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CHEMICAL AND CLINICAL PROBLEMS OF THE ADRENAL CORTEX*

BY

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It is difficult to assign precedence in the recognition of the association of changes in the adrenal gland and the occurrence of clinical symptoms and signs. Fortunately, I am relieved of duties in the early historical field, as the eminent founder of these lectures had himself lectured to this College on this subject in 1933 and 1934. As a result of this Sir Humphry Davy Rolleston (1936) has published a most valuable and full history of the subject up to that time. It is well to note that, historically, the importance of the adrenal emerged along two lines—in those cases associated with increased adrenal cortical activity and in those with deficiency of this gland. In the former group the earliest association that I am able to find is the occurrence of a probable tumour in a virilized girl described by Sampson in 1697. Knowledge of this phase gained impetus after 1900 (Bulloch and Sequeira, 1905; Apert, 1910; Broster and Vines, 1933) and had to be linked up with the work of Harvey Cushing in 1932. In the second group we know so well of the work of Thomas Addison in 1855, and it has been so ably reviewed again recently (Bishop, 1955), that I think I may be excused such a brief reference to an outstanding and fundamental contribution.

Following the phase of recognition of the problems, research rapidly developed along the classical lines in endocrinology, with the phase of pathological correlation and the attempt to obtain an active extract from the gland concerned. In the case of the adrenal cortex the stage had already been set by the pattern of events in development of our knowledge of the thyroid, although it is true that the synthesis of adrenaline had been made in 1904 by Stolz. As early as 1891 Murray pointed the way by relieving the symptoms of myxoedema by injection of a gland extract, and the synthesis of thyroxine was achieved by Harington and Barger in 1927. It was during the following decade that the chemical foundation-stones were laid in the elucidation of the problems of the adrenal cortex.

Adrenal Cortical Hormones

In 1930 the first active extracts of the adrenal gland were prepared, and, as is so commonly the case, almost simultaneously, by two groups of workers (Hartman and Brownell, 1930; Swingle and Pfiffner, 1930). The isolation of the active hormones from the adrenal began in 1934 by investigators led by Kendall, Reichstein, and Wintersteiner and Pfiffner. It was not until much later that the important new sodium-retaining steroid, aldosterone, was isolated and characterized (Simpson, Tait, Wettstein, Neher, von Euw, Schindler, and Reichstein, 1954).

Meanwhile work began on the problem of evaluating the adrenal cortical activity in patients, and, from the point of view of steroid analysis, started with the observations of Callow, Callow, and Emmens (1938), who found some correlation between estimates of 17-ketosteroids and the androgenic potency of urine extracts. Large amounts of ketosteroid were found in patients with carcinoma of the adrenal cortex (Callow, 1938; Crooke and Callow, 1939) and in increased quantities in patients with adrenal cortical hyperplasia (Callow, 1938; Talbot, Butler, and Berman, 1942; Patterson, McPhee, and Greenwood, 1942). It was quickly realized that the excretion of these substances was depressed in cases of Addison's disease and anterior hypopituitarism (Callow, Callow, and Emmens, 1940; Fraser, Forbes, Albright, Sulkowitch, and Reifenstein, 1941). As work proceeded on the isolation of compounds responsible for the reaction on the metabolites of administered steroids, many of these were seen to be identical with those of testosterone, the secretion of which is confined to the testicle. Search for a specific adrenal cortical ketosteroid revealed that in carcinoma patients much dehydroepiandrosterone could sometimes be obtained in the urine (Crooke and Callow, 1939), and this substance at least does not appear to be derived as a metabolite from testicular sources.

The isolation studies referred to above suggested that the substances now known as cortisone and hydrocortisone were important adrenal cortical hormones. Meanwhile, before its isolation from adrenal extracts, desoxycorticosterone had been synthesized by Steiger and Reichstein (1937), became commercially available, and transformed the prognosis of Addison's disease (Thorn, Dorrance, and Day, 1942). For chemical reasons the synthesis of cortisone and hydrocortisone was a more difficult task, the introduction of an oxygen function (=O or −OH) at position 11 being the stumbling-block. Cortisone was the first to be synthesized (Sarett, 1946), and the synthetic product was shown to have biological activity (Kendall, 1949; Dorfman, 1949; Venning, 1949). Its increased availability enabled careful evaluation of its metabolic effects to be made by Sprague, Mason, and Power (1951) in the first patients treated for rheumatoid arthritis at the Mayo Clinic. Only later did hydrocortisone become available, at first being prepared biosynthetically. It was first synthesized in 1950 by Wendler, Graber, Jones, and Tishler. In 1950 Sprague, Hayles, Power, Mason, and Bennett isolated 191 mg. of hydrocortisone from

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a 25-day urine sample in a boy with Cushing's syndrome, and this suggested its importance with respect to its origin from the human adrenal cortex.

The next line of attack on the problem of adrenal hormones was the biosynthetic one using adrenal gland preparations either for perfusion (Hechter, Jacobsen, Jeanloz, Levy, Marshall, Pincus, and Schenker, 1950) or as adrenal cortical tissue preparations (Plager and Samuels, 1952; Wettstein, 1954). The knowledge from this source has been reinforced by identification of steroids present in peripheral blood and more especially in adrenal vein blood of animals and man, with and without the prior stimulation of the gland by corticotrophin (Morris and Williams, 1953; Bush, 1953; Pincus and Romanoff, 1955). From all this work, tentative conclusions may be drawn about the nature of the substances actually secreted by the adrenal (Fig. 1). The main synthetic pathway of adrenal corticoids, derived ultimately from cholesterol, is by way of progesterone. Two steroids of great importance secreted by the gland are hydrocortisone and corticosterone, although the relative importance of the latter is much debated (see Pincus and Romanoff, 1955, and following discussion). These compounds after secretion are relatively easily convertible into cortisone and 11-dehydrocorticosterone. Aldosterone is probably derived via desoxycorticosterone, which is not itself secreted by the gland (Wettstein, 1954). Fig. 1 also indicates a separate synthetic pathway to the ketosteroids dehydroepiandrosterone, Δ^4-androstenedione, and 11-β-hydroxy-Δ^4-androstenedione, all of which may be found in the blood. From the physiological standpoint, hydrocortisone and aldosterone are at present best understood.

