THE EFFECTS OF PROLONGED ADMINISTRATION OF SOME ADRENOCORTICAL STEROIDS IN THE RAT

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An approximation has been made of the relative potency of some adrenocortical steroids to retard body growth and cause adrenocortical atrophy in chronically treated rats. The site of cortical atrophy and depletion of sudanophilic lipid varies with the nature of the steroid administered. The implications of these findings have been discussed with particular reference to the pituitary control of the zona glomerulosa. Corticosteroid induced adrenocortical atrophy is reversible on cessation of treatment. The effect of prolonged administration of cortisone on the weight of organs and other endocrine glands has also been studied.

THE prolonged administration of adrenocortical steroids to laboratory animals causes inhibition of normal growth (Silber and Porter, 1953; Shewell and Long, 1956; Hansen, Blivaiss and Rosenzweig, 1957; Goodlad and Munro, 1958) and adrenocortical atrophy (Ingle, 1938; Sayers and Sayers, 1949; Stebbins, 1950; Winter, Silber and Stoerk, 1950). The chronic administration of these steroids to man may suppress adrenocortical function resulting in the absence of a normal response to stress (Salassa, Bennett, Keating and Sprague, 1953; Nabarro, 1960; Stevens, 1960), and in particular to that of surgical trauma (*Lancet*, 1957; Bayliss, 1958; Shneewind and Cole, 1959). Severe shock and collapse from adrenal failure may occur in patients, who had ceased corticosteroid treatment weeks or months before the time of surgery (Lewis, Robinson, Yee, Hacker and Eison, 1953; Salassa and others, 1953; Hayes and Kushlan, 1956).

It was therefore thought of interest to determine whether there were any quantitative or qualitative differences between the effects of prolonged administration of the more commonly used corticosteroids on the adrenal glands of rats, and to determine whether adrenals which had atrophied as a result of such dosage regained their normal weight and histology when treatment ceased.

Organs and glands, other than the adrenals, were routinely examined, and the results included in this report.

Some preliminary results of this work have already been published (D'Arcy, 1958; D'Arcy and Howard, 1958a, 1958b, 1960, 1961).

MATERIALS AND METHODS

Male albino rats, from the Agricultural Research Council were used. The weights were 100–120 g.; they were kept in a thermostatically controlled room at 68–70° F, and were maintained on a cubed diet and tap water provided *ad lib.*

Adrenocortical steroids were injected intramuscularly, suspended in saline, with the exception of large doses of deoxycorticosterone acetate, which were injected subcutaneously because of the density of the suspension. Except where specifically mentioned, corticosteroids were

injected on alternate days, thrice weekly for 6 weeks; in each experiment, control groups of rats received injections of saline alone. The weight of each rat was recorded at regular intervals. At the end of each experiment, rats were killed with ether and the adrenal glands and other tissues were removed to formol saline. Subsequently these tissues were dissected from fat and other extraneous matter, dried between filter papers and weighed; adrenal glands were also examined histologically. Frozen sections of the adrenals were stained with Sudan III to show the distribution of sudanophilic lipid.

RESULTS

Effect on Body Weight and Adrenal Weight

The intramuscular injection of cortisone acetate (1·25 mg./rat with twofold increases in dose to 10 mg./rat), hydrocortisone acetate (1·25 to 5·0 mg./rat), prednisone acetate (0·63 to 2·5 mg./rat), prednisolone (0·63 to 2·5 mg./rat) and fludrocortisone acetate (0·31 to 2·5 mg./rat) retarded body growth and reduced the weights of the adrenal glands. Both effects increased with an increase in the dose of the steroid. An example of the effect of prolonged administration of adrenal steroids on body weight is shown in Fig. 1, which shows the effects of graded doses of cortisone acetate. Some deaths from infection occurred with high doses. Post-mortem examination of these animals revealed lesions of the lungs and liver and the presence of Gram-positive bacteria in the heart blood.

