# THE EFFECT OF CORTISONE AND HYDROCORTISONE ON THE PLASMA LEVELS OF CORTICOTROPHIN IN THE RAT, AFTER AN ACUTE STRESS

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Pretreatment of intact female rats with cortisone and hydrocortisone acetates produces a graded inhibition of ACTH release in response to acute stress as shown by changes in plasma ACTH levels. The inhibition was greater with hydrocortisone and was dependent upon the time and dose relations.

Until comparatively recently, most of the evidence that has been marshalled into the various theories of ACTH regulation has been of an indirect nature. The predominant type of experimental approach has been one in which the test animal is subjected to some stressful stimulus and then at an arbitrary interval secondary changes within the animal are examined. Such indices as eosinopenia (McDermott, Fry, Brobeck and Long, 1950), lymphocytopenia (Colfer, de Groot and Harris, 1950), thymic involution (Bruce, Parkes and Perry, 1952), altered urinary steroid excretion patterns (Liddle, Richard and Peterson, 1955), and adrenocortical changes (Sayers and Sayers, 1947) are all secondary or indirect indices of ACTH activity. Normally responses have been measured from 1 to 6 hours after the application of the stress: however, this time interval is even longer when morphological or histological changes have been used as indices of adrenocorticotrophic acitivity.

It cannot be denied that these indices reflect chages due to disturbed adrenocortical activity. But, they possess a distinct disadvantage in that they do not convey precise information on the dynamics of ACTH release from the adenohypophysis. With the development of techniques permitting the quantitative estimation of ACTH in plasma or blood, it is now possible to measure the rate and magnitude of ACTH release under a wide variety of experimental conditions.

The ability of adrenocortical hormones to inhibit the stress-induced release of ACTH has been observed in many laboratories (Barrett, 1959). However, there are various discrepancies in the literature over this phenomenon which may well be due to the fact that secondary indices of pituitary adrenocorticotrophic activity have been used. Barrett and Hodges (1956) reported the stress of laparotomy under ether anaesthesia to cause an elevation of plasma ACTH in the rat which was maximal ten minutes after the application of the stimulus. This finding has been confirmed in the present work and used to demonstrate the inhibitory action of cortisone and hydrocortisone on the release of ACTH in response to stress.

## Experimental

### *Materials and Methods*

Wistar rats were fed on a cube diet and water and maintained at a constant temperature of 70° F. Hypophysectomised rats were kept on a

normal diet with the addition of 5 per cent glucose in their drinking water. Male rats (120–140 g.) were used for assay purposes, and females (160–200 g.) for the plasma for assay.

Cortisone acetate (saline suspension) and hydrocortisone acetate were administered to female rats subcutaneously. Dilutions with normal saline were prepared immediately before use and injections were given in volumes of 1 ml. and in doses equivalent to 2 and 20 mg./100 g. weight. Control injections of normal saline were given in similar volumes. Deoxycortone acetate was dissolved in arachis oil and injected subcutaneously into male rats in volumes of 20 mg./100 g. weight. Deoxycortone (DCA) treated animals were used 16–24 hr. later for the assay of ACTH in plasma by the method of Hodges (1955).

Lyophilised adrenocorticotrophic hormone (Armour, 1 unit/mg.) was dissolved in normal saline immediately before use. ACTH was injected intravenously into both DCA-treated and hypophysectomised male rats in

TABLE I

THE CONCENTRATION OF ACTH (MU/100 ML.) IN THE PLASMA OF STRESSED NORMAL FEMALE RATS: COMPARISON BETWEEN ASSAYS PERFORMED ON DCA-TREATED AND HYPOPHYSECTOMISED ANIMALS

| Type of assay animal | Treatment | No. of donor rats | Dose per 100 g. assay rat | No. of assay rats | Mean ascorbic acid depletion (mg./100 g. adrenal ± S.E.) | Conc. ACTH mU/100 ml. plasma (95 per cent fiducial limits) |
|---|---|---|---|---|---|---|
| DCA-treated | Standard ACTH | — | 0·15 mU | 12 | 44 ± 9 | 14·6 (12·7–19·2) |
| | | — | 0·60 mU | 12 | 121 ± 6 | |
| | Plasma from intact female rats, 10 min. after stress | 32 | 3·0 ml. | 12 | 103 ± 14 | |
| Hypophysectomised | Standard ACTH | — | 0·15 mU | 12 | 53 ± 9 | 14·5 (12·5–17·8) |
| | | — | 0·60 mU | 12 | 133 ± 6 | |
| | Plasma from intact female rats, 10 min. after stress. | 32 | 3·0 ml. | 12 | 115 ± 7 | |

Depletion calculated from saline injected controls. DCA-treated 452 ± 7 mg. per cent; hypophysectomised 501 ± 6 mg. per cent.

doses of 0·15 and 0·60 milliunits (mU.) in volumes of 3 ml./100 g. weight. Control injections of normal saline were given intravenously in similar volumes.