Metabolites of Adrenal Cortical Hormones

It is now necessary to turn to the problem of the breakdown products of these substances found in the body. The corticoids, steroids with the side-chain at position 17, are metabolized according to current ideas along two main pathways. The first of these, and quantitatively the most important, is progressive reduction of the unsaturation in ring A, reduction of the ketone (=O) groupings and, finally, to a small extent, reduction of the terminal (21) hydroxyl group of the side-chain to a methyl group (CH_3). The 11-hydroxyl group, as already indicated, can easily undergo oxidation to a ketone. A mixture of compounds, of which at least 14 have been isolated, is excreted in the urine (Fig. 2).

An estimate of part of these metabolites is now obtainable for clinical and research purposes by various techniques. Two of these are best known. In America, modifications of the method of Reddy, Jenkins, and Thorn (1952), depending upon the Porter–Silber reaction, are usually employed The reaction depends upon the presence of a 17-hydroxyl group and an intact ketone group in the side-chain at position 20 (next to the terminal 21-hydroxylated group). In this country the method of Norymberski, Stubbs, and West (1953) is becoming frequently employed, and is alternatively known as the "17-ketogenic steroid" method. It estimates those compounds given in the Porter–Silber reaction and, in addition, those with 17-20-21 hydroxyl groups and 17-20 hydroxyls with a terminal 21-methyl group. It would therefore be likely to include a wider range of metabolites than the Porter–Silber reaction, and indeed, as will be seen, there is an important implication of this fact. Neither method estimates corticosterone or its metabolites. For purely chemical reasons, based on the chromogenicity of the final products, a strict parallelism between the values obtained by the two methods is not to be expected. Little is known of the metabolism of aldosterone, and the estimation of the microgram quantities excreted in the urine is in its infancy (Neher and Wettstein, 1955; Ayres, Simpson, and Tait, 1956).

The second important line of metabolism is that involving removal of the side-chain from the corticoid molecule and replacement by a ketone group giving rise to a 17-ketosteroid. The amount of the latter produced is small compared with the quantity of metabolites with intact side-chain. The 17-ketosteroids formed fortunately retain the oxygen at position 11 and are therefore recognizable from some other ketosteroid end-products of ketosteroid origin (see especially Dorfman and Ungar, 1953). The 11-oxygenated ketosteroids are divisible approximately equally into two isomeric groups, one of which is formed almost entirely from adrenal ketosteroid and the other largely derived from corticoid degradation (Dorfman, 1955; Kappas, Dobriner, and Gallagher, 1955). The determination of total ketosteroids in the urine includes metabolites of testosterone, the adrenal ketosteroids, and the ketosteroid metabolites derived from corticoids. In the analysis both of ketosteroids and of corticoid metabolites the problem is greatly complicated by the fact that they largely occur in the urine—not in the "free" state, but linked with sulphate or as glucuroniside. This factor is one of those responsible for multiplication of methods of analysis.

The metabolism of adrenal corticoid appears to be very rapid; for example, the administration of ^{14}C-labelled hydrocortisone results in the appearance of 15% in the urine in one to two hours and 56% in six hours. Of the initial 15%, half appears as reduction compounds (Hellman, Bradlow, Adesman, Fukushima, Kulp, and Gallagher, 1954). Even at the peak level at 15 minutes in the plasma only one-eighth of the radioactivity is present as free hydrocortisone, and conjugation is extremely rapid (Peterson, Wyngaarden, Guerra, Brodie, and Bunim, 1955). Since conjugation leads to loss of biological activity, from the point of view of the activity of the hormone in the peripheral tissues a knowledge of the blood level of the free substance is desirable. On the other hand, assessment of the free hormone in the blood will give only a poor indication of the output of the adrenal gland, if that is the aim of measurement. The most widely used methods of blood analysis for corticoids depend on extraction of free compounds and measurement of the extracts with the Porter–Silber reagent (Nelson and Samuels, 1952; Bayliss and Steinbeck, 1953). A more specific method can be based upon paper chromatographic separation of the hydrocortisone. This is done by Dr. Mills in our laboratory and the amount assayed fluorimetrically. It has been shown that the conjugated steroid in the plasma may be several times the free steroid (Bongiovani, 1954; Mills, 1955b). By the Porter–Silber technique an extreme range of 3 to 22 μg. of unconjugated steroids is present per 100 ml. of plasma. Mills finds values between 2 and 8 μg. per 100 ml.

In the case of ketosteroids the main substance present in peripheral blood is dehydroepiandrosterone together with androsterone, both apparently in conjugated form. The total amount of these compounds is approximately 50 μg. per 100 ml. (Migeon and Plager, 1955), so that their concentration is rather greater than the corticoids that can be accounted for even after hydrolysis. The ratio of ketogenic steroids to ketosteroids in the urine is usually found to be greater than 1, although this is by no means always the case. Examination of the adrenal vein blood in man after the administration of corticotrophin has given a valuable indication of the steroids actually secreted from the gland under these conditions. Pincus and Romanoff (1955) found from the two cases they examined that the ratio of hydrocortisone plus corticosterone to Δ^4-androstenedione plus 11-β-hydroxy-Δ^4-androstenedione is about 6 to 1. These investigators had difficulty in finding any dehydroepiandrosterone.

Corticotrophin and its Effects on the Adrenal Cortex

In the early 1920's it was realized that the adrenal was dependent on the activity of the anterior hypophysis (Evans, 1923–4; Smith, 1930); since that time developments in

knowledge have paralleled that of the cortical hormones, active pituitary extracts being obtained in 1933 by Collip, Anderson, and Thomson. In 1943 two sets of investigators again simultaneously announced the preparation of corticotrophin, which on the evidence then available appeared to be a pure protein (Li, Evans, and Simpson, 1943; Sayers, White, and Long, 1943). It was a short time before it was realized that it was possible to subject the material to hydrolysis with acid or pepsin and obtain components with greatly increased activity, and the idea evolved that corticotrophin activity resided in a peptide unit of the original protein (Li, 1953; Stack-Dunne and Young, 1954). It would seem that some modification of this view is necessary, as it is possible to separate an active peptide without the use of hydrolytic procedures (see Dixon, 1955; Dedman, Farmer, Morris, and Morris, 1953), the final activity residing in a basic polypeptide which itself may undergo partial hydrolysis with pepsin and acid.

During recent years several methods of corticotrophin preparation have been employed yielding crude protein, protein purified by oxycellulose (Astwood, Raben, Payne, and Grady, 1951), and, thirdly, products obtained after partial hydrolysis, particularly with pepsin. The use of varying preparations gave rise to confusion in several clinical laboratories about the response to be expected.

