steroid. First, and particularly important, a large local rise of Na^+ concentration, up to or above normal in a Na^+ -depleted sheep, did not produce an effect similar to a rapid correction of Na^+ deficiency, though the change was maintained for an adequate time. In raising the question of whether any misleading factor could have inadvertently entered into these experiments, the principal issue is that a normal response to rapid correction of Na^+ deficiency is prevented if an animal is disturbed or frightened. Now, these animals were trained and confident, and there was no evidence from demeanour, saliva secretion rate, or cardiac rate that they were in any way disturbed. In fact, it was in the control experiments where the 4 M NaCl raised systemic Na^+ rapidly by 20 mEq that there was some restlessness and evidence of thirst, yet the response occurred normally. It seemed legitimate to suppose that if a simple rise of Na^+ concentration or Na^+/K^+ ratio of the adrenal arterial blood caused this response, we would have reproduced it by these experiments.

The experiments involving decreased Na^+ and increased K^+ to the adrenal indicated that this change could stimulate electrolyte-active steroid production if continued long enough, and that progressive reduction of Na^+ and increase of K^+ could prevent inhibition of it during rapid change of Na^+ balance. In this latter series, the fact that the normal response did occur about 90–100 minutes after stopping the adrenal infusion was strong evidence that the local ionic change at the adrenal level had prevented adrenal inhibition. Conversely, in the experiments where the normal response to commencing Na^+ deficiency was prevented by gradually increasing adrenal arterial Na^+ concentration (Section IV), there would have been an abrupt fall of adrenal arterial Na^+ concentration when the infusion stopped. A response occurred shortly afterwards, though the procedure of abruptly lowering adrenal Na^+ concentration (Section I) of itself did not stimulate the adrenal.

These findings, along with those of Section II, suggested the possibility that alteration of a standing state at the adrenal level involved concurrent action of at least two components of a causal system. That is, the results recorded led to a conclusion that appropriate local change of the Na^+ and K^+ concentrations of adrenal arterial blood did not cause the same extent and time relationship of change of adrenal secretion which occurred during alteration of Na^+ balance. However, there was evidence that such local ionic changes affected the adrenal secretory responses which occur in these circumstances.

The local ionic changes may be a contributory condition in a set of jointly sufficient and severally necessary conditions which operate at the adrenal level to cause the physiological response to change of Na^+ balance. Thus in relation to the question of whether the adrenal responds directly to local changes in its ionic environment or whether it responds to a stimulus from a distance, it is possible that there is a causal system involving both, and, moreover, that stimuli to the receptor controlling the humoral stimulus may be physiological effects of Na^+ deficiency other than change of systemic ionic concentrations. In the experiments cited above we may have dissociated to some extent the two stimuli, which usually work concurrently and synergistically.

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

STEROID THERAPY IN HIRSUTISM AND VIRILISM

BY

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The effects of steroid therapy in hirsute women with increased excretion of 17-ketosteroids have been reported by Spence *et al.* (1956), who described the use of cortisone acetate by the oral and intramuscular routes, hydrocortisone acetate by intramuscular injection, and oral prednisone and prednisolone. Further observations are reported here on the use of oral prednisone and prednisolone, and of intramuscular hydrocortisone acetate, and on a trial of a new preparation, prednisolone trimethylacetate, which consists of a microcrystalline suspension of 200 mg. of the hormone in 2 ml. of an aqueous isotonic buffered solution.

Prednisolone Trimethylacetate

The effects of prednisolone trimethylacetate on the urinary excretion of 17-ketosteroids were studied in five patients. One of these was a female pseudo-hermaphrodite aged 15 years (Case 5); the other four were hirsute women with oligomenorrhoea (Cases 1–4). Each patient was given one or more intramuscular injections of 200 or 400 mg. of prednisolone trimethylacetate. The urinary 17-ketosteroid output was estimated by the standard M.R.C. method over at least two 24-hour periods before injection and thereafter on successive days or at intervals of up to one week.