Deoxycorticosterone acetate (DCA) in doses of 1·25 to 5·0 mg./rat intramuscularly failed to influence either body or adrenal weights;
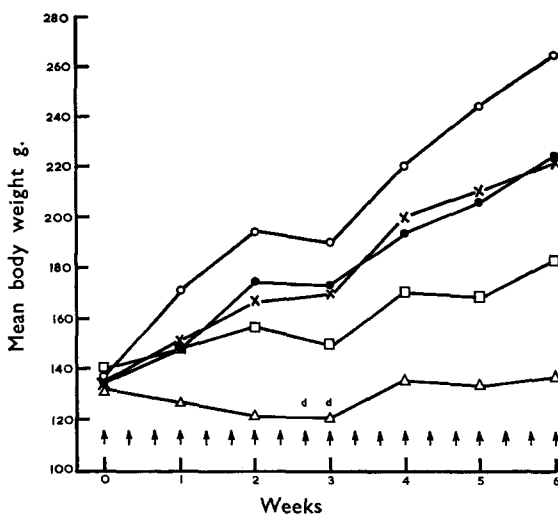

FIG. 1. The effect of prolonged administration of cortisone acetate on body weight. The steroid was injected 3 × a week, i.m. (at arrows) for 6 weeks to groups of 6 male rats at doses of 1·25, (●——●); 2·5, (X——X); 5·0, (□——□), and 10 mg./rat (△——△). The control group (○——○), received saline injections. Mortality is indicated by "d."

however, larger doses of this steroid (10 to 50 mg./rat subcutaneously) retarded growth and caused adrenal atrophy. The effects of other steroids on body weight and adrenal weight are compared with the effects of cortisone acetate in Table I.

An attempt has been made to evaluate the approximate potencies of these steroids, relative to cortisone acetate, in causing adrenal atrophy, retardation of body growth and also for their relative toxicity (Table II). Relative toxicity was assessed by comparing the maximum tolerated doses of each steroid, when dosage was increased by twofold stages. Fludro-cortisone is the most active and DCA the least active; also with increase in potency there is an accompanying increase in toxicity.

TABLE I

THE EFFECT OF PROLONGED ADMINISTRATION OF ADRENAL STEROIDS ON BODY AND ADRENAL WEIGHTS

| | | | | Adrenal atrophy | |
Steroid	Dose*	No. of rats	Mean body weight per cent of control	Mean adrenal weight (mg.) per cent of control	Mean adrenal weight (mg./100 g. body wt.) per cent of control
Cortisone acetate	1·25	6	84·6	65·8	80·4
	2·5	6	83·9	66·3	79·0
	5·0	6	69·2	40·3	59·4
	10·0	6	51·7	25·5	57·2
Hydrocortisone acetate ..	1·25	6	89·2	68·4	77·3
	2·5	6	62·5	34·8	55·5
	5·0	6	55·8	31·6	60·9
Prednisone acetate	0·63	6	93·1	78·5	81·1
	1·25	6	89·7	76·0	82·9
	2·5	6	47·2	47·3	103·7†
Prednisolone	0·63	6	76·7	74·0	98·5
	1·25	6	54·1	37·8	67·4
	2·5	6	33·5	25·8	68·8
Fludrocortisone acetate ..	0·31	6	71·5	58·7	79·3
	0·63	6	65·9	28·2	43·1
	1·25	6	42·3	29·1†	69·1†
	2·5	6	40·8	29·8†	73·4†
Deoxycorticosterone acetate	1·25	6	101·1	102·1	80·4
	2·5	6	104·2	100·0	99·1
	5·0	6	98·4	75·6	103·7
	10·0	6	81·9	34·2	40·8
	25·0	6	61·1	26·9	40·8
	50·0	6	49·1	28·6	57·7

* mg./rat, i.m. × 3 per week for 6 weeks.
† Apparent increase in adrenal weight from early mortality at these doses.

TABLE II

RELATIVE POTENCIES OF ADRENAL STEROIDS IN CAUSING ADRENAL ATROPHY, RETARDATION OF BODY GROWTH, AND DEATH DURING CHRONIC ADMINISTRATION*

Steroid	Adrenal atrophy	Retardation of growth	Toxicity (death)
Cortisone acetate	1·0	1·0	1
Hydrocortisone acetate	2·2	2·2	2
Prednisone acetate	1·6	2·8	4
Prednisolone	3·9	6·7	8
Fludrocortisone acetate	11·5	9·9	16
Deoxycorticosterone acetate ..	0·5	0·3	Non toxic at doses of 50 mg./rat × 3 per week.

