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A COMPARISON OF THE PITUITARY INHIBITORY EFFECTS OF PREDNISONE, PREDNISOLONE, AND HYDROCORTISONE

BY

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Prednisone, prednisolone and hydrocortisone, administered in doses of 1 mg. daily for thirty days, depressed the growth rate of rats. Prednisolone and hydrocortisone caused atrophy of the adrenal glands and increased the relative weights of the thyroid glands and testes. The apparently similar effects produced by prednisone were not significant. The seminal vesicles were not affected significantly by prolonged administration of prednisone, prednisolone or hydrocortisone. Pretreatment of rats with these steroids prevented the adrenal ascorbic acid depletion normally caused by laparotomy under ether anaesthesia. Prednisolone was slightly more potent and prednisone was considerably less potent than hydrocortisone in producing these effects.

It is well known that elevated blood levels of corticosteroids depress the secretion of adrenocorticotrophic hormone (corticotrophin, ACTH) by the anterior pituitary gland. This has been shown by many laboratory workers (Ingle and Kendall, 1937; Ingle, Higgins, and Kendall, 1938; Sayers and Sayers, 1947). Clinicians have also found that corticoids depress adrenocortical activity and that abrupt cessation of corticoid therapy may result in the signs of transient adrenal insufficiency. The development of prednisone (Δ'cortisone) and prednisolone (Δ'hydrocortisone) has provided clinical workers with corticosteroid-like substances more potent than cortisone and hydrocortisone and lacking the undesirable mineralocorticoid effects. Clinically these compounds also appear to depress the secretion of ACTH (Bunim, Black, Bollet, and Pechet, 1955; Pechet, 1955; Kupperman, Blatt, Vesell, Gagliani, Weisbader, and Vosburgh, 1955). Therefore, it was of interest to compare the pituitary inhibitory actions of these synthetic corticoid analogues with that of hydrocortisone. The experiments described in this paper were performed to study some effects of prolonged administration of these compounds and also to investigate their action on the secretion of ACTH which normally follows stress.

MATERIALS AND METHODS

The experiments were performed on male albino Wistar rats fed on a diet of cubes (diet 41, Lane-Petter and Dyer, 1952) and water and kept at a constant temperature of 70° F.

Prednisone (Merck), prednisolone (Merck) and hydrocortisone free alcohol (Glaxo) were suspended in normal saline. Lyophilized adrenocorticotrophic hormone (ACTH, Armour) was dissolved in normal saline to prepare solutions containing 1 International Unit/ml. which were kept at room temperature for not more than one hour. Solutions for injection were prepared immediately before use by diluting the 1 i.u./ml. solutions with normal saline.

Experimental Procedures. — (1) Fifty-one rats, weighing from 110 to 130 g., were divided into eight groups. Two groups served as controls, the rats in one group receiving no treatment and those in the other daily injections of normal saline only. Prednisone, prednisolone or hydrocortisone was administered to the remaining groups daily in doses of 0.1 or 1.0 mg. All the injections were made subcutaneously in volumes of 0.1 ml./rat. The animals were weighed frequently, and after 30 days' treatment they were killed with ether, and their adrenal glands, testes, seminal vesicles and thyroid glands were removed, dissected free from adhering tissue and weighed as quickly as possible to minimize the lost of moisture. (2) Four hundred and forty-eight rats, weighing from 120 to 160 g., received subcutaneously normal saline only or doses of 1.25 or 5 mg./100 g. body weight of a normal saline suspension of prednisone, prednisolone or hydrocortisone. The injections were given in volumes of 0.5 ml./100 g. The animals were anaesthetized with ether and mock adrenalectomized (Hodges, 1953) 1, 2, 4, 8, 16, and 32 hr. after receiving the injections. They were allowed to recover from

