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THE EFFECT OF INJECTED CORTICOSTERONE ON THE RELEASE OF ADRENOCORTICOTROPHIC HORMONE IN RATS EXPOSED TO ACUTE STRESS

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The mechanisms by which stress causes the release of adrenocorticotrophic hormone (corticotrophin, ACTH) from the adenohypophysis have not been established. The suggestion that the secretion of ACTH is regulated by changes in the concentration of corticoids in the blood (Sayers & Sayers, 1947) is difficult to reconcile with many recent observations (Cox, Hodges & Vernikos, 1958; Fortier & de Groot, 1959; Hodges & Vernikos, 1960). However, the work of Yates, Leeman, Glenister & Dallman (1961) has re-emphasized the possible importance of the blood corticoids in exerting a negative feed-back control on pituitary adrenocorticotrophic activity. Yates *et al.* (1961) injected corticosterone intravenously into rats and immediately subjected them to the stress of either laparotomy or the intravenous injection of histamine. They found that the corticosterone in the blood of the stressed animals rose no higher than in control animals given the same doses of corticosterone but without the stress. Yates *et al.* (1961) interpreted their results as implying that the mechanism of the release of ACTH involves a negative feed-back by corticosterone in the blood and that the immediate effect of stress is to 'increase the set point of the controller'.

The results of their experiments are obviously of great interest in the understanding of the mechanisms which control the release of ACTH, and we have therefore attempted to repeat them with some minor modifications. Under the different conditions of our experiments we have not confirmed the results of Yates *et al.* (1961).

METHODS

Animals. Male albino Wistar rats obtained from the Agricultural Research Council were kept in stock cages in a room where the experiments were performed, at a constant temperature of 22° C for at least 5 days. They were fed on a diet of cubes (diet 41, Lane-Petter & Dyer, 1952) and water. Animals, weighing 100–160 g, were transferred to separate cages and left undisturbed until the commencement of the experiments. Each individual experiment was performed on rats from the same shipment.

Stress. Rats were taken from their cages, anaesthetized with ether and 'sham-adrenalectomized', as described by Hodges & Vernikos (1959). Blood corticotrophin, plasma corticosterone and adrenal ascorbic-acid concentrations were estimated 2½, 30 and 60 min respectively after the operation.

Collection of blood samples. Blood for corticosterone determinations was obtained under ether anaesthesia from the abdominal aorta. The samples were collected within 2 min of the removal of the animals from their cages for anaesthesia. Plasma was separated and stored at 4° C. Plasma corticosterone levels were determined by the method of Zenker & Bernstein (1958). Blood for ACTH estimations was collected and assayed for corticotrophin content as described by Hodges & Vernikos (1959).

Analysis of adrenal glands. The adrenal glands were removed from freshly killed rats, dissected free from peri-adrenal fat and connective tissue, weighed on a torsion balance and transferred to trichloroacetic acid solution for the determination of ascorbic acid content by the method of Roe & Kuether (1943).

Corticosterone in concentrations of 125–500 μg/ml. was injected as solution in 7 % ethanol in normal saline and in concentrations of 4–16 mg/ml. as suspension in normal saline. All the injections were made subcutaneously in volumes of 1 ml./100 g body weight.

RESULTS

The effect of stress on the concentration of corticosterone in the plasma of normal rats is shown in Fig. 1. Rats were anaesthetized with ether, 'sham-adrenalectomized' and allowed to recover. Blood samples were removed for corticosterone determination 15, 30, 60 or 120 min after the operation. The plasma corticosterone concentration in normal control

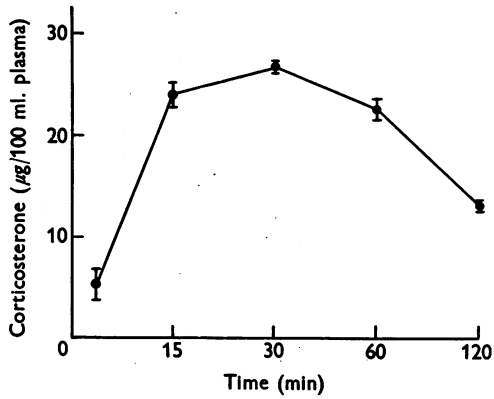

Fig. 1. Plasma corticosterone concentrations in male rats at various intervals of time after sham-adrenalectomy at zero time. Each point is the mean of six determinations. The vertical lines indicate the standard errors of the means.

animals was 5·6 ± 1·5 μg/100 ml. Stress resulted in a very rapid rise in the concentration of corticosterone in the blood. The concentration reached a maximum 30 min after the operation and subsequently declined towards the control value.