*Experimental procedures.* The stressful stimulus was laparotomy under ether anaesthesia. Ten min. after commencement of exposure to ether the animals were decapitated and blood collected from the trunk portion into heparinised tubes by a funnel which had been rinsed in heparin solution. The blood was centrifuged at 3,000 r.p.m. for 15 min. and the plasma collected.

Plasma obtained from each group of stressed rats was pooled and injected intravenously into groups of either DCA-treated or hypophysectomised rats. Hypophysectomy was by the parapharyngeal approach. The hypophysectomised rats were used for ACTH assay 24 hr. after

removal of their pituitary glands according to the method of Sayers, Sayers and Woodbury (1948), as modified by Munson, Barry and Koch (1948).

The concentration of adrenal ascorbic acid in all DCA-treated and hypophysectomised rats was determined (Roe and Kuether, 1943) 1 hr. after the intravenous injections had been administered. The ACTH activity was estimated from the adrenal ascorbic acid depletion produced in test and standard ACTH injected groups obtained by subtraction of these values from that for the control, saline injected group. The results and

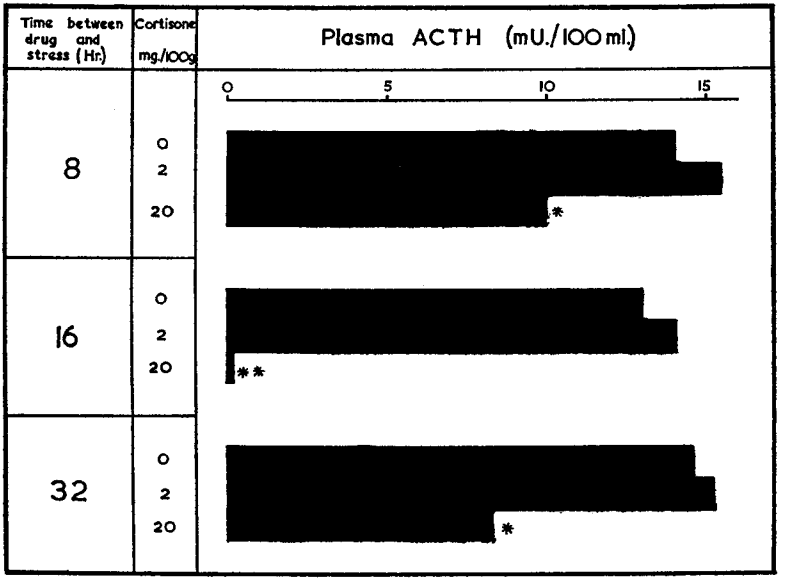


FIG. 1. ACTH concentrations in the plasma of normal rats and rats pre-treated with cortisone acetate, 10 min. after stress. The value expressed by each horizontal column represents an estimate of the ACTH concentration in the pooled plasma from 16 animals assayed in a group of six DCA-treated rats. An asterisk indicates that the result is significantly ($P = 0.05$) different from non-steroid injected control value: a double asterisk indicates that the difference is highly significant ($P = 0.01$).

their fiducial limits ($P = 0.95$) were calculated by the method of Gaddum (1953) for a $2 + 1$ assay and were expressed as mU. ACTH/100 ml. plasma.

*The effect of cortisone and hydrocortisone.* Six groups of 16 female rats were injected with cortisone. Three of these groups received a dose of 2 mg./100 g. and three groups a dose of 20 mg./100 g.; similar groups of animals were injected with the same dose levels of hydrocortisone. These rats were exposed to the stressful stimulus 8, 16 or 32 hr. after the steroid injection. Further groups of 16 female rats, which had received control injections of saline alone at the appropriate time intervals, were stressed in a similar manner. Pooled plasma from each group of rats was assayed for ACTH activity, and the plasma ACTH concentrations were calculated.

## RESULTS

The summarised results for two methods for the bioassay of ACTH are compared in Table I. When mobilisation of endogenous ACTH in the assay animal was prevented by pre-treatment with DCA the concentration of ACTH in the plasma of stressed female rats was found to be 14·6 mU. ACTH/100 ml. With hypophysectomised assay animals a value of 14·5 mU. ACTH/100 ml. of plasma was obtained. The results showed that DCA-treated animals compared favourably with hypophysectomised rats in the bioassay of plasma ACTH.

