of 40 mg. per day divided into four doses over a period of four days produced as much as a fivefold increase in the excretion of these steroids in a normal male, in a male with mild pituitary deficiency, and in a girl with asthenia due to a neurosis (Fig. 5). Doses of 240 mg. and more have been found to reproduce an increased excretion of glycocorticoids (Venning, Kazmin, Ripstein, McAlpine, and Hoffman, 1948), in as brief a period as 12 hours. There is a rapid return to normal levels after withdrawal of ACTH.

* By courtesy of Dr. J. R. Mote, of the Armour Laboratories, Chicago.

Alterations in Carbohydrate Metabolism.—Doses of 40 mg. ACTH daily produce small but significant rises in the fasting blood sugar level, from approximately 80 to 120 mg. %. In the experiments of Forsham *et al.* (1948) this tended to be

FIG. 5.—Typical responses obtained on prolonged stimulation of the adrenal cortex with 40 mg. daily ACTH. (Reprinted from Forsham *et al.*, 1948.)

maximal in the first 24 hours after the beginning of ACTH administration and then gradually to decline. No glycosuria occurred in these experiments, but with doses of 52 mg./day Conn, Louis, and Johnston (1949) obtained truly diabetic-like changes in blood glucose levels and glucose tolerance, with pronounced glycosuria, and the latter is being widely observed (Elkinton, Hunt, Godfrey, McCrory, Rogerson, and Stokes, 1949). It was possible to observe in one of our patients

changes in liver glycogen following 48 hours' treatment with 80 mg. ACTH, an increase of 2.2 g. % (wet weight) being produced. Such a change is characteristic of the action of 11-oxygenated steroids (Reinecke and Kendall, 1943). Diabetic patients are liable to show much greater elevations of fasting blood sugar than are normal individuals during the use of ACTH (Thorn and Forsham, 1949).

Increased Nitrogen Excretion.—Such a change is known to be a constant effect of the administration of 11-oxygenated compounds to animals (Long, Katzin, and Fry, 1940) although variable in amount, being due to the catabolic effect of these hormones (Albright, 1943). The response in nitrogen excretion to ACTH is, however, less constant than might be expected from the results of animal experiments and is more in accord with results observed with administration of "Compound E" in man (Perera, Pines, Hamilton, and Vislocky, 1949).

Uric Acid Metabolism.—After the administration of ACTH there is a consistent increase in the amount of uric acid excreted, though it may decline after several days' treatment (Fig. 5). This phenomenon is also seen as a result of adrenal stimulation due to stress (Pincus *et al.*, 1949) and after adrenal cortical extracts (Babad, 1939). It is thought that uric acid excretion is dependent upon increased production from broken down lymphoid tissue (q.v.) and increased uric acid clearance (Forsham *et al.*, 1948; Hellman, Weston, Escher, and Leiter, 1948), and it has also been suggested by Pincus *et al.* (1949) that under certain conditions brain cell

nucleoprotein may be an additional source. In gouty subjects ACTH is found greatly to increase the clearance of uric acid (Thorn, Bayles, Mansell, Forsham, Hill, Smith, and Warren, 1949). Metabolic data of Forsham *et al.* (1948) have shown that creatinine excretion is not appreciably altered by ACTH, and it has proved convenient to express changes in uric acid excretion as changes in the ratio of uric acid to creatinine (see below).

Changes in Blood Cell Counts.—Following on the extensive work of Dougherty and White (1947) on the lysing action on lymphoid tissue of 11-oxygenated steroids and ACTH in animals, observations in man confirmed these findings (Forsham *et al.*, 1948; Hills *et al.*, 1948). With prolonged administration of ACTH there is a large and sustained fall in eosinophil counts and an initial decrease in lymphocyte counts, although the latter is not always maintained. The magnitude of these effects is dependent upon the dosage of ACTH used, but with 40 mg. per day, near zero levels of eosinophils may be attained (Fig. 5). Simultaneously with these changes there is an elevation in the numbers of polymorphonuclears, which may be doubled. This last change was considered to be partly a non-specific one, for some elevation in this count with ACTH was also observed in patients with Addison's disease.