Table 1 shows the plasma corticosterone concentration in rats at various times after the subcutaneous administration of solutions and suspensions of corticosterone in doses ranging from 125 μg to 16 mg/100 g body weight. Control injections of the vehicle produced initial slight increases in plasma corticosterone, but the concentration of the steroid returned almost to the resting level within 30 min. Subcutaneous injections of solutions of corticosterone in doses ranging from 125 to 500 μg/100 g produced rapid increases

TABLE 1. Plasma corticosterone concentrations (μg/100 ml. $\pm$ S.E.) in rats at various intervals of time after subcutaneous injections of corticosterone

		Time after injection (min)			
		7½	15	30	60
Vehicle alone		6·8 ± 1	16 ± 1	10 ± 0·5	8·8 ± 1
Corticosterone					
(μg/100 g)	(mg/100 g)				
125	—	56 ± 5	62 ± 7	36 ± 3	16 ± 3
250	—	60 ± 6	75 ± 2	47 ± 5	23 ± 2
500	—	77 ± 6	86 ± 6	70 ± 2	42 ± 2
—	4	—	96 ± 2	98 ± 7	52 ± 3
—	16	—	100 ± 10	120 ± 6	68 ± 4

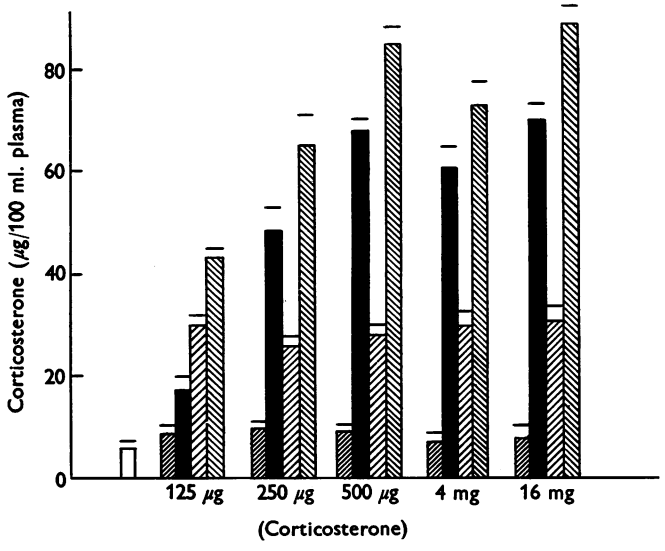

Fig. 2. The effect of stress on plasma corticosterone concentrations in corticosterone-pretreated rats. The rats were stressed 15 min (125–500 μg) or 30 min (4 and 16 mg) after receiving subcutaneous injections of corticosterone or vehicle, and plasma was removed 30 min later. The open column indicates the resting level. Each column indicates the mean of six determinations and the horizontal line above it the standard error of the mean. ▨ saline; ■ corticosterone; ▨ saline + stress; ▧ corticosterone + stress.

in the concentration of the steroid in the plasma with the maximal values 15 min after administration. The blood corticosterone concentrations reached 98 and 120 μg/100 ml. after the injection of suspensions of corticosterone in normal saline in doses of 4 and 16 mg/100 g body weight. Maximal values were attained 30 min after the injections had been given.

Figure 2 compares the plasma corticosterone concentrations 30 min after sham-adrenalectomy in control and corticosterone-pre-treated rats with those in similar rats which had not been subjected to the stress. The

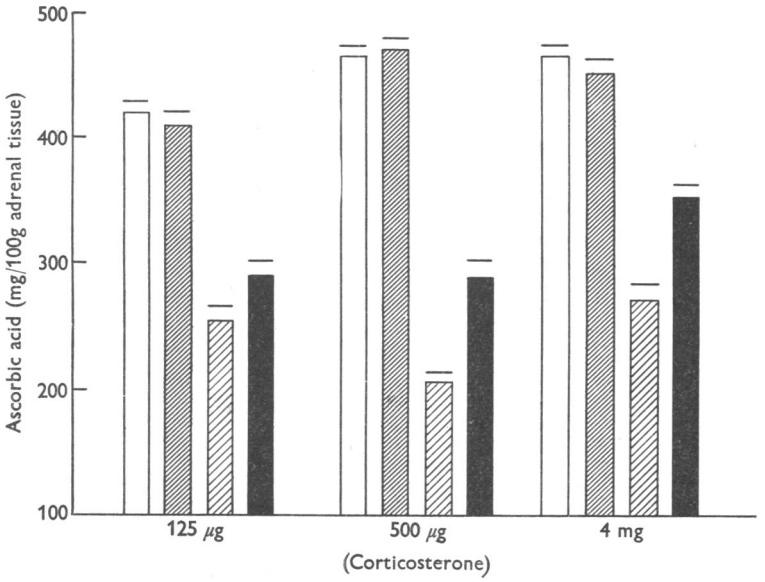

Fig. 3. The effect of stress on the adrenal ascorbic-acid concentrations in corticosterone-pretreated rats. The rats were stressed 15 min (125 and 500 μg) or 30 min (4 mg) after receiving subcutaneous injections of corticosterone or vehicle, and the adrenals were removed 1 hr later. Each column indicates the mean of twelve determinations and the horizontal line above it the standard error of the mean. □ saline control; ▨ corticosterone control; ▧ saline stress; ■ corticosterone stress.