The resting 17-ketosteroid excretion varied between 35.6 and 62.3 mg. (mean 46.8 mg.) per 24 hours in Case 5, and the mean output in Cases 1–4 ranged from 17 to 25 mg. per 24 hours.

Prednisolone trimethylacetate was originally given in a single intramuscular dose of 200 mg. to two hirsute women (Cases 1 and 2). This produced no significant

FIG. 1.—Effect of prednisolone trimethylacetate (P.T.M.A.) injected intramuscularly and of oral prednisone on urinary 17-ketosteroids. (Case 1.)

FIG. 2.—Effect of prednisolone trimethylacetate (P.T.M.A.) injected intramuscularly and of oral prednisolone on urinary 17-ketosteroids. (Cases 2-5.)

FIG. 3.—Effect of oral prednisone on the urinary 17-ketosteroids (Case 6).

FIG. 4.—Effect of hydrocortisone acetate injected intramuscularly on urinary 17-ketosteroids (Cases 7 and 8).

suppression of the 17-ketosteroid output, and these two patients and the other three studied were subsequently given single injections of 400 mg. intramuscularly. The larger dose was followed by an immediate and striking fall in 17-ketosteroid output in Case 5 (Fig. 2), and in Case 2, a less striking fall in Cases 3 and 4, and no significant change in Case 1 (Fig. 1). A considerable degree of suppression was maintained for between three and four weeks in Case 5, but it was found necessary to repeat the intramuscular injections of 400 mg. at weekly intervals in order to maintain the 17-ketosteroid output below the level of 8 mg. per 24 hours advocated by Wilkins *et al.* (1952). After weekly injections had been given for a period of 13 weeks, supplies of the material temporarily ran out and the patient was left without further treatment; to our surprise, suppression of the 17-ketosteroid output persisted for as long as five months after discontinuing treatment, the total excretion amounting to only 1 mg. daily at one stage and being increased only temporarily by stimulation with corticotrophin-zinc, 40 units daily intramuscularly, over a period of three days.

Oral Prednisone and Prednisolone

In three of the four hirsute women treated with prednisolone trimethylacetate intramuscularly the effects of oral prednisone or prednisolone were studied (Figs. 1 and 2). The initial dosage was 20 mg. daily in all cases. Cases 3 and 4 were given prednisolone, and Case 1 was given prednisone. Immediate and profound suppression of the urinary 17-ketosteroid output was produced in all three patients. Steroid therapy was then gradually withdrawn over periods of four to seven weeks. Some degree of suppression was maintained for many weeks after withdrawal. In Case 4 the 17-ketosteroid excretion was still within the normal range (less than 18 mg. per 24 hours), and well below the resting level, 21 weeks after the withdrawal of steroid therapy, and in Case 3 as long as 43 weeks after stopping treatment; in Case 1 it had returned to the original level (within normal limits) 20 weeks after the withdrawal of steroid therapy.

Further follow-up of two of the three hirsute women reported by Spence *et al.* (1956) has shown that the suppression produced by oral prednisone and prednisolone persisted for long periods of time. In these two women the 17-ketosteroid output remained well within normal limits and much below the resting level for as long as 15 months after the withdrawal of steroid therapy. In a further hirsute patient with amenorrhoea (Case 6, Fig. 3) profound suppression of 17-ketosteroid output was obtained by the administration of 20 mg. of prednisone daily for two weeks, followed by 15 mg. daily for a further 26 weeks, and has already persisted for as long as 15 months after stopping treatment.

Intramuscular Hydrocortisone Acetate

In one of the hirsute women studied by Spence *et al.* (1956) it was reported that the suppression induced by daily intramuscular injections of cortisone acetate was subsequently maintained by fortnightly injections of hydrocortisone acetate, 250 mg. intramuscularly. When, later, we observed the long duration of suppression after withdrawal of steroid therapy, it occurred to us that the apparent maintenance of suppression by hydrocortisone acetate might have represented merely the continued effect of the previous cortisone therapy. We therefore