With the advent of these preparations of corticotrophin attempts were made to correlate the response of the human adrenal with the bioassay method almost universally employed in its preparation. This was the ascorbic acid depletion method of Sayers, Sayers, and Woodbury (1948), which is still widely used, although other methods were later developed (Clayton and Prunty, 1951; Bruce, Parkes, and Perry, 1952). In an investigation on six medical student volunteers it was established that with two preparations of corticotrophin a parallelism could be shown between ascorbic acid depletion in the rat and the response of the human adrenal (Prunty, Clayton, and McSwiney, 1951). It was found that the four-hour eosinophil depression after injection of corticotrophin gave better statistical results than measurement of ketosteroids over a single 24-hour period. The finding of a wide scatter of ketosteroid excretions with those early lyophilized preparations was substantiated by observations in patients.

A study of 21 patients, using 14 different batches of cortico-

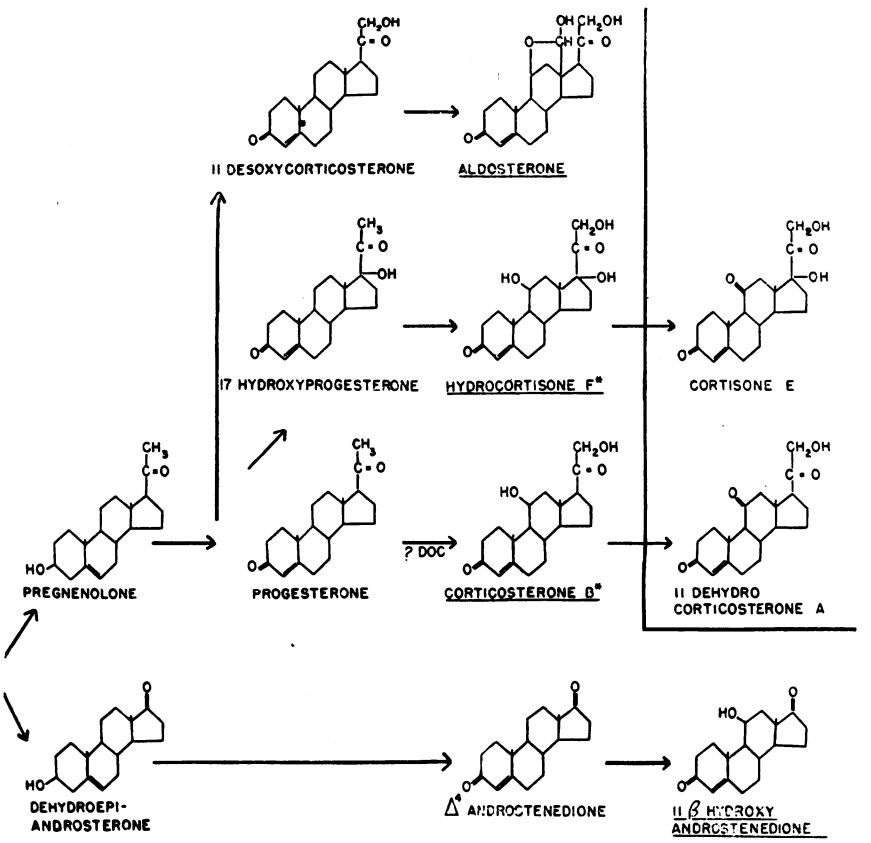

FIG. 1.—Synthesis of hormones in adrenal cortex.

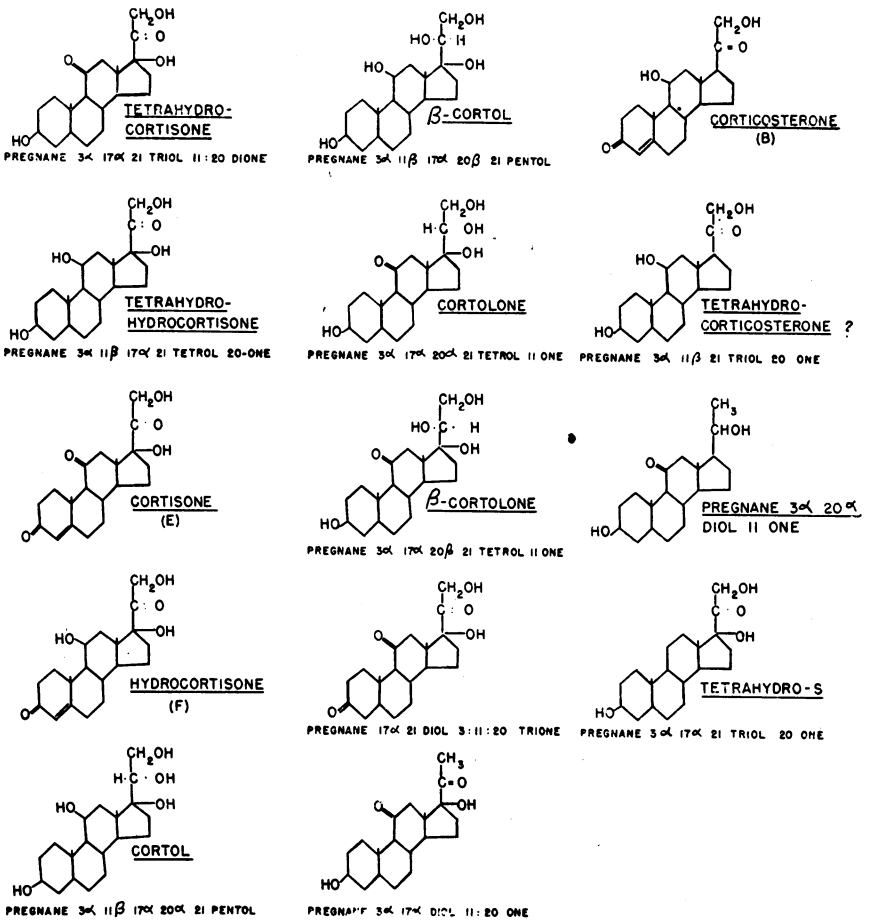

FIG. 2.—Metabolites (21-carbon compounds) found in urine.

trophin for periods exceeding five days, showed a very wide variation of ketosteroid excretion over a dose range of 10 to 400 I.U. daily in spite of the fact that nine of the batches were re-assayed by Dr. Clayton in our laboratory, in rats, and necessary corrections made for dosages. For example, one batch at a dosage of 150 units daily produced a greater ketosteroid excretion in a single patient than 300 units of a different batch which by rat assay was twice as potent. Very varying responses with the same batch in different patients were also noted, and some patients tended to fall into extreme groups; Querido, Kassenaar, and Cats (1955) have also commented on the great ketosteroid variation encountered in 13 subjects. Nevertheless there was a general tendency for a straight-line relation between the dose of corticotrophin and the response obtained.