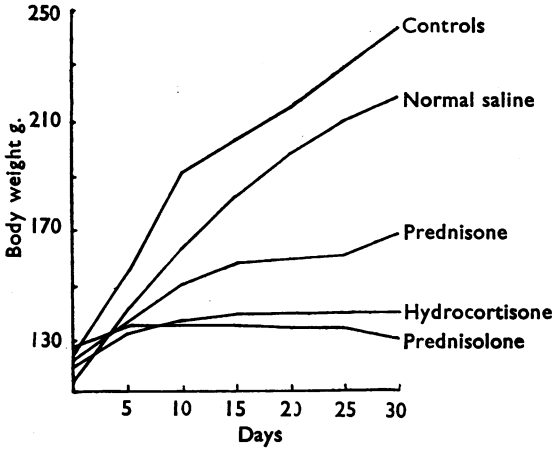

FIG. 1.—Growth rates of rats receiving daily subcutaneous injections of 1 mg. prednisone, prednisolone or hydrocortisone.

the operation and were killed 1 hr. later. Control rats were killed without any previous surgical interference. The adrenal glands were removed, dissected free from periadrenal tissue, weighed rapidly and transferred to 4% trichloracetic acid solution for estimation of their ascorbic acid contents by the method of Roe and Kuether (1943).

The animals were given access to food and water throughout the experimental periods.

RESULTS

Effects of Prolonged Administration of Prednisone, Prednisolone, and Hydrocortisone.—Doses of 0.1 mg./day/rat of the three steroids produced no significant differences between the growth rates of the treated animals and those of the controls and caused no morphological changes in the endocrine glands and accessory sex organs which were examined. However, a 10-fold increase in dosage produced marked changes in growth rate. Rats treated with prednisone gained weight less rapidly than the control animals. This effect was more marked with hydrocortisone, which completely arrested growth after it had been administered for about five days, and even greater with prednisolone, which caused some loss of weight after a similar period. These results are shown in Fig. 1. The steroid-treated rats were in poor condition and exhibited pronounced muscular weakness after about five days' treatment. Post-mortem examination of the animals showed that the steroids had caused adrenal atrophy (Table I). The effect of hydrocortisone was more marked than that of prednisolone. The effect of prednisone on adrenal weight was not significant. The weights of the testes and thyroid glands expressed in terms of body weight were significantly ($P<0.05$) greater in the animals which had received prednisolone or hydrocortisone than in the controls. Prednisone also increased the relative weights of the thyroid glands but did not increase significantly the testis weight. The apparent differences between the weights of the seminal vesicles of the treated groups and those of the controls were not significant. The *absolute* weights of these organs were in every case less than in the controls, but this is not surprising since prolonged steroid treatment inhibited the general growth rate so markedly.

Effect of Laparotomy on the Ascorbic Acid Concentration in the Adrenal Glands of Rats Pretreated with Prednisone, Prednisolone or Hydrocortisone.—Subcutaneous injections of normal saline, or saline suspensions of the steroids, resulted in no detectable changes in adrenal ascorbic acid concentration. Mock adrenalectomy under ether anaesthesia produced marked adrenal ascorbic acid depletion in the rats which had been pretreated with normal saline only. Previous treatment with prednisone, prednisolone or hydrocortisone diminished the fall in adrenal ascorbic acid concentration normally caused by the operation (Fig. 2). A dose of 1.25 mg. prednisolone

TABLE I

ADRENAL, THYROID, TESTIS AND SEMINAL VESICLE WEIGHTS IN RATS AFTER 30 DAYS' TREATMENT WITH PREDNISONE, PREDNISOLONE AND HYDROCORTISONE

The asterisk indicates that the difference from controls is statistically significant ($P<0.05$).

Treatment	No. of Animals	Adrenal Weight mg./100 g. Body Weight		Thyroid Weight mg./100 g. Body Weight	Testis Weight g./100 g. Body Weight		Seminal Vesicles g./100 g. Body Weight
		Left	Right		Left	Right	
Prednisone, 1 mg./day	6	6·1±0·5	6·1±0·7	8·1±0·2*	0·7±0·04	0·69±0·05	0·49±0·03
Prednisolone, 1 mg./day	6	5·6±0·4*	5·2±0·4*	10·9±0·4*	0·88±0·05*	0·92±0·05*	0·52±0·07
Hydrocortisone, 1 mg./day	6	4·6±0·4*	4·35±0·4*	9·6±0·7*	0·81±0·04*	0·84±0·04*	0·43±0·07
Saline, 0·1 ml./day	9	8·0±0·4	7·0±0·3	7·1±0·4	0·57±0·04	0·59±0·05	0·39±0·03
Uninjected controls	6	6·7±0·5	6·1±0·3	7·0±0·2	0·54±0·01	0·55±0·01	0·36±0·02