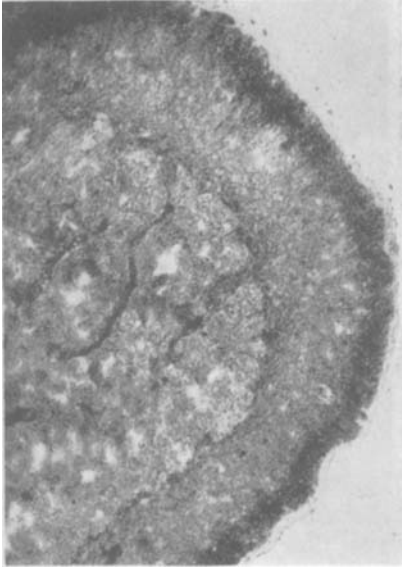* Intramuscular injections × 3 per week for 6 weeks.
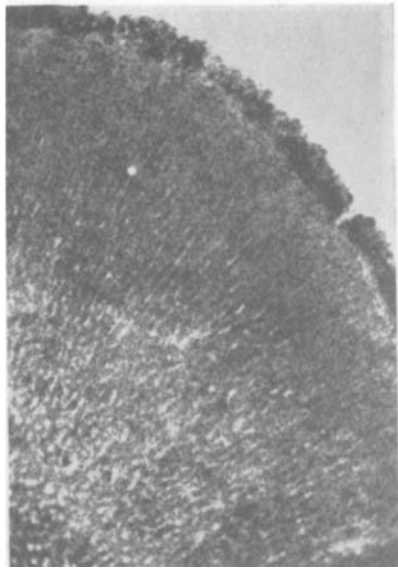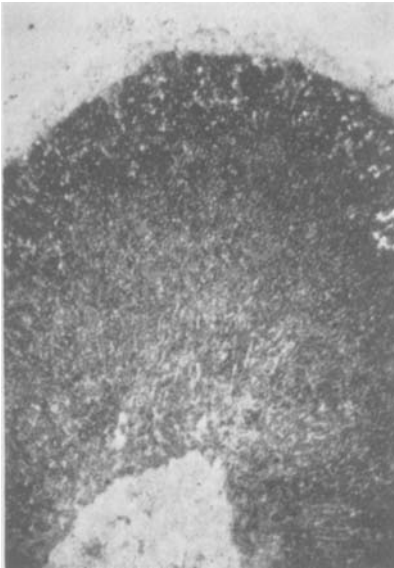
PLATE 1

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 1. Adrenal gland of rat treated with cortisone acetate (10 mg./rat 3 × a week, i.m., for 6 weeks). Marked atrophy of the zona fasciculata, but the zona glomerulosa is normal and contains abundant sudanophilic lipid. × 110.

FIG. 2. Adrenal gland of saline treated control rat. Normal morphology and distribution of sudanophilic lipid. × 110.