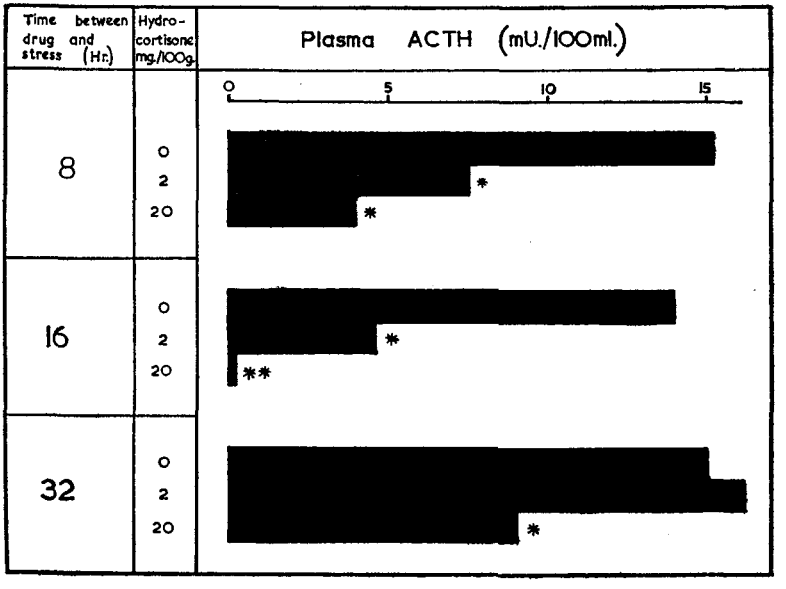


FIG. 2. ACTH concentrations in the plasma of normal rats and rats pre-treated with hydrocortisone acetate, 10 min. after stress. The value expressed by each horizontal column represents an estimate of the ACTH concentration in the pooled plasma of 16 animals assayed in a group of six DCA-treated rats. An asterisk indicates that the result is significantly ($P = 0.05$) different from non-steroid injected control value: a double asterisk indicates that the difference is highly significant ($P = 0.01$).

DCA-treated animals were then used for ACTH estimations as this technique prevented the release of ACTH in the assay rat due to any stressful stimuli associated with the assay technique.

The effects of pre-treatment with cortisone and hydrocortisone on plasma ACTH concentrations 10 min. after stress are summarised in Figs. 1 and 2. In all experiments where rats were subjected to stress, without pre-treatment with either cortisone or hydrocortisone, a steady value of about 14 mU./100 ml. of plasma ACTH was found. As shown in Fig. 1, cortisone at a dose of 20 mg./100 g. produced a significant reduction in the plasma ACTH level after stress at 8, 16 or 32 hr. after administration whereas at a dose of 2 mg. there was no significant change. But, as shown in Fig. 2, hydrocortisone was active at the lower dose at 8 and 16 hr.

but not at 32 hr. The high dose of hydrocortisone was active at all times. Both cortical hormones afforded a complete inhibition of ACTH release 16 hr. after injection, at the higher doses. Eight hr. after administration hydrocortisone appeared to be twice as effective as cortisone. In general hydrocortisone was more active and quicker acting than cortisone.

## DISCUSSION

A comparison has been described between two methods for the assay of plasma ACTH. Results obtained in DCA-treated animals were in close agreement with those found using hypophysectomised rats. It has been argued that the DCA-blocking technique is neither specific nor fully effective since the ability of corticosteroids to inhibit pituitary adrenocorticotrophic activity is relative rather than absolute (Miahle-Voloss and Stutinsky, 1956). But, with a dose of DCA of 20 mg./100 g. the release of endogenous ACTH was totally inhibited in the assay animal.

In the present work it has been shown that under certain conditions complete inhibition of ACTH discharge in response to stress can be obtained with both cortisone and hydrocortisone. However, the effective doses were large, confirming earlier observations from indirect indices of ACTH release (Hodges, 1954; Hodges and Vernikos, 1958). Hydrocortisone was active earlier than cortisone and was about twice as potent as a pituitary inhibitor. This is in contrast to the report of Sayers and Sayers (1947) which ascribed equal potency to the two compounds in this respect.

There has been much controversy about the amounts and relative potencies of the various steroids necessary to inhibit pituitary ACTH discharge. This may well be due to the widely different techniques used. Routes of administration, time intervals between injection and testing, stressful stimuli, and acetate or free alcohol steroid preparations have all varied and contributed to the confusion. The relative potencies must also be influenced by the relative solubilities of the steroids in the vehicle used compared with the tissue fluids surrounding the site of administration.