Increased Production of Sodium-retaining Steroids.—Prolonged treatment with ACTH frequently produces extracellular water retention, and sodium and chloride retention (Prunty, Forsham, and Thorn, 1948; Forsham *et al.*, 1948). The retention of sodium may be profound; for instance, on an intake of 74 milliequivalents per day, 64 milliequivalents a day were retained during six days' treatment with 40 mg. ACTH daily. Increased excretion of potassium may occur, due to intracellular loss of this mineral, especially in the early phase after the injection of ACTH. It is considered that these effects on electrolyte balance are indicative of the stimulation of salt-retaining steroids of the adrenal cortex. There are differences observed in the behaviour of different patients in these respects, and it has already been indicated that the response of individuals to desoxycorticosterone may be dependent upon the existing electrolyte and water balance of the body. Potassium excretion may be particularly variable (Elkinton *et al.*, 1949), and ACTH may even produce signs of potassium deficiency.

Increased 17-ketosteroid Excretion.—Large increases in adrenal 17-ketosteroid excretion are observed on prolonged treatment with ACTH (Fig. 5). In a case of mild hypopituitarism the increase in four days on 40 mg. ACTH daily amounted to 500%. This patient showed an increase in the rate of growth of his beard, and had nocturnal erections which had not previously occurred. The nature of the 17-ketosteroids excreted is not likely to lead to increases in the 3 β -hydroxy fraction (Mason *et al.*, 1948).

Failure of Typical Response to ACTH in Adrenal Cortical Insufficiency

In severe adrenal cortical insufficiency none of the changes enumerated above can be expected to occur. That this is the case can be seen from the data in Fig. 6 which is typical of patients with Addison's disease.

FIG. 6.—Absence of typical responses on prolonged stimulation of the adrenal cortex with ACTH in a patient with Addison's disease. (Reprinted from Forsham *et al.*, 1948.)

Effects of Single Doses of ACTH

On account of the briefer period of stimulation of the adrenal cortex with a single dose of ACTH the response obtained differs in some respects from that with more prolonged administration. Unless large doses are used (Venning *et al.*, 1948) changes in steroid excretion are not measurable. The following paragraphs summarize the effects discernible with a single dose of 25 mg. ACTH in subjects with normally responding adrenal cortices.

Changes in Blood Count.

—The characteristic changes enumerated above are also

to be seen under these conditions. It has been found that the most uniform results are obtained when the observed changes in blood count are expressed as a percentage variation from the initial count. In patients with intact adrenal cortices the average variation from the resting level four hours after the injection of 25 mg. ACTH were found to be an increase of 100% in polymorphonuclears, a decrease of 40% in lymphocytes (Fig. 7), and a decrease of 75% in eosinophils, with a range from 52 to 98% in 50 individuals, including normal persons and hospital patients. The mechanism of these changes in cell count is not understood. In the case of lympholysis *in vitro* it seems that some component of lymphoid tissue itself is required for the effect to be observed (Hechter and Johnson, 1949). *In vivo* experiments with adrenal steroids have shown that the effect on eosinophils is due to secretion of 11-oxygenated steroid by the adrenal cortex in response to ACTH. The administration of approximately 20 mg. of "Compound F" to seven patients with Addison's disease resulted in an average fall of eosinophils of 61%, whereas neither desoxycorticosterone nor testosterone in the form of easily absorbable water soluble compounds had any appreciable effect in doses of approximately 30 mg. The time relationship of the response in blood count to the administration of ACTH is shown in Fig. 7. The maximum fall in lymphocytes is seen to occur four hours after the dose of ACTH has been given, and this too is the time of maximum response to eosinophils. The same finding was observed after the administration of "Compound F." This suggests that the time interval involved in the response of the adrenal cortex to ACTH is small and that most of the intervening period is concerned with the action of the steroid on the cells. It is difficult to press this argument too far, however, for ACTH is known to be very rapidly absorbed, whereas the absorption of "Compound F" is more prolonged.

FIG. 7.—The time relations of lymphocyte response and uric acid excretion to injection of 25 mg. ACTH I.M. (Reprinted from Thorn and Forsham, 1949.)