FIG. 3. Adrenal gland of rat treated with DCA, (5 mg./rat 3 × a week, i.m., for 6 weeks). Marked depletion of sudanophilic lipid in the zona glomerulosa; no evidence of cortical atrophy. × 110.
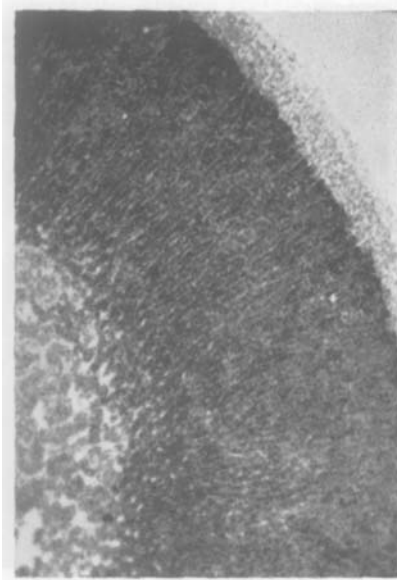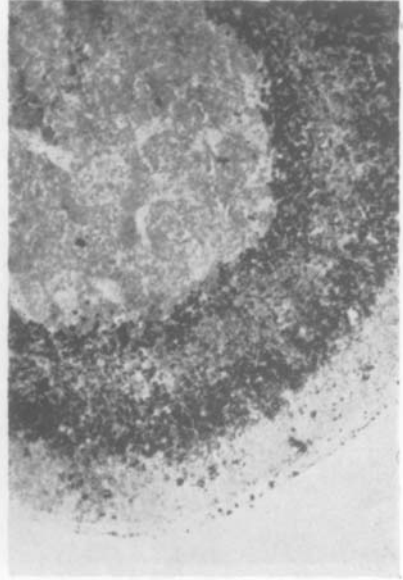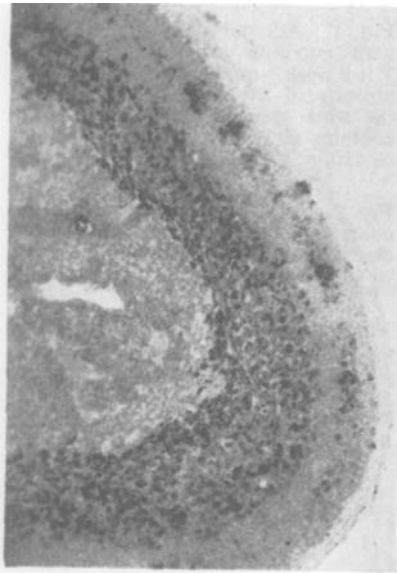
PLATE 2

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 1. Adrenal gland of rat treated with fludrocortisone acetate (0·31 mg./ rat 3 × a week, i.m., for 6 weeks). Marked depletion of sudanophilic lipid in the zona glomerulosa; slight evidence of cortical atrophy. × 110.

FIG. 2. Adrenal gland of rat treated with DCA (50 mg./rat 3 × a week, s.c., for 6 weeks). Depletion of sudanophilic lipid in the zona glomerulosa and in outer region of the zona fasciculata, together with extensive atrophy of cells in the middle and inner regions of the zona fasciculata. × 110.

FIG. 3. Adrenal gland of rat treated with fludrocortisone acetate (0·63 mg./ rat 3 × a week, i.m., for 6 weeks). Depletion of sudanophilic lipid in the zona glomerulosa and in outer region of the zona fasciculata, together with extensive atrophy of cells in the middle and inner regions of the zona fasciculata. × 110.
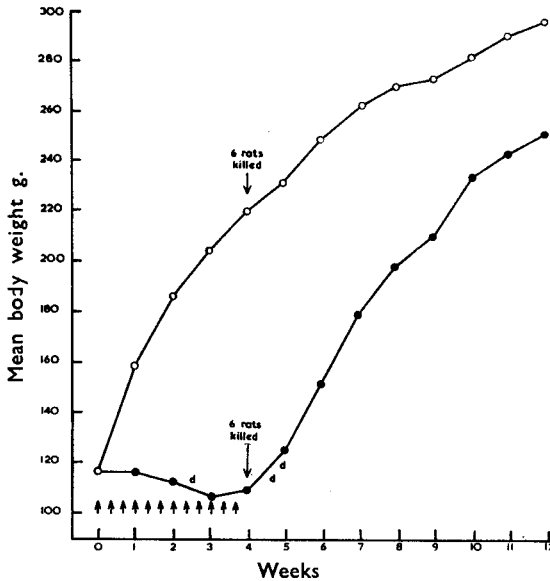
FIG. 2. The effect of prolonged administration of cortisone acetate and the sudden cessation of this treatment on body weight. The steroid was injected at a dosage level of 10 mg./rat 3 × a week, i.m. (at arrows) for 4 weeks to a group of 12 male rats (●———●), control animals received injections of saline (○———○). Mortality is indicated by "d."