Further observations on this question, using different methods, gave similar results. A patient received, according to our assay, 125 I.U. of a batch of corticotrophin daily and gave a much smaller response when urinary corticoids and eosinophil depressions were measured than she did with 16 I.U. daily of a different batch. The experiment was carried out on crossover lines, and the corticoids were measured by the old method of formaldehydogenic steroids extractable from the urine at *p*H 1 (Daughady, Jaffe, and Williams, 1948). On the other hand, similar measurements in two other patients gave good correlation with rat assays. These experiences dictated a policy of great caution in interpretation of corticotrophin responses, even if a careful check was made by bio-assay of the batch being used.

Others were having similar difficulties and the situation became more complex. It was possible to obtain quite different responses in adrenal ascorbic acid depletion in the rat, depending on the route of administration. In 44 rats given a lyophilized preparation a mean depression of 60 mg. per 100 g. of tissue in adrenal ascorbic acid was obtained after one hour when the hormone was given subcutaneously, and 270 mg. when given intravenously. The maximum response occurred at one hour (Clayton, 1954). A long-acting gel preparation, on the other hand, gave a maximum depression of 200 mg. by either route of administration, although the maximum response was delayed for two hours by the subcutaneous route, as might be expected from the slower release of this type of hormone. This observation was in agreement with the findings of Thompson and Fisher (1953). The interpretation of these puzzling phenomena is complex. In the case of the earlier lyophilized preparations it was thought that destruction in the patient's tissues occurred (Geschwind and Li, 1952) probably owing to the presence of hydrolytic enzyme contaminants in them.

Later developments in preparation, using the oxycellulose technique, gave products with a relatively much more potent action when given intramuscularly (Rosenberg, Cleroux, Raben, Payne, and Astwood, 1951), although intravenous potency was similar, when measured in international units, to that of the older preparations. Unhydrolysed preparations in gelatin, being commonly used intramuscularly, have been restandardized in terms of "clinical units" which are three times the international or U.S.P. unit (see Jenkins, Forsham, Laidlaw, Reddy, and Thorn, 1955). Some preparations in which hydrolysis is employed prove to be unstable in the tissues, in contrast to the unhydrolysed long-acting gel preparations which are relatively stable (Hays and White, 1954; Forsham, Raimondo, Island, Rinfret, and Orr, 1955).

Corticotrophin Gels

In spite of the obvious inadequacies of the older formaldehydogenic method for analysis of corticoid metabolites extracted from the urine at *p*H 1, which estimates only a very small proportion of the total substances present, it was found that such a measurement gave a relatively far greater response to corticotrophin than measurement of ketosteroids (Prunty, Brooksbank, Clayton, and McSwiney, 1951). The increase in formaldehydogenic steroid excretion was approximately four times that of ketosteroids. This emphasized the potential value of a satisfactory method of assaying corticoid metabolites, and the method of Norymberski *et al.* (1953), being the best available, was used in all later studies. The more ready availability of corticotrophin gels enabled their activity to be investigated, and this was largely done during the course of treatment of patients with rheumatoid arthritis and other non-endocrine conditions. The log dose-response curve for ketosteroid excretion followed a similar pattern to that with the older lyophilized preparations (Fig. 3). According to our results with ketosteroids about 40 "clinical units" of corticotrophin gel gives an activity corresponding to 100–150 I.U. of the old preparations when the latter were administered in four equal doses during 24 hours. They appear to be of even greater potency than suggested by Jenkins *et al.* (1955). This observation may be biased to some extent by the finding that one of the batches of gel used was probably of greater potency by the rat assay than the labelled potency. Although the values were very scattered in the group of patients given the gel preparation, in the individual patient a surprisingly good log dose-response curve could be obtained.

Five of these gel batches were also assessed in terms of ketogenic steroid excretion in seven patients. As expected, the response tended to be greater, but the scatter in this case was even wider and two of the patients gave very brisk responses. The administration of the gel is more effective in 12-hourly doses than when the same dose is given 24-hourly.

Testing for Adrenal-Cortical Function

It seemed that the methods of testing adrenal cortical function with corticotrophin were not entirely satisfactory and an investigation of the best criteria to be employed was planned. It had been our experience that there was a considerable day-to-day variation of resting ketosteroid and ketogenic steroid excretions, and from the sample of patients subsequently tested this proved to be as follows: the mean maximal variation between day-to-day excretions ranged from 21% in 34 patients with normal ketosteroids to 34% in 11 males and 60% in 9 female patients with low ketosteroids (less than 8 and 5 mg. daily respectively). For ketogenic steroids the variations proved to be 27% in 22 patients at levels of excretion between 9 and 20 mg. per day and 48% for levels below 9 mg. per day in 32 patients. It was considered that these variations were sufficient to render unreliable any test based upon comparison of a single control 24-hour period and a 24-hour period on corticotrophin, the so-called 48-hour regime of Thorn and Forsham (1949). Hence the first necessity of the method was the establishment of an adequate baseline. Since it is impracticable to prolong the observation in many patients, a minimum of two 24-hour periods was considered necessary.

The next problem concerned the duration of observation. Experience with the treatment of patients with rheumatoid arthritis showed that it might take several days to elicit a near maximal response, and little was known of the factors governing the rate of its achievement, with the exception that patients with hypopituitarism might respond slowly (Prunty, 1950; Gordon, Horwitt, and Segaloff, 1954). The duration of corticotrophin stimulation was therefore selected as four days. In order to get a pronounced response a large dosage level of 20 "clinical units" was administered twice daily. The previous experience described showed that careful attention to batch variation was probably still essential in comparison of results, and Dr. Clayton has kindly assayed some of the batches used. During the course of the observations to be described, other methods of assessing the response to corticotrophin appeared and must be briefly reviewed. They were all based on shorter-term observation. Thorn and his colleagues reported on three methods (Jenkins *et al.*, 1955).

First of these was the injection of 25 I.U. of lyophilized short-acting corticotrophin intravenously and observation of the four-hour eosinopenia. A total of 545 patients had been tested, and these workers concluded that this method may

still be useful as a preliminary screening test, provided the proper precautions are taken in its interpretation. Secondly, they reported on the use of 20 to 25 I.U. intravenously over eight hours and measured the steroid excretion during 24 hours compared with a baseline 24-hour excretion. The third test was conducted for 24 hours with the injection of 20 "clinical units" intramuscularly at the beginning of the 24 hours and 12 hours later. Again a 24-hour baseline period was used for comparison. In the last method they also measured eosinophil cells 8 to 12 hours after the first injection. The best criterion of measurement proved to be the measurement of corticoid metabolites by means of the Porter–Silber reagent, and the response was found to be more sensitive than that of ketosteroids.