decided to observe the effect of hydrocortisone acetate *de novo* in two patients. This preparation was given to a hirsute woman aged 28, with regular menses (Case 7, Fig. 4) in two doses of 250 mg. intramuscularly at an interval of one week. This was followed by a gradual fall in 17-ketosteroid output from a mean figure of 20.2 mg. per 24 hours before treatment to a level of 8.5 mg. per 24 hours five days after the second injection. The output was still slightly below the initial mean figure as long as 43 weeks after the second injection. The second patient (Case 8, Fig. 4), a woman aged 26 with obesity, amenorrhoea, and slight hirsutism, was given an initial intramuscular injection of 250 mg. of hydrocortisone acetate, followed by an injection of 125 mg. two weeks later and a third injection of 250 mg. after a further two weeks. This was followed by a gradual fall in 17-ketosteroid output from a mean figure of 21.2 mg. per 24 hours to a level of 10.5 mg. three weeks after the third injection. The output had returned to the resting level six weeks after the last injection.

Effects on Hirsutism and Menstruation

Our impression has been that no striking improvement in the hirsutism has resulted from prolonged steroid therapy in any of the patients, as previously observed by Spence *et al.* (1956). In two patients with oligomenorrhoea regular menses have been restored. In another patient (Case 6) amenorrhoea has persisted despite prolonged suppression with prednisone. In the remainder the menses were regular before treatment.

Discussion

The oral administration of prednisone or prednisolone appears to be an effective method of suppressing the output of 17-ketosteroids in the urine and is more convenient than repeated intramuscular injections of cortisone acetate or hydrocortisone acetate. Prednisone and prednisolone are more effective by the oral route than is cortisone acetate, probably because they are more slowly metabolized and may therefore maintain a more constant inhibition of pituitary-adrenal function (Slaunwhite and Sandberg, 1957). Prednisolone trimethylacetate by the intramuscular route seems to be less reliable than oral prednisolone, even when given in doses of 400 mg., possibly as a result of its very slow absorption from the site of injection, although the cumulative effect of repeated injections was shown by excessive and prolonged adrenal cortical suppression in Case 5.

Our results emphasize that suppression of adrenocortical function by steroid therapy may be prolonged and may persist in some degree for many weeks or months after withdrawal of therapy. The degree and duration of suppression obtained in Case 5 were of a dangerous order and might have led to adrenocortical failure under the stress of an intercurrent infection or surgical operation.

The effects on the hirsutism in these patients is extremely disappointing, which suggests either that the hirsutism is not endocrine in origin or that the hair-follicles have become abnormally sensitive to androgens. Steroid therapy is clearly not justifiable on the grounds of hirsutism alone. Amenorrhoea or severe oligomenorrhoea, if due to adrenocortical hyperfunction, constitutes a stronger indication for treatment. Whether a raised 17-ketosteroid output is in itself an indication for steroid therapy is debatable, but it certainly demands

a diagnostic suppression test in order to exclude the possibility of an adrenocortical tumour. If steroid therapy is undertaken it would appear from our results that short intermittent courses of steroid therapy would be as effective as prolonged treatment and much safer, at any rate in cases of simple hirsutism and mild degrees of virilism. Continuous treatment is usually necessary in cases of female pseudohermaphroditism, and prolonged suppression of the degree shown in our patient does not appear to have been reported previously. Prednisone and prednisolone were given orally in doses of 20 mg. daily in this series of hirsute patients in order to ensure adequate suppression, but it is likely that this could be achieved with smaller doses.

Investigations are now in progress to determine the minimal effective dose for this purpose.

Summary

Eight female patients with adrenal virilism, all adults except one pseudohermaphrodite and all with a raised 17-ketosteroid excretion, were treated with a crystalline suspension of prednisolone trimethylacetate injected intramuscularly, with oral prednisone or prednisolone, or with intramuscular injection of hydrocortisone acetate.

As much as 400 mg. of prednisolone trimethylacetate at weekly intervals was required to effect suppression of corticotrophin, judged by depression of the 17-ketosteroid excretion, due no doubt to its very slow absorption, whereas daily doses of 20 mg. of prednisone or prednisolone by mouth caused an immediate and profound suppression, and two injections of 250 mg. of hydrocortisone acetate at an interval of a week were also effective. With all three methods suppression persisted for many weeks or months after withdrawal of therapy. For this reason short intermittent courses are advocated except in female pseudohermaphroditism.