Effect on Histology of the Adrenal Glands

Prolonged administration of cortisone acetate (10 mg./rat) caused marked atrophy of the zona fasciculata but the zona glomerulosa remained unaffected (Plate 1, Fig. 1); this is shown in comparison with a normal adrenal gland from a saline treated control rat (Plate 1, Fig. 2). The prolonged administration of hydrocortisone acetate (5 mg./rat), prednisone acetate (2·5 mg./rat) and prednisolone (2·5 mg./rat) resulted in qualitatively similar patterns. However, relatively low doses of DCA (5 mg./rat) and fludrocortisone (0·31 mg./rat) caused a specific and marked depletion of sudanophilic material in the zona glomerulosa (Plate 1, Fig. 3, and Plate 2, Fig. 1 respectively); with the latter steroid there was, in addition, a slight cortical atrophy. Larger doses of DCA (50 mg./rat subcutaneously) and slightly larger doses of fludrocortisone (0·63 mg./rat) caused a depletion of Sudan III staining material in the zona glomerulosa and in the outer region of the zona fasciculata, together with an extensive atrophy of cells in the middle and inner regions of the zona fasciculata (Plate 2, Figs. 2 and 3 respectively).

Regeneration of Adrenal Glands after Cortical Atrophy

Cortisone acetate (10 mg./rat) was injected intramuscularly thrice weekly to a group of 12 rats; a similar control group was injected with the same volume of saline. Cortisone treatment was stopped after 4 weeks and 6 animals were killed from each group. The adrenals from these
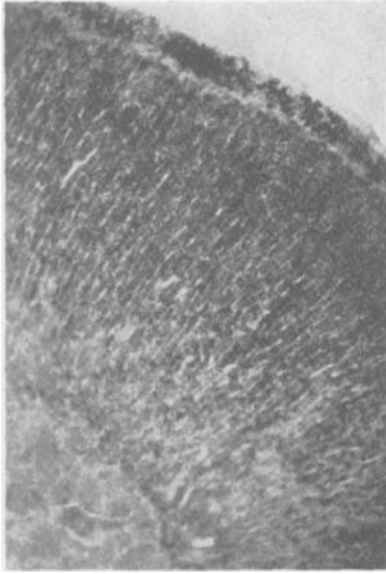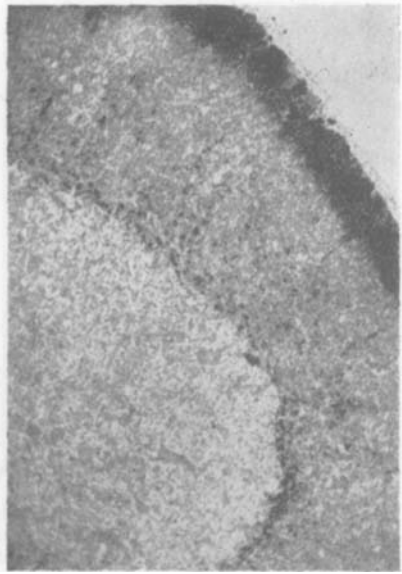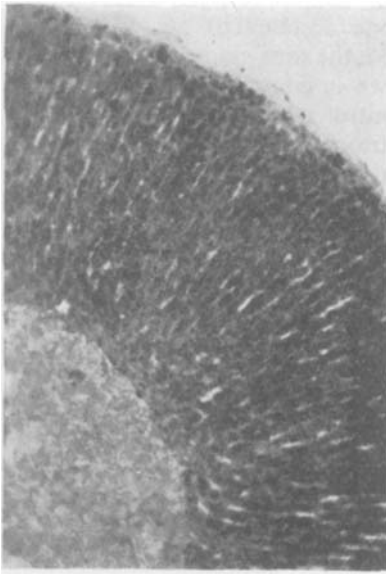
PLATE 3

Fig. 1.

Fig. 2.

Fig. 3.

FIG. 1. Adrenal gland of saline treated control rat, killed 12 weeks after start of experiment. Normal morphology and distribution of sudanophilic lipid. × 110.

FIG. 2. Adrenal gland of rat treated with cortisone acetate (10 mg./rat 3 × a week, i.m.), for 4 weeks and then killed. Extensive and typical cortical atrophy; depletion of sudanophilic lipid. × 110.

FIG. 3. Adrenal gland of rat treated with cortisone acetate, 10 mg./rat, 3 × a week, i.m.), for 4 weeks and then allowed to live until 12th week without any further treatment. Normal morphology and distribution of sudanophilic lipid. × 110.

rats were weighed and examined histologically. The remaining rats in the treated and control groups were then kept without further treatment for an additional 8 weeks; at this time they were killed and the adrenals weighed and sectioned.