Nabarro (1954), employing ketosteroids as the basis of measurement, used a similar test but prolonged the time of 48 hours to increase sensitivity and reliability. Forsham *et al.* (1955) suggested that an eight-hour urine collection could be utilized after intramuscular injection of gel in special circumstances. Other methods proposed have been based upon the alteration of blood corticoid levels as determined by the Nelson and Samuels (1952) method. A six-hour corticotrophin infusion, with determination of the change in blood level of steroid at the end of that time, was employed by Eik-Nes, Sandberg, Nelson, Tyler, and Samuels (1954) and by Bayliss and Steinbeck (1954). The former authors found a wide variation in response; in a single subject the six-hour response differed by as much as 100% on two occasions.

Types of Response to Corticotrophin

A typical normal response obtained by the method investigated by us is shown in Fig. 4. It demonstrates the variation in baseline levels and the greater response of ketogenic steroids than ketosteroids, although in this particular patient there is no increase in ketogenic steroids on the first day of giving corticotrophin. Such discrepancies may arise partly from technical sources as well as idiosyncrasies of the patient. In this instance, maximal levels of steroid excretion were reached on day 4, but it often happens that the third or even the second day shows the maximal response. This is a justification for prolonging the test over a four-day period. The fall in the eosinophil count is typical, the counts being made approximately seven hours after the morning dose of corticotrophin. Before reviewing the findings in a group of patients, using this test, it is necessary to draw attention to the problem of batch variation of corticotrophin which still seems to exist. One example may be seen in a patient with hypopituitarism when, after adrenal cortical stimulation had been obtained by five consecutive days' treatment with intravenous corticotrophin, she responded as follows: on 40 "clinical units" twice daily intramuscularly of batch A, maximum ketogenic steroid excretion obtained was 21 mg. per 24 hours, and immediately after, on the same dose of batch B, the maximum was 6 mg. On 40 "clinical units" of batch B three times daily she excreted 16 mg., and on the same dose of batch C 30 mg. of ketogenic steroid. Batch C (89754) by our rat assay exceeded the makers' labelled potency. A second example led to an unfortunate error in diagnosis.

In addition to the "standard test" described above, using intramuscular corticotrophin, we have also used corticotrophin intravenously. There are four reasons for doing this. In the first place we have had only limited supplies of corticotrophin of a single batch, which we knew to be reliable and potent by evidence both from administration to patients and by our own bio-assay. Secondly, in view of the earlier experiences we have felt that failure to respond by

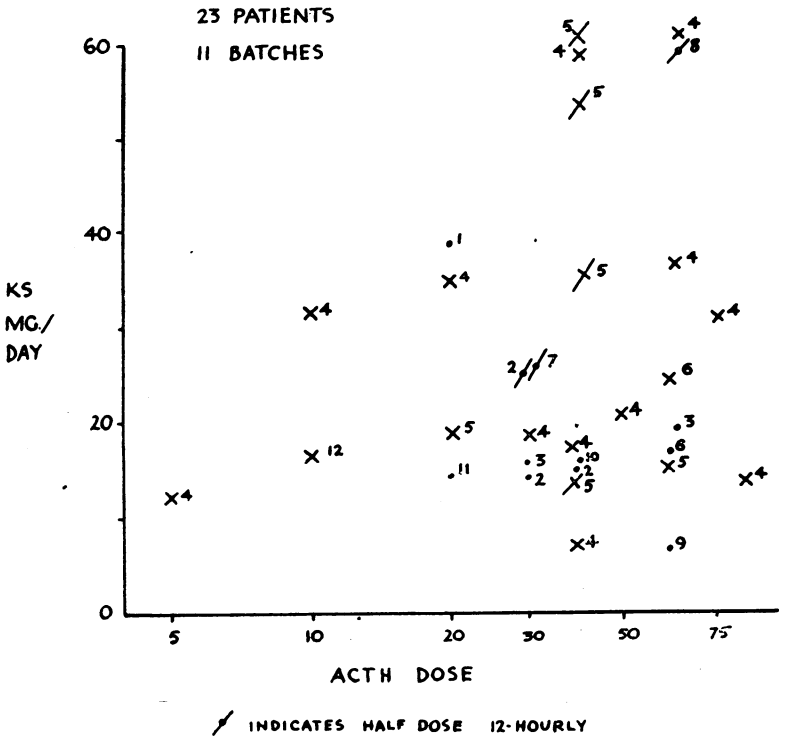

FIG. 3.—Response of 17-ketosteroids to corticotrophin gel preparations given once daily intramuscularly except where indicated. Dose in "clinical units."

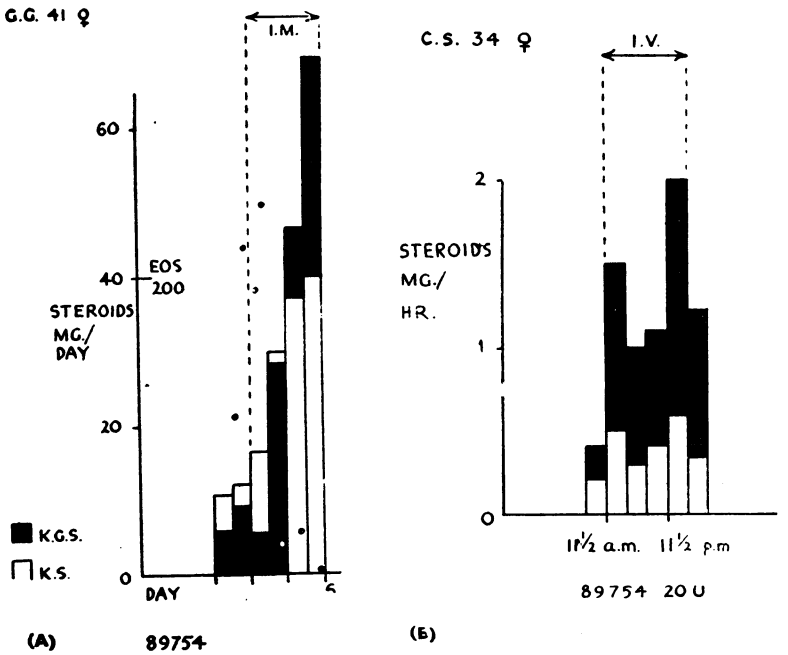

FIG. 4.—(A) Typical normal response of urine ketogenic steroids and 17-ketosteroids to intramuscular corticotrophin gel (20 clinical units twice daily). Eosinophil counts per c.mm. marked ●. (B) Typical normal response to corticotrophin intravenously, 20 units administered continuously.