operation caused marked increases in plasma corticosterone concentration and the stress-induced rises were of the same magnitude in both the control and corticosterone-pre-treated groups. Thus the increases were independent of the pre-existing concentration of the steroid in the blood.

The results of a similar experiment, in which adrenal ascorbic-acid contents 1 hr after stress were determined, are shown in Fig. 3. Subcutaneous injections of corticosterone solution or suspension or the vehicle alone produced no change in adrenal ascorbic-acid concentration. Sham-adrenalectomy under ether anaesthesia produced marked adrenal ascorbic-acid depletion in both the control and test animals. However, the fall in

adrenal ascorbic acid was slightly but significantly ($P < 0{\cdot}05$ (125 μg), $P < 0{\cdot}01$ (500 μg and 4 mg)) reduced by corticosteroid pre-treatment.

Blood corticotrophin was estimated before and 2½ min after stress in control and corticosterone-treated rats. The results are summarized in Fig. 4, which shows the mean adrenal ascorbic-acid depletions produced by the blood samples when administered to cortisol-treated assay rats (Hodges & Vernikos, 1959). The blood of unstressed rats possessed no detectable adrenocorticotrophic activity: it produced no fall in adrenal

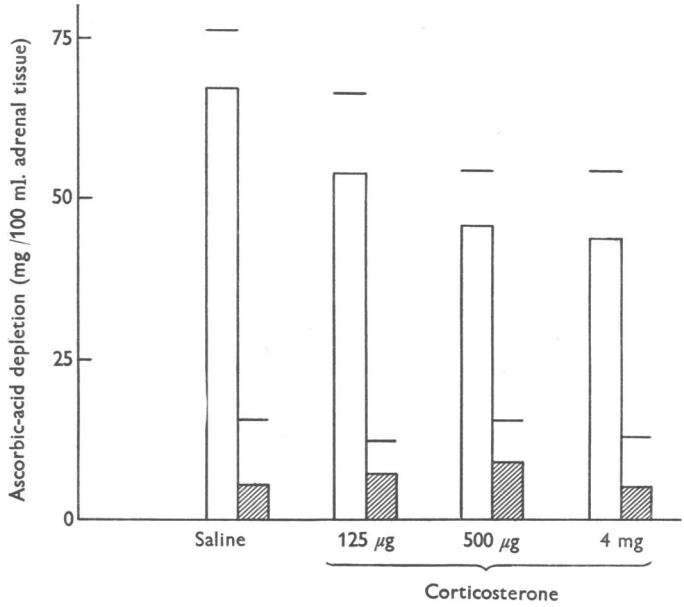

Fig. 4. Adrenal ascorbic-acid depletions in assay rats after injections of blood from stressed □, and unstressed ▨, rats pre-treated with corticosterone. Donor rats were given subcutaneous injections of corticosterone or vehicle 15 min (125 and 500 μg) or 30 min (4 mg) before stress, and the blood samples were collected before, or 2½ min after, sham-adrenalectomy. Each column represents the mean result obtained by injecting blood from ten donor animals into groups of six assay rats. The horizontal lines above the columns indicate the standard errors of the means.

ascorbic acid in the assay animals. Sham-adrenalectomy under ether anaesthesia raised the ACTH content of the blood to easily detectable concentrations in both the control and corticosterone-treated groups, and the rise in circulating corticotrophin was not reduced significantly ($P > 0{\cdot}05$) by pre-treatment with corticosterone in the doses used.

The results obtained with plasma corticoid and circulating ACTH changes as indices of pituitary adrenocorticotrophic activity indicate that

the release of ACTH in response to sham-adrenalectomy is not suppressed by blood corticosterone concentrations at least four times as great as those which occur in normal rats exposed to a similar stress. However, the adrenal ascorbic-acid changes indicate that such blood concentrations of corticosterone produce some depression of ACTH release, but the degree of pituitary inhibition is only very slight indeed.