The effect of this régime of treatment on body weight is shown graphically on Fig. 2. Cortisone caused the familiar retardation of body growth and death of some animals.

When this treatment was stopped two rats died but the remaining animals continued to grow rapidly. However, the mean body weight of

TABLE III

THE REGENERATION OF ADRENAL GLANDS AFTER THE CESSATION OF CHRONIC DOSES OF CORTISONE ACETATE

Treatment	No. of rats	Mean adrenal weight (mg.) ± S.E.	Mean adrenal weight (mg./100 g. body weight) ± S.E.
Controls, saline i.m. × 3 per week for 4 weeks. No further treatment, killed at 12 weeks	6	31·8 ± 2·0	10·7 ± 0·5
Controls, saline i.m. × 3 per week for 4 weeks then killed	6	22·0 ± 1·7	9·6 ± 0·6
Cortisone acetate, 10 mg./rat i.m. × 3 per week for 4 weeks. No further treatment, killed at 12 weeks	6	25·7 ± 2·3*	10·2 ± 0·7*
Cortisone acetate, 10 mg./rat i.m. × 3 per week for 4 weeks then killed	6	6·5 ± 0·2	6·3 ± 0·7

* Value from 3 surviving animals.

$$\frac{\text{Mean weight tissue treated rats (mg.)}}{\text{Mean weight tissue control rats (mg.)}} \text{ per cent} - 100 \text{ per cent.}$$
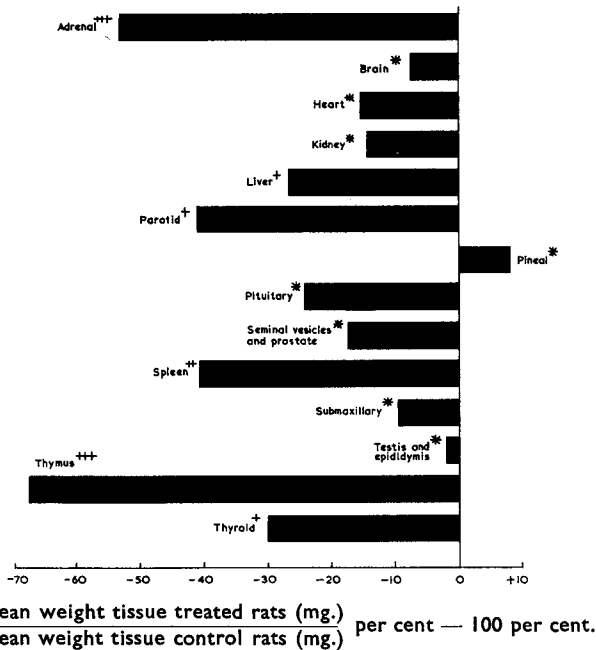
FIG. 3. The effect of prolonged administration of cortisone acetate (5 mg./rat 3 × a week, i.m., for 6 weeks) on the absolute weight (mg.) of specific tissues in groups of 6 rats. The symbols denote the levels of significance of the differences between mean weights of tissues from control and treated rats; + + + = P < 0·001, + + = P < 0·01, + = P < 0·05, * = P > 0·05.

the survivors did not equal that of the controls at any time during the remaining 8 week period of the experiment. Table III shows the mean adrenal weight for each group of rats expressed both in terms of absolute weight and as adrenal weight per 100 g. body weight.

These results suggest that the adrenal atrophy induced by cortisone is a reversible process. After treatment was stopped, the glands of the surviving rats gained in weight and at 12 weeks from the start of the experiment they did not differ appreciably in weight from those of