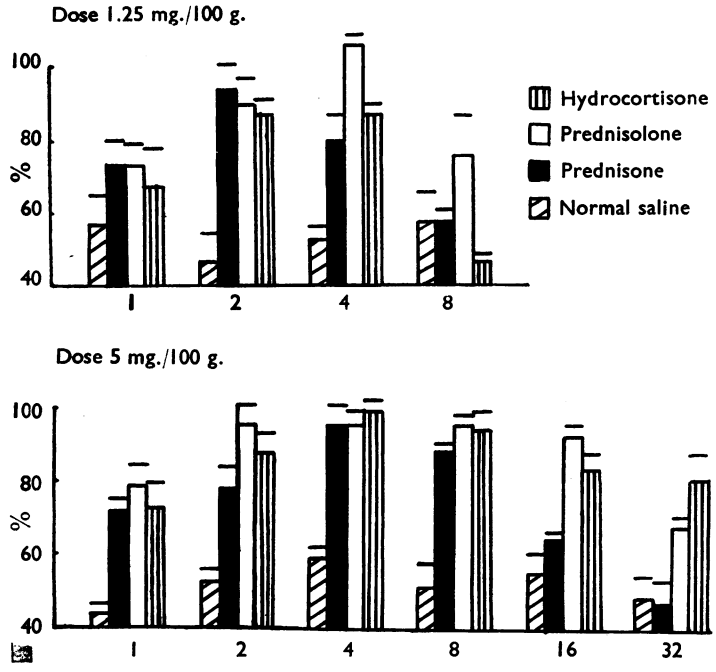

FIG. 2.—The effect of prednisone, prednisolone and hydrocortisone on the adrenal ascorbic acid depletion caused by mock adrenalectomy under ether anaesthesia. Ordinate: adrenal ascorbic acid concentration, 1 hr. after the operation, expressed as a % of the control level in unoperated rats. The numerals below the columns represent the time intervals in hours between the administration of the steroids and the performance of the operations. The lines above the columns represent the standard error.

prevented completely the adrenal ascorbic acid depletion only when the stress was applied 4 hr. after its administration. At other time intervals this dose of prednisolone was only partially effective. The same dose of prednisone or hydrocortisone was never more than partially effective. In a dose of 5 mg. prednisolone blocked completely the fall in adrenal ascorbic acid for a period of up to 16 hr. after its administration. The same dose of hydrocortisone produced a similar but less prolonged action. The action of prednisone was only transient and its effect in preventing adrenal ascorbic acid changes completely persisted for only a few hours.

It was probable that the steroids prevented the stress-induced depletion in adrenal ascorbic acid by interfering with the release of ACTH rather than by acting directly on the adrenal cortex. This was confirmed by the following experiment. Thirty rats weighing 120 to 150 g. were injected subcutaneously with 7.5 mg. hydrocortisone/100 g. and the animals were divided into five equal groups. One group served as controls. Four hours later the rats in the other four groups were anaesthetized with ether and injections were made into their exposed right femoral veins as follows: group (1) normal saline, group (2) heparinized blood from normal rats, group (3) heparinized blood from adrenalectomized rats 22 days after the removal of their adrenal glands, and group (4) normal saline containing 0.2 mu. ACTH/ml. All the intravenous injections were made in volumes of 3 ml./100 g. body weight. The animals were killed 1 hr. later and their adrenal ascorbic acid contents were determined. The mean adrenal ascorbic acid levels in each group were subtracted from the mean level in the control animals. The results are given in Table II. Normal saline and blood from normal rats produced no significant change in adrenal ascorbic

TABLE II

ADRENAL ASCORBIC ACID DEPLETION IN RATS

The rats were injected subcutaneously with 7·5 mg. hydrocortisone 4 hr. before and were killed 1 hr. after the intravenous injection of various fluids (3 ml./100 g. body weight). Adrenal ascorbic acid level in the controls was 524±11 mg./100 g. adrenal tissue.