Effect on Uric Acid Excretion.—In Fig. 7 the results of the administration of 25 mg. of ACTH to a normal individual are shown. Uric acid and creatinine excretion were measured in urine specimens obtained at half-hourly intervals. It will be seen that there is relatively small change in the creatinine, but a sharp increase in the uric acid excretion, which was maximal at four hours, coincident with the greatest change in blood count. It takes at least one hour before any measurable change occurs. In the same way that blood eosinophils are affected by “Compound F” so is the increase in uric acid excretion. Twenty mg. of “Compound F” given to two patients with Addison’s disease caused an increase over the pre-injection level of 48% and 45% in the ratio uric acid: creatinine (uric acid and creatinine in the urine being expressed in mg. %). In a third patient the ratio increased 180% during seven hours following injection of 17 mg. of “Compound F.” Desoxycorticosterone in a dose of 30 mg. produced a small but definite increase in the ratio in two patients (25%).

Alterations in Electrolyte Excretion.—Increases in the amounts of sodium, chloride, and potassium excreted during the four hours following injection of 25 mg. of ACTH have consistently been observed and have been related to the similar effect produced by 11-oxygenated steroids (Forsham *et al.*, 1948). These observations form a contrast to those with the more prolonged administration of ACTH considered above. Apparently in short term observations the effect of 11-oxygenated steroids is predominant.

The Use of ACTH in Testing Adrenal Cortical Function

The use of a single test dose of ACTH provides information about the capability of the adrenal to give an immediate response, while the more prolonged 48-hour test to be described allows the adrenal a greater opportunity of giving a measurable response under certain conditions. From the data reviewed above it will be seen that the two most useful and convenient measurements that can be made in a short test are the change in eosinophil count and increase in uric acid excretion. The former has the advantage, unlike so many other indices of adrenal cortical function, that it is independent of the kidney as a mediator of the response. It suffers from the disadvantage that certain individuals with allergic eosinophilia may fail to respond (Thorn and Forsham, 1949) and occasionally patients are encountered with eosinopenia due to bone marrow depression. The measurement of uric acid excretion has been found to be less reliable than changes in eosinophil count. If the uric acid excretion is approaching a maximum due to the presence of gout, leukaemia, or decrease of renal function, or in some instances to an increase of adrenal cortical secretion, it may be difficult further to increase the excretion of uric acid. Secondly, it seems that other factors which are not understood may at times interfere with the uric acid responses. Increased uric acid excretion may, therefore, be regarded as valuable corroborative evidence of adequate adrenal cortical activity, if it is present. The response in uric acid excretion is also probably a less sensitive index of adrenal stimulation than changes in the eosinophil count.

The Four-hour ACTH Test.—The technique adopted for the four-hour ACTH test by Forsham *et al.* (1948) may be restated as follows:—

The patient is allowed no breakfast (glucose ingestion is known to increase uric acid excretion) but water is freely permitted, and 200 ml. are given at the start of the test. At this time urine is voided and two hours later voided again and this control specimen kept. Then 25 mg. ACTH (equivalent to the Armour Laboratory standard) are injected intramuscularly, 200 ml. water given again, and the latter is repeated two hours later. One hour after the ACTH injection the patient voids and the urine is rejected. The urine passed during the subsequent three hours is collected. Eosinophil counts are carried out immediately before the injection of

TABLE IV
DEGREES OF RESPONSE TO ACTH

Patient			Diagnosis	Chemical Degree of Pituitary Insufficiency	Eosinophil Change (%)	Uric Acid Creatinine Change (%)
No.	Sex	Age				
* 1	F	42	Simmonds's disease	Severe	+24	+1
2	F	33	" "	"	-4	+18
3	F	61	" "	Moderate	-17	+22
4	M	58	Chromaphobe adenoma	Severe	-15	+32
5	M	60	" "	Mild	-27	+49
6	M	46	" "	"	-45	+90
7	M	40	" "	"	-32	+39

* The data on Cases 1 to 6 are taken from the article by Forsham *et al.* (1948); Case 7 is unpublished.

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ACTH and at the end of four hours after this time. Uric acid and creatinine are determined in the two urine specimens and expressed as mg.%.*

In patients with normal adrenal cortical function a fall of 50% or more in the eosinophil count is observed, and an increase of 50% or more in the uric acid: creatinine ratio indicates a good response (Fig. 8). In patients with Addison's disease these minimum changes are not attained. Patients having adrenal cortical failure secondary to anterior pituitary deficiency may show varying degrees of response (Table IV) depending upon the severity of their condition and the degree of secondary adrenal cortical atrophy.