$$\frac{\text{Mean weight tissue treated rats (mg./100g.)}}{\text{Mean weight tissue control rats (mg./100g.)}} \text{ per cent} - 100 \text{ per cent.}$$
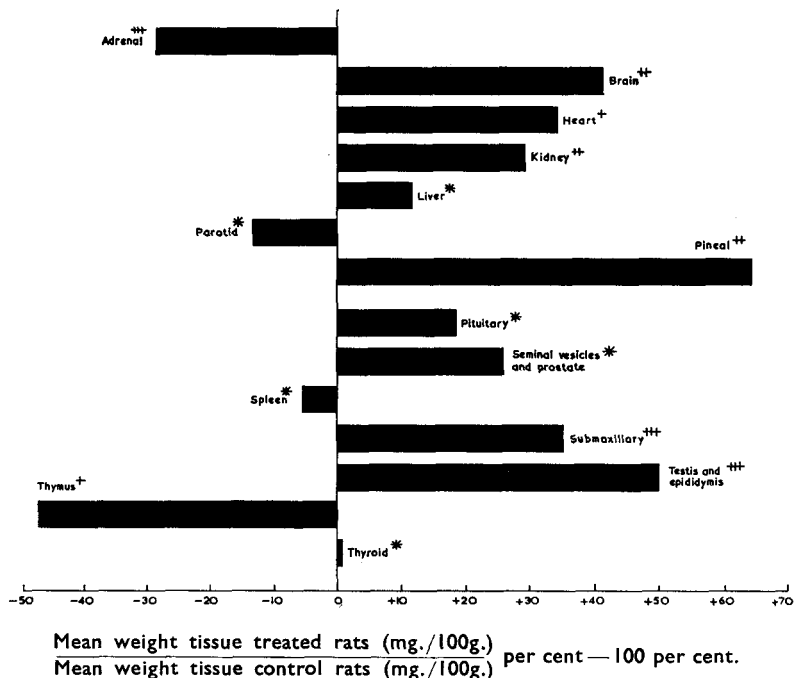
FIG. 4. The effect of prolonged administration of cortisone acetate (5 mg./rat 3 × a week, i.m., for 6 weeks) on the weight (mg./100 g. body weight) of specific tissues in groups of 6 rats. The symbols denote the levels of significance of the differences between mean weights of tissues from control and treated rats; $+++ = P < 0.001$, $++ = P < 0.01$, $+ = P < 0.05$, $* = P > 0.05$.

the untreated controls. When examined histologically, the adrenal glands from rats after 4 weeks treatment showed extensive and typical atrophy (Plate 3, Fig. 2). However, the adrenals from rats surviving the 8 week period after the cessation of treatment (Plate 3, Fig. 3) did not differ in morphology and distribution of sudanophilic lipid from those of the control animals (Plate 3, Fig. 1).

Effect on Weights of Other Tissues

Since the chronic administration of corticosteroids to rats caused such marked changes in the weight and histology of the adrenal glands, it was thought of interest to examine the effect of such treatment on the weights

of various organs and tissues and also on endocrine glands other than the adrenals. These tissues were brain, heart, kidney, liver, parotid salivary gland, pineal, pituitary, seminal vesicles and prostate, spleen, submaxillary salivary gland, testis and epididymis, thymus and thyroid.

In this experiment, cortisone acetate (5 mg./rat) was injected intramuscularly thrice weekly for 6 weeks. Fig. 3 compares the absolute weights of the tissues with those from the control animals; all tissues from the treated rats were smaller than those from the controls; the pineal gland was the sole exception, this appeared to be slightly larger in the treated rats although the difference was not statistically significant (P>0·05). However, since cortisone treatment also affected the body weight of the rats, the tissue weights were expressed in terms of proportion of body weight to exclude the influence of the steroid on the animals' general body growth. The presentation of experimental results in this manner (Fig. 4) suggests that a significant atrophy is present with the adrenal glands (P<0·001) and the thymus (P <0·05) but not with the spleen or the parotid gland. The pineal gland showed the most marked relative increase in weight while the submaxillary gland, the testis and epididymis, the brain, heart and kidney showed a less marked but still significant increase in weight over corresponding tissues from the control rats.

DISCUSSION

All the corticosteroids examined caused an inhibition of the animals' normal rate of growth. For example with high doses of cortisone acetate (10 mg./rat) this inhibition was almost complete. Fludrocortisone, the most potent of the steroids tested, is more active in producing adrenocortical atrophy than in causing a retardation of body growth. Prednisone and prednisolone, on the other hand, appear to be more potent in their effects on body weight than on adrenal weight, whilst hydrocortisone has equal activity on both.