DISCUSSION

The results of the present series of experiments are in complete contrast with those of Yates *et al.* (1961), and do not support their hypothesis that the secretion of ACTH in response to stress is due to a rapid resetting of a negative feed-back mechanism. Yates *et al.* (1961) administered corticosterone to rats in doses calculated to match the stress-induced increase in the blood concentration of the steroid, and found that subjection of the animals to stress 15–30 sec after the steroid treatment produced no greater increment in plasma corticosterone than that observed in stressed untreated control animals. They argued that, since the increments in plasma corticosterone concentration produced by the exogenous corticosteroid and by the stress did not sum partially or completely, the release of ACTH had been suppressed entirely by a blood corticoid concentration no greater than the maximal level resulting from stress. Yates *et al.* (1961) used changes in corticosterone blood levels as the only index of pituitary adrenocorticotrophic activity. In the present work the same index was employed in addition to direct estimates of circulating ACTH and changes in adrenal ascorbic-acid concentration. Our results with all three parameters indicate that the release of ACTH in response to sham-adrenalectomy is not suppressed by blood corticosterone concentrations at least four times as great as those which occur in normal rats exposed to a similar stress.

It remains to consider the possible reasons for the difference between the results of Yates *et al.* and ours. In our experiments the corticosterone was administered subcutaneously, and the stress was applied 15–30 min later. On the other hand, Yates *et al.* (1961) stressed their animals 15–30 sec after the *intravenous* administration of corticosterone. Consideration of their dosage and the blood volume of the rat leads to the conclusion that, at least for a very short period, their blood corticosterone concentrations must have been considerably greater than those ever attained physiologically. No estimates of the plasma corticosterone concentration between 0 and 3 min after its intravenous administration are given, but Smelik (personal communication) found that during this period it may fall from 300 to 50 μg corticosterone/100 ml. plasma. Therefore it is

probable that Yates *et al.* (1961) applied the stress stimuli to their experimental animals when the blood corticoid concentrations were considerably higher than those ever reached physiologically. The probability that such blood concentrations of corticoid would suppress effectively the release of ACTH is in accordance with the previous work of Hodges & Vernikos (1958). Thus it appears that the discrepancy between the findings may be explained on the basis that, when Yates *et al.* (1961) applied the stress stimuli, the blood corticosterone concentration was falling rapidly, but was still considerably higher than the concentration reached in conditions of stress. In our experiments, the stress was applied when the corticosterone blood concentration was constant and close to the physiological range.

It seems most likely that the release of ACTH in response to acute stress is not affected by any feed-back mechanism. The hypothesis that stress results in increased utilization of corticoids by the peripheral tissues and that the resulting low blood corticoid concentrations stimulate the pituitary gland to increase its output of corticotrophin (Sayers & Sayers, 1947) appeared to be invalidated when it was shown that stress causes only *increases* in plasma corticoid concentrations, and that animals with adrenocortical insufficiency exhibit high circulating ACTH concentrations only after a considerable time lapse (Cox *et al.* 1958). These observations made it evident that the secretion of ACTH is not the result of a fall in the plasma corticoid concentration but did not rule out the possibility, suggested by Yates *et al.* (1961), that stress-induced ACTH secretion is due to a rapid resetting of a negative feed-back mechanism and that 'the increase of the set point of the controller creates a virtual drop in plasma corticosteroid concentration which then provides a signal indistinguishable to the controller from the absolute drop in concentration such as required by the original view of Sayers & Sayers'. Our failure to confirm the experimental findings of Yates *et al.* (1961) appears to invalidate the 'reset' hypothesis and provides further evidence that the secretion of ACTH in response to stress is independent of changes in the level of corticosteroids in the blood.

SUMMARY

1. The time relationships of the changes in plasma corticosterone were studied in male rats after stress and after subcutaneous injections of various doses of the steroid.

2. Maximal plasma corticosterone levels were found 30 min after sham-adrenalectomy, 15 min after the administration of corticosterone solutions and 30 min after corticosterone suspensions.

3. Rats pre-treated with corticosterone were subjected to the stress of

sham-adrenalectomy. Plasma corticosterone was estimated 30 min, adrenal ascorbic acid 1 hr and blood ACTH 2½ min after the stress.

4. Corticosterone in doses sufficient to raise the blood concentration of the steroid to up to four times the maximal level induced by stress did not diminish the further rise in corticosterone or the elevation in blood ACTH and only slightly reduced the adrenal ascorbic-acid depletion normally caused by sham-adrenalectomy.

5. Since the release of ACTH is not suppressed by blood levels of corticosterone greater than those reached in conditions of stress, it is considered that the secretion of corticotrophin is independent of changes in blood corticoid levels within the physiological range, and that the 'reset' hypothesis is untenable.

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