Treatment	Adrenal Ascorbic Acid Depletion. mg./100 g. Adrenal Tissue. ± Standard Error
Normal saline	6±8
Heparinized blood from normal female rats	21±9
Heparinized blood from female rats 22 days after adrenalectomy	70±12
Normal saline containing 0·2 mu. ACTH/ml.	100±7

acid. However, ACTH and blood from rats adrenalectomized 22 days previously, which contains approximately 10 mu. ACTH/100 ml. (Cox and Hodges, 1958), produced significant adrenal ascorbic acid depletion.

Discussion

The results of experiments performed in many laboratories indicate that prolonged administration of corticoids causes marked adrenocortical atrophy and depresses the growth rate of rats (Ingle *et al.*, 1938; Ingle, 1941; Ingle and Meeks, 1952; Wells and Kendall, 1940; Winter, Silber, and Stoerk, 1950). Prednisolone produces a similar effect and the results described above indicate that it is more potent in inhibiting growth but less potent in causing adrenal atrophy than hydrocortisone. Prednisone is less potent than hydrocortisone in both respects. Prednisolone and hydrocortisone increased significantly the weights of the thyroid and testes relative to the total body weight. However, the body weights of the steroid treated animals, at the end of the experimental period, were considerably less than those of the controls. It can only be concluded that the growth of the thyroid glands and testes was not inhibited as markedly as was the general body growth of the animals.

There is little doubt that the adrenal cortical steroids depress the secretion of ACTH by the pituitary gland. The results of the experiments described in this paper indicate that prednisone and prednisolone produce a similar effect. However, in the rat, prednisolone is only slightly more potent than hydrocortisone and prednisone has a considerably weaker action in suppressing stress-induced ACTH secretion. It has been established that, in the rat as in other species, prednisone and prednisolone are considerably more active biologically in, for example, suppressing inflammation, maintaining life in adrenalectomized animals, depositing glycogen in the liver, promoting the uptake of [^{32}P] by adrenals, pituitary and thymus glands, etc., than the naturally occurring corticoids (Ducommun, Ducommun, and Baquiche, 1955; Donato, 1955). It appears, therefore, that the pituitary inhibitory activity of these compounds is not always related to their potencies in other respects. Thus it might be expected that the synthetic corticoid analogues could possess an additional advantage over cortisone and hydrocortisone when used therapeutically that they should not tend to depress endogenous ACTH production to such an extent. However, many clinical workers (Bunim *et al.*, 1955; Pechet, 1955; Kupperman *et al.*, 1955), using 17-ketosteroid excretion as an index of adrenocortical activity, have found that prednisone and prednisolone are considerably more active than cortisone and hydrocortisone in suppressing ACTH secretion in man.

Corticoids cause adrenocortical atrophy and prevent the adrenal ascorbic acid depletion normally caused by stress by interfering with the release of ACTH from the pituitary gland rather than by acting directly on the adrenal cortex. This was demonstrated by Ingle and Kendall (1937), who found that the atrophy of the adrenal cortex normally caused by the chronic administration of adrenocortical extracts could be prevented by the simultaneous administration of ACTH. Similar evidence was obtained by Sayers and Sayers (1947) and in the present work from the results of experiments which showed that ACTH still caused adrenal ascorbic acid depletion in rats pretreated with corticosteroids.

Since hydrocortisone and prednisolone were so effective in inhibiting ACTH release, the possibility was investigated of modifying the technique of Sayers, Sayers, and Woodbury (1948) for the bioassay of ACTH by substituting, for hypophysectomized animals, rats in which pituitary adrenocorticotrophic activity had been effectively blocked. Hodges (1955) described a method using rats previously treated with deoxycorticosterone acetate (DCA). The method suffered from the disadvantage that it lacked precision, and, owing to the sparingly soluble nature of DCA in arachis oil, large volumes of solution had to be injected subcutaneously to produce complete inhibition of ACTH release. The solution frequently tended to leak from the site of injection. The hydrocortisone or prednisolone treated rat appears to be more suitable and, at the present time, experiments are in progress using such animals for the estimation of ACTH in blood.

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