intramuscular injection might still occur and yet the patient respond with intravenous administration. Thirdly, occasionally the clinical circumstances limit the time available for carrying out the intramuscular test, and the intravenous test may be carried out in 24 hours. Lastly, we have wished to study the dynamics of the response elicited. We have not as yet finally standardized the technique. Doses of 20 or 40 " clinical units " have been administered over periods varying from 8 to 20 hours and the urine has been collected over four-hour periods, a comparison being made between the ketogenic steroids and ketosteroids before and during the infusion. Fig. 4 shows a typical normal response. In this case corticotrophin, 20 " clinical units," was given in 5% glucose over a period of 14 hours. There is a sharp rise in ketogenic steroids when the infusion is started, and a very sharp fall at the end of the infusion. The maximum rate of ketogenic steroid excretion rose fivefold to 2 mg. per hour. The ketogenic steroid excretion exceeds that of ketosteroids, which show a threefold rise to a rate of 0.6 mg. per hour.

We have found it quite satisfactory to administer purified gel preparations in this way and have not encountered any reactions of a serious nature, but it is surely correct to be extremely cautious and to avoid the method for general use. It has been necessary to do this in order to compare the responsiveness to the intramuscular and intravenous routes of a given batch of corticotrophin in instances where the response by the former route has not attained expectation. The diagnostic error arose in a woman of 42 years who over a period of a few months developed a vague illness suspected to be Addison's disease. At this time administration of 20 " clinical units " of corticotrophin intravenously produced no elevation of ketogenic steroids or ketosteroids above the baseline levels of 0.3 and 0.4 mg. per hour respectively. We had not at that time carried out rat assay of the batch employed, but it was later found to have a very low potency. Three months later it was clear that the patient had developed acute lymphoblastic leukaemia, with a normal white count and primitive cells in the blood. Retesting with the standard intramuscular routine, and using a batch which was assayed to be at least as potent as the makers' estimate, gave a completely normal response. Incidentally, there was a striking decrease in the number of immature cells. These findings call for a note of caution in interpreting the effects of an unknown batch of corticotrophin.

Eosinophil Counts as a Measure of Response

It has been our continued experience that eosinophil counts correlate very well with sufficiently increased activity of the adrenal cortex during the short-term tests described. The results in 48 patients given intramuscular corticotrophin are given in Table I.

TABLE I.—*Eosinophil Counts and Ketogenic Steroid Response During Four Days' Intramuscular Corticotrophin (20 Clinical Units Twice Daily) in 48 Patients*

A. Initial eosinophil count exceeding 100 per c.mm. (morning counts)

(*a*) Eosinophils in 22 patients decreased to fewer than 50 per c.mm. in later afternoon counts,* while ketogenic steroids were simultaneously greater than 25 mg. per 24 hours on one of the four days of the test.

(*b*) In 5 patients eosinophils fell to fewer than 50 per c.mm., but when ketogenic steroid levels were below 25 mg. per day they reached the high level of 50 mg. or more per day on one of the next two days.

(*c*) In one patient eosinophils fell to 19 per c.mm., with a maximum ketogenic steroid excretion of 19 mg. per 24 hours. In a subsequent test eosinophils did not fall below 50 per c.mm.

B. Initial eosinophil count fewer than 100 per c.mm.

In 6 patients the eosinophils fell to below 50 per c.mm., with ketogenic steroids exceeding 17 mg. per day. In 4 of the patients the ketogenic steroids exceeded 25 mg

C. Initial eosinophil count exceeding 100 per c.mm.

In 12 patients the eosinophils did not fall below 50 per c.mm. and the ketogenic steroids did not reach 28 mg. per day. Three of these patients had Addison's disease.

D. Initial eosinophil count fewer than 100 per c.mm.

In 2 patients the eosinophils did not fall below 50 per c.mm. and the ketogenic steroids did not exceed 20 mg. per day.

* Afternoon counts made in the late afternoon after injection of corticotrophin gel at 10 a.m

In intravenous tests eosinophils were counted at the end of the infusion. Near zero levels (highest count 31 per c.mm., with an initial count of 350 per c.mm.) were reached in eight patients and corresponded to a ketogenic steroid excretion rate of at least 1 mg. per hour. This result and those from the intramuscular tests show a strong probability that the ketogenic steroid excretion rate reaches or exceeds 25 mg. per 24 hours if a satisfactory fall in eosinophils occurs. It would seem that the cell count will serve as a first approximation to adequate stimulation, but it gives no indication if higher excretion rates are reached. It is well known that eosinophil depression tends to fail after prolonged stimulation with corticotrophin, and under these circumstances higher counts are not necessarily indicative of failure of adrenal cortical activation.

Series of 61 Cases

In 61 patients suffering from a variety of diseases the ketogenic steroids have been assayed during the four-day intramuscular test. The results of these tests are summarized in Table II. The responses of the patients have been classified according to the initial steroid and ketosteroid

TABLE II.—*Summary of Responses to Corticotrophin*

Corticotrophin Ketogenic Steroid Responses (mg./Day)[1]	Low Resting Ketosteroid		Normal Ketosteroid			High Ketosteroid	
	Low Ketogenic	Normal Ketogenic	Low Ketogenic	Normal Ketogenic	High Ketogenic	Normal Ketogenic	High Ketogenic
	2 Children 2 Malnutrition 1 ? Cushing child 1 Male obese child Subacute nephritis Turner's syndrome	Acute leukaemia	2 Rheumatoid Secondary amenorrhoea	Hirsute Thyrotoxic Gastrectomy syndrome Diabetes Polyarthritis Normal ? Cushing Male obese Anorexia nervosa	Cushing* ? Cushing	5 Hirsute	Adrenal cortical tumour
40							40
	Hypophysectomy Carcinoma pituitary Male obese Scurvy Bronchiectasis	Carcinoma lung	2 Rheumatoid 2 Hirsute	Hirsute Pyelonephritis* Acromegaly	Thyrotoxic*	Hirsute Acromegaly* Gynaecomastia* Hyperparathyroid*	
25							25
		Delayed male puberty Encephalitis	Hypophysectomy Primary amenorrhoea Normal	3 Male obese		Male obese	
10							10
	4 Addison 1 Ant. hypopituitary						

