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Hypothalamo-pituitary-adrenal function in the rat after prolonged treatment with cortisol

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1. Cortisol, administered subcutaneously every day for long periods, caused growth retardation, adrenal atrophy and impaired hypothalamo-pituitary-adrenal (HPA) function in the rat.

2. The growth rate and the functional activity of the HPA system gradually returned to normal after steroid withdrawal.

3. Normal adrenal sensitivity to corticotrophin returned more rapidly than normal pituitary corticotrophic function, suggesting that the initial impairment of HPA function was due both to reduced responsiveness of the adrenal gland to corticotrophin and failure of the pituitary gland to secrete the hormone.

It is well established that corticosteroid treatment causes atrophy of the adrenal cortex in the rat (Ingle & Kendall, 1937) and that the pituitary-adrenal response to operative, anaesthetic and other stressful stimuli is impaired in patients who have been treated with steroids over long periods (Fraser, Preuss & Bigford, 1952; Salassa, Bennett, Keating & Sprague, 1953; Livanou & Ferriman, 1965). With the exception of those of Shuster & Williams (1961), and Livanou, Ferriman & James (1967), few attempts have been made to correlate the degree of inhibition of hypothalamo-pituitary-adrenocortical (HPA) activity with the dose and duration of steroid treatment. It is also not yet clear whether the impaired activity is due to a failure of the hypothalamo-pituitary complex to secrete corticotrophin (ACTH), of the adrenal to respond to the hormone, or both. The present work was done to study hypothalamo-pituitary-adrenal function in rats after long-term cortisol treatment and to investigate the recovery of the system after withdrawal of the steroid.

Methods

Approximately nine hundred albino Wistar rats weighing 160-200 g (Oxford Laboratory Animal Centre) were used over a period of 2 years. The animals were housed in groups of four per cage in a quiet room with a constant air temperature of 23° C, and were allowed food (diet 41, Lane-Petter & Dyer, 1952) and water *ad lib.* They were handled and weighed daily for several days before treatment. The animals were divided into three groups; one was injected daily with 2·0 mg cortisol (Ef-cortelan, hydrocortisone free alcohol, Glaxo)/100 g body weight; another similarly with 0·2 mg cortisol, and a control group with 0·9% sodium

chloride solution. The cortisol was suspended in 0·9% sodium chloride solution and the injections were administered subcutaneously in volumes of 1 ml./100 g. The rats were weighed three times a week. Care was taken not to expose the animals to unnecessary infection, although they were not kept under barrier conditions. The treatment lasted for 20 days and was terminated abruptly. After cessation of the treatment the animals were given 0·9% sodium chloride solution to drink. Body weight during and after treatment, and pituitary and adrenal weights after treatment were measured. Assessment of hypothalamo-pituitary or adrenocortical function was made by measuring the plasma corticosterone and adrenal ascorbic acid changes caused by stress or corticotrophin injections respectively. All the tests were done at the same time of day (14·00 to 16·00 hours).

Stress consisted of exposing the animals to ether vapour for 2 min during which time the animals became completely anaesthetized.

Corticotrophin (porcine ACTH Organon) was injected subcutaneously in a submaximal dose of 0·05 i.u./100 g body weight to assess adrenal sensitivity, or in a supramaximal dose of 0·5 i.u./100 g body weight to test adrenal reserve.

Blood samples were removed half an hour after subjection to stress or the injection of ACTH. The rats were decapitated and the blood was rapidly drained into heparinized glass tubes. The samples were centrifuged within a few minutes of collection, and the plasma was removed immediately and stored for up to two weeks at $-10°$ C. Corticosterone concentrations were determined by the method of Zenker & Bernstein (1958).

Adrenal glands were removed 1 hr after subjection to stress or the injection of ACTH. They were dissected free from fat and connective tissue on a dry tile, weighed on a torsion balance and ground singly with the aid of a glass rod in a hard glass tube containing acid washed sand and 8 ml. 4% trichloracetic acid solution. The tubes were stored overnight at 4° C and analysed for ascorbic acid by the method of Roe & Kuether (1943) on the following day.

Pituitary glands were rapidly removed intact and weighed on a torsion balance.

Results

The growth rates of the steroid-treated and control rats are shown in Fig. 1. Although postpubertal rats tolerated cortisol in a dose of 2 mg/100 g body weight per day fairly well, they failed to grow and then lost weight after the second day. Loss of weight continued for about 5 days after withdrawal of the steroid. In contrast, the animals on the low dose of cortisol grew at the same rate as the controls. Post mortem examination showed that the weight loss was due mostly to wasting of the dorsal muscle mass surrounding the vertebral column. Some sudden deaths occurred in the animals on the high dose. There was also an occasional intercurrent *Klebsiella* infection which was associated with a copious nasal discharge. However, the remaining animals appeared to be in good health. After the injections had been stopped, the growth rate of the rats which had received the high dose ultimately tended to exceed that of the other rats.