The 48-hour ACTH

Test.—This test, described by Thorn and Forsham (1949), has the advantage that it allows a longer time for stimulation of the adrenal cortex. It is also favoured by the fact that measurable changes in 17-ketosteroid excretion can be observed and the specificity of the test thereby improved. The patient receives a total dose of 40 mg. of ACTH per day in doses of 10 mg. at six-hourly intervals, day and night for two days. The eosinophil count is measured before the first dose of ACTH and four hours after the last dose. A 24-hour specimen of

FIG. 8.—Scatter diagram of responses of patients with normal adrenal cortical function and patients with Addison's disease to 25 mg. ACTH. (Reprinted from Prunty, 1949b.)

urine is collected the day preceding the first dose of ACTH and a second one during the last 24 hours of ACTH injection. The authors state that a low initial 17-ketosteroid excretion which fails to rise after ACTH accompanied by a poor fall in eosinophils (less than 50%) indicates absence of adrenal cortical reserve as is seen in Addison's disease and hypopituitarism. A low initial 17-ketosteroid excretion which rises after ACTH with a progressive fall in eosinophils is suggestive of moderate adrenal cortical deficiency due to pituitary failure, for example, while a normal 17-ketosteroid excretion followed by a rise to the upper limit of normal indicates a normal reserve function of the cortex. A fall of 50% or more in the eosinophils is corroborative evidence for this. They cite examples of cases illustrating these points, and it is interesting to note that a patient with acromegaly and two patients with Cushing's syndrome gave supernormal responses, the 17-ketosteroid levels rising to 31, 53, and 119 mg. per day respectively. This is good evidence for the claim that the rise in 17-ketosteroids can be regarded as an index of cortical capacity.

* The method used for uric acid determination is given in the appendix.

Responses of the Adrenal Cortex to Adrenaline and to Stress Stimuli

The eosinopenic action of adrenaline was first noted by Bertelli, Falta, and Schweegee (1910) in dogs. In the light of the experiments reviewed earlier in this paper it can be seen how this effect is likely to be mediated through the secretion of adrenal cortical 11-oxygenated steroids. For this reason a test for the integrity of the adrenal cortex, combined with that of the anterior pituitary, based on the action of adrenaline has been proposed (Recant, Hume, Forsham, and Thorn, 1950 ; Thorn and Forsham, 1949 ; Thorn, Bayles, *et al.*, 1949). A subcutaneous dose of 0.3 mg. of adrenaline is injected and the eosinophils counted immediately before the dose and four hours later. Normal individuals respond with a fall of 50% or more in the count. Neither patients with hypopituitarism nor Addison's disease respond to this test in the normal way. The data given by the authors show good correlation with the 4-hour ACTH test, but the latter is preferred. Clearly with the scarcity of ACTH in England this test is worth further trial.

The characteristic response of the adrenal to stress is also accompanied by eosinopenia. This may prove of value in assessing the response of patients to trauma and operations. Poor responses may be expected with anterior pituitary or adrenal cortical insufficiency, and in poor states of nutrition (Venning and Browne, 1949). Thorn and Forsham (1949) have found that after severe pain, injury, infection, and operation there is normally a large depression in eosinophil counts. It would be expected that patients with Addison's disease and hypopituitarism would fail to show this change. In patients with mild degrees of the latter post-operative reactions may be poor, but administration of ACTH for 48 hours should bring about a normal response. Such tests may prove of considerable value in assessing the condition of these patients and in determining in which instances the adrenal cortex has become exhausted and fails to respond.

Summary

Abnormalities of adrenal cortical function may be found in a wide range of conditions. These abnormalities may be detected by tests designed to assess the resting function of the gland, or tests based upon the capacity of the gland to react to various stimuli, which give an indication of the functional reserve. These latter tests depend upon the stimulation of the cortex either by exogenous or endogenous ACTH. The physiological properties of ACTH have been reviewed and it has been shown how these may be applied in the use of ACTH as a test for adrenal cortical functional reserve. Brief consideration has also been given to the behaviour of the adrenal cortex in conditions of stress, such as the post-operative state.