[1] Irrespective of age. * Less active corticotrophin batches. All other patients received the same batch (89754).

excretion. They are first subdivided into three groups according to the resting ketosteroid level—low, normal, or high—and each of these groups is subdivided according to the level of ketogenic steroids. The patients have also been classified according to their response to corticotrophin with respect to ketogenic steroid excretion, the highest level attained during the four-day test being recorded. At the bottom left-hand corner of the table are four patients with Addison's disease and one with Simmonds's disease. At the top right-hand corner is a patient with adrenal cortical carcinoma (J. H.) who responded to corticotrophin. At the top of column 5 is a patient with Cushing's syndrome and another suspected of it. In the case of the patients with Addison's disease, basal ketosteroid levels varied between 2 and 6.8 mg., whilst the ketogenic steroids varied between 4.5 and 7.5 mg.—that is to say, ketogenic steroids were present and had not reached zero levels as might, in theory, be expected. This phenomenon has been commented upon by various investigators (Dustan, Mason, and Corcoran, 1953; Liddle, Island, Rinfret, and Forsham, 1954; Laidlaw, Reddy, Jenkins, Haydar, Renold, and Thorn, 1955). The last group of workers have confirmed by paper chromatographic methods that they were dealing with hydrocortisone metabolites. Similarly, if blood corticoid levels are considered, the occurrence of levels above zero in Addison's disease has been noted (Eik-Nes *et al.*, 1954; Bayliss, 1955; Christy, Wallace, and Jailer, 1955).

It is well established that, although corticotrophin cannot be certainly detected in the blood of normal individuals, large amounts are circulating in patients with Addison's disease (Sydnor and Sayers, 1952). The commonly accepted explanation of the phenomenon of the presence of adrenal cortical hormone in these patients is that they have a small amount of functional adrenal cortical tissue which is constantly subjected to maximal stimulation by endogenous corticotrophin and is unable to give further response to administered hormone. According to this view it would be necessary to postulate that the circulating corticoid level in the blood of these patients is just low enough to fail to prevent the inhibition of pituitary secretion by the feed-back mechanism (Sayers, 1950). Patients with hypopituitarism, in whom partial-to-complete failure of corticotrophin secretion presumably occurs, would be expected to have adrenal cortices of varying grades of atrophy and therefore varying grades of potential response to corticotrophin.

There is a group of patients at the top left-hand corner of Table II who deserve special comment. These are patients who have low levels of ketosteroids and ketogenic steroids in the basal state and are very responsive to corticotrophin. It might be expected that they include the two "normal" children in the group who were under treatment with coticotrophin. There seem to be no comparable data for a larger group of children in the literature, but the low basal levels of ketosteroid excretion in them are well known (Mason and Engstrom, 1950). The other patients in this group have various diagnoses. One of these was a boy aged 7 with suspected Cushing's syndrome who in fact excreted excessive amounts of ketosteroid for his age. Two patients appear under the heading of "malnutrition." One of these was a woman of 52 with a history of pellagra followed by pulmonary tuberculosis. She was suspected of having anterior hypopituitarism, but her ^{131}I excretion proved to be normal and other tests were negative. Her adrenal was very responsive to corticotrophin, the maximum ketogenic steroid excretion reaching 63 mg. per 24 hours. She had many of the characteristics of anorexia nervosa. The second of these patients was a man aged 52 with body pigmentation, excluding the mouth, loss of weight, and asthenia for a prolonged period of years. He had diminution of beard and sexual hair. All tests for Addison's disease and hypopituitarism proved to be negative. He was, however, known to have diverticulitis of the sigmoid colon. His blood pressure was usually 220/120 mm. Hg. The maximum ketogenic steroid response in this patient was 68 mg. per 24 hours.

Attention should also be drawn to a woman with prolonged subacute nephritis, a scorbutic man, and a man with bronchiectasis. The last had been suspected for five years of Addison's disease due to amyloid deposit in the adrenal cortex, but he did not develop other clinical evidence of amyloid disease. Other tests for Addison's disease were negative; he had previously failed to respond to corticotrophin of dubious activity.

"Basal Hypo-adrenal Corticalism"

Patients of the type described who are suspected of Addison's disease or hypopituitarism but respond well to corticotrophin are of especial interest. Selye has produced evidence that pituitary function, with respect to corticotrophin, can be influenced by dietary means (Henriques, Henriques, and Selye, 1949). These patients seem to have in common a low level of nutritional status for one reason or another, a condition which can itself, if severe enough, lead to decreased steroid excretion (see discussion after Prunty, Clayton, McSwiney, and Mills, 1955; Clayton and Hammant, 1955a; Perloff, Lasché, Nodine, Schneeberg, and Vieillard, 1954). The potentially good adrenal cortical response to corticotrophin, in spite of depletion of adrenal ascorbic acid in scurvy, has been previously discussed (Prunty *et al.*, 1955). It must be supposed that the level of circulating corticotrophin is too low to maintain normal steroid excretion and not low enough to lead to cortical atrophy sufficient to produce failure of response to corticotrophin; indeed, they appear to be able to respond very strongly, and they fall in a group in which there are two patients who almost certainly have some reduction of pituitary function. It is proposed to use the term "basal hypo-adrenal corticalism" to distinguish them from patients with Addison's disease or anterior hypopituitarism.

Further analysis of the patients in Table II brings out three points to which attention should be drawn. Firstly,

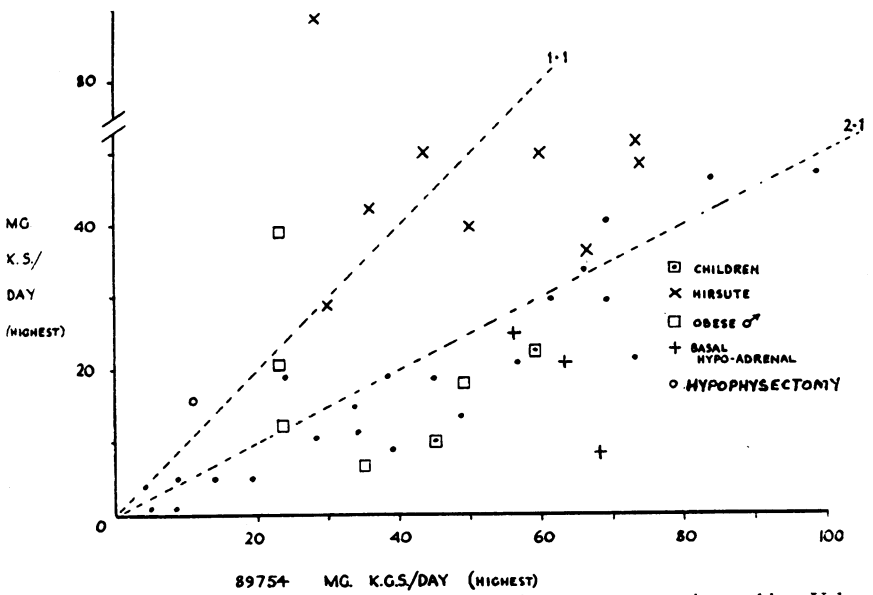

FIG. 5.—Ratio of ketogenic steroids to 17-ketosteroids in response to corticotrophin. Values are highest marked during four days' intramuscular administration (corticotrophin 89754). The normal adult responses are marked ●.