No significant improvement in hirsutism was obtained and therefore steroid therapy is not justifiable on the grounds of hirsutism alone. In two patients with oligomenorrhoea normal menstruation was restored, in one patient amenorrhoea persisted, and in the remainder menstruation was normal before treatment.

We are indebted to Ciba Laboratories Ltd. for generous supplies of prednisolone trimethylacetate.

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In spite of the rise in the prison population, from 20,900 at the end of 1956 to 25,798 at the end of 1958, health has been well maintained, reports the director of medical services. Although the number of those remanded in custody for medical reports has greatly increased, the numbers found insane and mentally defective are the lowest recorded for many years. The director says that the figures taken together suggest not only that cases of mental illness which would otherwise have appeared in court and have been remanded to prison may have been saved from the committal of offences by mental treatment but also that courts are increasingly interested in the mental disabilities or maladjustments of those who appear before them even when this falls short of certifiable disease or defect. (*Report of the Commissioner of Prisons, for the Year 1958*. H.M.S.O., 9s. 6d. net.)

MULTIPLE SERIAL ENZYME STUDIES IN ACUTE MYOCARDIAL INFARCTION

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Elevation of the level of serum glutamic oxalacetic transaminase (S.G.O.T.) follows necrosis of cardiac muscle, and we have recently published our experiences in the use of this enzyme in the diagnosis of acute myocardial infarction (Dewar *et al.*, 1958). The serum levels of other enzymes are also transiently raised after myocardial infarction, and in this communication we report the results of serial estimations of serum glutamic pyruvic transaminase (S.G.P.T.), serum aldolase, and serum oxidase, and the relation to similar estimations of S.G.O.T.

S.G.O.T. catalyses the reversible transfer of the α -amino nitrogen of aspartic acid to α -oxo-glutaric acid, with the production of glutamic and oxalacetic acids. Elevated levels are found in about 92% of cases of myocardial infarction. The level rises within three hours after infarction, usually reaches a maximum on the second day, and falls to normal in three to five days. The peak level is roughly related to the extent of the myocardial infarction in the absence of centrilobular necrosis of the liver due to cardiac failure.

S.G.P.T. reversibly catalyses the conversion of alanine and α -oxo-glutaric acid to pyruvic and glutamic acids. Wróblewski and LaDue (1956) found the mean level to be 16 ± 9 units per ml. in 260 normal subjects. S.G.P.T. failed to rise significantly in eight out of nine of their cases of acute myocardial infarction. They considered that only when the infarct was sizable was the amount of S.G.P.T. liberated great enough to raise transiently the serum level. They concluded that the S.G.P.T. appeared to be elevated above normal only when necrosis is great enough to raise the level of the S.G.O.T. above 150–200 units. Chinsky *et al.* (1957) found the S.G.P.T. elevated in 9 out of 24 cases of acute myocardial infarction, but in only one of these was the rise greater than the rise in the S.G.O.T.

Serum aldolase catalyses the splitting of fructose 1,6-diphosphate to D-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. It can be estimated by the method of Sibley and Lehninger (1949), and the normal average is 5 units per ml. of serum, with a range of 2.5–8.5 units per ml. (White, 1956). Experimentally, it has been shown that coronary artery ligation in dogs results in a considerable and rapid rise in serum aldolase and that there is a semi-quantitative relationship between the area of myocardial necrosis and serum aldolase (Volk *et al.*, 1956). The rise starts within three hours after infarction, reaches a maximum in about 24 hours, and then falls rapidly by 48 hours (Siegel and Bing, 1956), becoming normal in five days (Losner *et al.*, 1957). When pathological changes are correlated with plasma enzyme activity it is found that coagulation necrosis coincides with the peak of enzyme activity, and as reparative processes in cardiac muscle take place enzyme activity declines (Bing *et al.*, 1956).