Continued high dosage of some steroids caused mortality, from the sudden flare up of a dormant infection. Post-mortem examination of these animals revealed lesions of the lungs and liver and the presence of Gram-positive bacteria in the heart blood. Selye (1955) has reported a similar pattern of infection in rats given large doses of cortisone and Ingle, Prestrud and Li (1951) have described a severe infection in rats in which hypercorticalism was induced by a continuous injection of adrenocorticotrophic hormone. In man, also, there have been many reports of a decreased resistance to infection accompanying continued treatment with large doses of cortical steroids or corticotrophin (Kass and Finland, 1953; British Medical Journal, 1954; Robinson, 1956).

The present studies indicate that adrenocorticoids may be divided into two classes from their effects on the adrenal cortex. Firstly, steroids like cortisone, hydrocortisone and their $\triangle^1$- analogues, which produce extensive atrophy of the zona fasciculata, without affecting the cellular histology or lipid distribution within the zona glomerulosa, even when administered repeatedly at high dosage. Secondly, those like fludrocortisone and DCA which in low doses, produce a specific depletion of the

sudanophilic material in the zona glomerulosa with little or no accompanying adrenocortical atrophy. It would seem likely that the first group of steroids act by reducing the secretion of corticotrophin from the anterior pituitary and so produce adrenocortical atrophy, whereas the second group influence the secretion of lipid in the zona glomerulosa by a predominant effect on the animals' electrolyte balance. At the same time this latter group of steroids may also have an effect on the pituitary output of corticotrophin since higher doses of both fludrocortisone and DCA cause atrophy of the zona fasciculata as well.

Deane and Greep (1946) and Greep and Deane (1949) observed that the zona glomerulosa of the hypophysectomised rat maintains its morphological integrity and a readily demonstrable lipid content while the zonae fasciculata and reticularis atrophy, observations which led these and other workers to suggest that the zona glomerulosa of the rat adrenal cortex is independent of the pituitary gland (Greep and Deane, 1949; Yoffey and Baxter, 1949). The results of this present series of experiments are in agreement with this concept since steroids administered in doses which inhibit the anterior pituitary and produce cortical atrophy do not affect the zona glomerulosa, whereas steroids, known to have potent effects on electrolyte metabolism, specifically deplete the zona glomerulosa at doses which do not produce cortical atrophy. On the other hand, exogenous corticotrophin has been reported to cause marked activity in the zona glomerulosa of the rat adrenal (Cater and Stack-Dunne, 1953; Wexler and Rinfret, 1955), an observation that has been confirmed in our own unpublished experiments; it may be therefore that this zone is not entirely autonomous.

The present studies also indicate that the steroid induced adrenocortical atrophy is a reversible process, since when treatment ceased the glands gradually recovered their normal weight, histology and distribution of sudanophilic lipid, although the body weight of the treated rats did not attain that of the controls.

During these studies, it was also observed that prolonged treatment with cortisone acetate had a marked effect on the weight of various tissues other than the adrenals. Cortisone has a marked effect on body growth and also inevitably a general, but non-specific, catabolic effect on many of the tissues of the body. The change in weight of a tissue may be due to a specific effect on that tissue, alternatively it may reflect the general catabolic effect of the steroid on body growth. For this reason comparison between the weights of tissues from control and treated rats was made in terms of the absolute weights of the tissues and also in terms of the weight of the tissue per 100 g. body weight.

In the absence of a more reliable index, specific atrophy has been assumed when a tissue shows a statistically significant decrease in weight when expressed either as absolute weight or weight related to body weight. A true hypertrophy exists when the steroid exerts a direct effect on the tissue and increases its weight; however, hypertrophy may also seem to be present when the weight of the particular tissue is independent of the catabolic effect of the steroid on general body growth. Histology is

perhaps the only simple way of distinguishing between true and apparent hypertrophy.

The adrenal glands, the thymus but not the spleen showed specific atrophy. Shewell (1957) reported that thymic involution was a more sensitive index of the biological activity of corticosteroids than was atrophy of the spleen. Only the pineal gland was larger in the treated animals, and when its weight was related to the animal's body weight there was a greater difference between pineal weight from treated and control rats than there was for any other tissue examined. The significance of this finding is the subject of current investigation.

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