a group of 10 adult hirsute women who responded well to corticotrophin. Secondly, certain patients, marked with an asterisk in Table II, may have given submaximal responses, since they received a batch of corticotrophin of poor potency by rat assay. The remainder of the patients all received very potent batches. It is therefore possible that the position of the former patients should be moved vertically upwards when compared with the remainder. In the third place, there are five adult obese males all of whom received potent corticotrophin and four responded feebly, although basal steroid excretion was very adequate. These cases will be discussed further.

Interesting results have been obtained when the ratio of the increase in ketogenic steroid excretion is compared with the increase in ketosteroid excretion (Fig. 5). For further reasons, which will become apparent, data in this chart have been confined to patients receiving a single very potent batch of corticotrophin (89754). The figures plotted represent the highest levels of ketogenic steroid or ketosteroid attained during the four days of the test. The majority of the observations fall near or below a line indicating a ratio of ketogenic steroids to ketosteroids of 2 to 1. The two normal children are below it. This indicates a relatively greater responsiveness of corticoids. Ferrazzini, Borth, and Mach (1952) have published data on 10 children, using older methods of assessment, indicating that they are very responsive in this respect, whereas the level to which ketosteroids rise is low. In one of our children, a girl aged 9, ketosteroids reached a level of 10 mg. per day, and in the other, a boy aged 11 and therefore nearing puberty, they reached 22 mg. per day. Also in this area of the chart are the patients with basal hypo-adrenal corticalism. There are nine women with post-pubertal hirsutism, all of whom have responded well to corticotrophin. Their response will be examined in greater detail.

The responses of the entire miscellaneous group of patients to corticotrophin may be summarized as follows: (1) Low ketosteroids and ketogenic steroids with no response to corticotrophin—Addison's disease. (2) Low resting steroid excretion with rapid and often high response in ketogenic steroids—basal hypo-adrenal corticalism. (3) Normal resting steroids with normal response to corticotrophin, the ketogenic steroid excretion then exceeding the ketosteroid excretion. (4) Normal to high resting ketogenic steroids, ketosteroids not usually elevated, with rapid and large response of ketogenic steroids to corticotrophin—Cushing's syndrome (see remainder of lectures, to appear next week). (5) Normal ketogenic steroids with possibly some elevation of resting ketosteroids, a good response of ketogenic steroids to corticotrophin, and a supernormal response of ketosteroids —for example, in post-pubertal hirsutism.

It is therefore meaningless to express the increase in steroid excretion due to corticotrophin as a percentage of the basal level, as has often been done in the past.

[*The conclusion of these lectures, with a list of references, will appear in our next issue.*]

In his search through documents stored in Guy's Hospital strong-room Dr. Hector C. Cameron has found information on the previously unknown ancestry of Thomas Guy, the founder of the hospital (*Guy's Hosp. Rep.*, 1956, **105**, 151). The document pertains to a meeting of Thomas and his brother, with two other members of the family, on May 6, 1687, to discharge a trust instituted by "Thomas Guy of Heckfield in the County of Southton, Clothier." He was the founder's great-grandfather. The Trust directed that £5 should be paid every feast of St. Michael the Archangel to some descendant of the same "Stock-birth." In contrast, the founder himself distributed £92,400 among people not his "Stock-birth," being chiefly the humble relatives of his mother, among whom he was brought up. Of the 50 families mentioned in his will two only had the surname Guy. The governors of the hospital paid the annuitants regularly until the last of them, Mary Hill, died in 1770.

A CLASS EXPERIMENT ON GANGLION BLOCK IN HUMAN SUBJECTS

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The recent development of ganglion-blocking agents has led to their use principally in patients suffering from hypertension and a number of other disorders. Their effects in normal subjects have not been examined in the same detail, although numerous scattered observations are recorded and an attempt has been made with a group of normal subjects to correlate action with plasma concentration (Morrison and Paton, 1953). The human pharmacology class at University College provided an opportunity whereby some knowledge about the action of these drugs could be obtained in a population of healthy young adults, at the same time helping to train the students in making a number of clinical observations and in rendering these observations quantitative in a way already found valuable in a previous class (Wilson, Crockett, Exton-Smith, and Steinberg, 1950).

An experiment of this type naturally requires stringent safeguards, and in no case was a drug administered without the consent of the student. Demonstrators were always at hand to supervise the observations. Furthermore, a dose of drug and a route of administration were chosen which were known to produce only mild or moderate effects of reasonably brief duration. The class has proved successful, and has yielded valuable information on the individual variation of response to a ganglion-blocking agent. The class has now been running for four successive years, enabling observations to be made on more than 70 students, without any mishap. The development of the experimental procedure has also prompted us to devise some methods for measuring autonomic function which may be of use to others.

Methods

Systemic Blood Pressure.—This was measured, using the conventional cuff, by auscultation at the elbow, combined with palpation of the radial pulse when difficulty was found in hearing the sounds in the brachial artery. The blood pressure was taken in the supine position and then standing. To estimate the standing blood pressure sixty seconds, timed with a stop-clock, was allowed to elapse after the subject had got to his feet, and the systolic and diastolic pressures were recorded as near as possible to this time. Where postural hypotension made it impossible to obtain a reading, the subject was made to lie down despite the absence of a reading. Under these circumstances it was usually possible to state that the blood pressure was at least below a certain level.

Pulse Rate.—This was measured in the supine position and in the standing position at the same time as the blood pressure was measured, the observations being made by a second observer.

Skin Temperature.—This was measured using a thermocouple and galvanometer, with a reference junction in ice. It was usual to place a thermocouple on the plantar surface of the right big toe, marking the region with a spot of ink

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