Sayers and Sayers (1947) have shown that subcutaneous administration of both cortisone and hydrocortisone was effective in reducing the adrenal ascorbic acid depletion observed in untreated rats after various stimuli. They used doses less than 1 mg./100 g. weight and gave the drugs immediately before stressing. Another report described similar experiments but was unable to confirm that cortisone had any effect on the release of ACTH in response to stress, at similar dose levels (Fortier, Yrarrazaval and Selye, 1951). Other workers have found that the stress-induced secretion of ACTH can be prevented only by very large doses of cortical hormones and by allowing a longer time interval between injection and submission to stress (Hodges, 1954; Abelson and Baron, 1952). Hodges (1954) showed that cortisone was most effective as a pituitary inhibitor at the same time and dose relations as have been found in the present experiments. But using adrenal ascorbic acid depletion as a secondary index, he also found that cortisone did not completely prevent the depletion observed in untreated controls. The technique described here does not necessarily provide evidence that ACTH discharge is completely inhibited by cortisone

and hydrocortisone at the high dose, 16 hr. after administration. At present the method is not sufficiently sensitive to detect the normal circulating level of ACTH in the non-stressed animal. There may therefore be a large increase in plasma ACTH levels of acutely stressed rats which had pre-treatment with cortisone 16 hr. previously which is not detectable by this method. Very low concentrations of ACTH are effective in producing a response in the adrenal cortex (Renold, Jenkins, Forsham and Thorn, 1952) and this may account for the discrepancy between the two investigations.

In the present experiments DCA was used successfully to produce complete inhibition of pituitary adrenocorticotrophic activity. The dose used was 20 mg./100 g. administered at least 16 hr. before testing. By the same route of administration it was necessary to give the same doses of both cortisone and hydrocortisone in order to effect complete inhibition of ACTH 16 hr. later. ACTH is primarily concerned with the control of the secretion of glucocorticoids and it is surprising that DCA is equally effective at the same dose as those of cortisone and hydrocortisone. The experiments suggest that the ability of cortical hormones to suppress ACTH release is not necessarily related to their physiological activity. Dr. Farrell tells me that the glucocorticoid activity of hydrocortisone is approximately one hundred times that of DCA whereas for electrolyte balance DCA is twenty-five times more potent than hydrocortisone. The relation between physiological activity and pituitary adrenocorticotrophic inhibitory activity of the various steroids is not clear and deserves further study.

## REFERENCES

Abelson, D. and Baron, D. N. (1952). *Lancet*, **2**, 663–664.
Barrett, A. M. (1959). Ph.D. Thesis, University of London.
Barrett, A. M. and Hodges, J. R. (1956). *J. Endocrinol.*, **14**, 292–296.
Bruce, H.M., Parkes, A. S. and Perry, W. L. M. (1952). *Ibid.*, **8**, xvi.
Colfer, H. F., de Groot, J. and Harris, G. W. (1950). *J. Physiol.*, **111**, 328–334.
Fortier, C., Yrarrazaval, S. and Selye, H. (1951), *Amer. J. Physiol.*, **163**, 466–468.
Gaddum, J. H. (1953). *J. Pharm. Pharmacol.*, **5**, 345.
Hodges, J. R. (1954). *J. Endocrinol.*, **10**, 173–178.
Hodges, J. R. (1955). *Ibid.*, **12**, 152–158.
Hodges, J. R. and Vernikos, J. (1958). *Brit. J. Pharmacol.*, **13**, 98–102.
Liddle, G. W., Richard, J. E. and Peterson, R. E. (1955). *Endocrinology*, **57**, 594–598.
McDermott, W. V., Fry, E. G., Brobeck, J. R. and Long, C. N. H. (1950). *Yale J. Biol. Med.*, **23**, 652–666.
Miahle-Voloss, C. and Stutinsky, F. (1956). *C.R. Acad. Sci., U.R.S.S.*, **243**, 189.
Munson, P. L., Barry, A. G. and Koch, F. C. (1948). *J. clin. Endrocrinol.*, **8**, 586.
Renold, A. E., Jenkins, D., Forsham, P. H. and Thorn, G. W. (1952). *Ibid.*, **12**, 763.
Roe, J. H. and Kuether, C. A. (1943). *J. biol. Chem.*, **147**, 399–407.
Sayers, G. and Sayers, M. A. (1947). *Endocrinology*, **40**, 265–273.
Sayers, M. A., Sayers, G. and Woodbury, L. A. (1948). *Ibid.*, **42**, 379–393.