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APPENDIX

Eosinophil Counts

Venous blood, 5 ml., is drawn with a syringe and placed in an oxalate bottle and gently mixed. The bottle is prepared by drying off in it 0.5 ml. solution containing 0.8 g. potassium oxalate and 1.2 g. ammonium oxalate per 100 ml. The blood is drawn up to the 0.5 mark in a white cell pipette and diluted with fluid to the 11 mark. The diluting fluid (Dunger, 1910) contains 5 ml. 2% aqueous eosin, 5 ml. acetone, and 90 ml. distilled water, and is filtered before use. The pipette is gently agitated for 30 seconds and some of the fluid, discarding the first few drops, placed on a Fuchs-Rosenthal chamber. The cells covering the field are counted after 2 minutes. As many counts as possible should be made, employing freshly diluted blood for each. If four chambers are counted the number of eosinophils per mm.³ is given by the total count multiplied by 0.625.

Uric Acid Estimation in Blood and Urine

This has been modified from the method described by Kern and Stransky (1937) (see also Krebs and Örström, 1949).

Reagents

The reagents present are listed below, and it should be specially noted that the solution of sodium silicate and glycerol is critical since some brands lead to the formation of opalescence during colour development.

1. 0.25 N Sodium Hydroxide.

2. Uric Acid Reagent.—Sodium tungstate, 50 g., which must be molybdenum-free,* is dissolved in 400 ml. water; 40 ml. phosphoric acid (gr. 1.75) are added from a cylinder and the cylinder washed out. The mixture is boiled under reflux for two hours, cooled, and made up to 500 ml. It should be stored in a brown bottle.

3. Silicate-glycerol Reagent.—Sodium silicate,† 230 g., Sp.g.1.7, is dissolved in 300 ml. hot water and filtered. Glycerol, 85 ml., is added (B.D.H.—“reagent grade,” not AR) and made up to 500 ml.

4. Liquoid-uric Acid Reagent.—Liquoid, 2.78 g., is dissolved‡ in 139 ml. water and 111 ml. uric acid reagent added. Solution is made up to 500 ml. with water.

* Eimer and Armand, N.Y. † General Chemical and Pharmaceutical Co., Ltd. ‡ Roche.

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5. Uric Acid Standard Solution (1 mg./1 ml.).—Dry, pure uric acid, 1 g., is put into a 1,000 ml. flask through a dry funnel, and 0.6 g. lithium carbonate dissolved in 150 ml. water by warming to 40° C. and filtered. The 1,000 ml. flask is warmed to 50°–60° C. and the lithium carbonate poured in and washed in. It is kept warm for 5 minutes until dissolved. It is cooled at once and 20 ml. 40% formalin added. The flask is half filled with water and a few drops methyl orange added. *N* sulphuric acid, 25 ml., are run in until methyl orange goes pink. (There should be 2–3 ml. of acid left in the pipette.) The remainder of the 25 ml. of acid is then run in, mixed, and made up to 1,000 ml. For a working standard 1 ml. is diluted to 200 ml.

Technique

Plasma.—To 1 ml. of plasma is added 8 ml. water and 0.4 ml. NaOH; 0.6 ml. uric acid reagent is added slowly, the solution allowed to stand 10 minutes and spun; 5 ml. supernatant is taken and treated as for urine.

Urine.—Dilute 1 ml. to 100 ml. (or 1/200 or 1/50 as necessary). Uric acid solution 5 ml. (standard, plasma filtrate, or diluted urine) are treated with 2.5 ml. silicate-glycerine solution and 2 ml. liquoid-uric-acid-reagent, the mixture allowed to stand for 6 minutes, and read in the photoelectric-colorimeter. Optimum absorption is at 700 mμ.

Calculation

$$\text{Plasma } 5 \times \frac{U}{S} = \text{mg. \% uric acid}$$

$$\text{Urine } 50 \times \frac{U}{S} = \text{mg. \% for 1/100 dilution.}$$

With this technique normal plasma values vary between 2.5 and 6 mg. %, and fasting levels of uric acid: creatinine ratio average 0.46, S.D. ± 0.092. The specificity for this reaction in urine has been shown to be over 90% when uricase is employed to destroy the uric acid in urine specimens (Forsham *et al.*, 1948).

For methods of creatinine estimation in urine see Folin (1914).