Table 1 shows adrenal and pituitary weights and plasma corticosterone concentrations 48 hr after the last cortisol injection. The adrenal and pituitary weights are expressed both in absolute values and in terms of body weight. The adrenal atrophy induced by 2·0 mg cortisol per day was highly significant ($P<0·001$) but the 0·2 mg

dose had no effect. Pituitary weight expressed in terms of body weight appeared to be greater in rats treated with the larger dose of cortisol than in the controls, but the absolute weights of the glands were less. This is not surprising, since the steroid treatment inhibited the general growth rate so profoundly. The resting plasma corticosterone concentration was unchanged by treatment with the low dose of cortisol, but significantly ($P<0{\cdot}01$) depressed by the high dose and it returned only slowly to normal (Table 2). Figure 2 shows plasma corticosterone and adrenal ascorbic acid changes in rats subjected to stress or injected with ACTH 48 hr after the last cortisol injection. The response to stress, the adrenal sensitivity and the adrenal reserve were considerably reduced in both steroid-treated groups. Daily treatment with 2·0 mg cortisol almost completely obliterated the response to stress and to both doses of ACTH. The 0·2 mg dose produced a smaller reduction in the stress response and in adrenal sensitivity, and only a slight reduction in adrenal reserve.

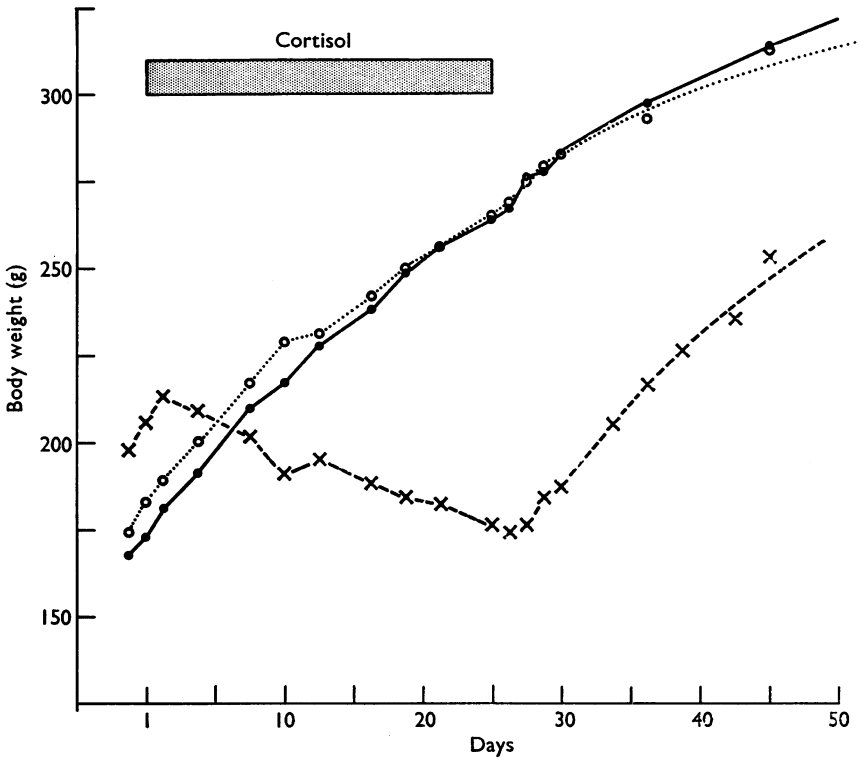

FIG. 1. Effect of prolonged cortisol treatment on growth rate in the rat. Each point on the graph represents a minimum of twenty-four animals. ×---×, 2·0 mg cortisol subcutaneously; ○- - -○, 0·2 mg cortisol subcutaneously; ●—●, saline control.

TABLE 1. *Adrenal and pituitary weights and resting plasma corticosterone concentrations (mean±S.E., n=12) in rats 48 hr after cessation of prolonged cortisol treatment*

Cortisol mg/day	Adrenal weight		Pituitary weight		Plasma corticosterone
	mg	mg/100 g	mg	mg/100 g	μg/100 ml.
2·0	8·1±0·4	4·0	9·0±0·4	4·3	7·0±0·6
0·2	20·1±0·8	6·3	10·1±0·4	3·2	13·0±1·0
Saline controls	19·8±0·6	5·9	9·9±0·5	3·2	13·0±2·0

Rats were killed 2, 4, 8, 16 and 32 days after cessation of cortisol treatment. There was a gradual recovery of the cortisol-induced adrenal atrophy and 32 days after steroid withdrawal the differences between the adrenal weights in the control and cortisol-treated groups were no longer significant (Fig. 3). The same tests for hypothalamo-pituitary-adrenal function were repeated at the same time intervals during the period of recovery from the steroid treatment. The results are shown in Fig. 4a and b. Hypothalamo-pituitary-adrenal function in rats treated with 0·2 mg cortisol appeared to be normal within 4–8 days of stopping the treatment. However, the larger dose produced a greater and longer lasting impairment of the functional activity of the system. The plasma corticosterone changes indicated that HPA function had recovered fully in 32 days, although the adrenal ascorbic acid changes suggested only a partial recovery. The return to normal of both adrenal sensitivity and reserve was more rapid than the recovery of the hypothalamo-pituitary-adrenal response to stress. The plasma corticosterone and adrenal ascorbic acid changes in the test animals after ACTH injections were not significantly different, 16 days after cessation of steroid treatment, from the changes in the controls but the responses to stress were still reduced.

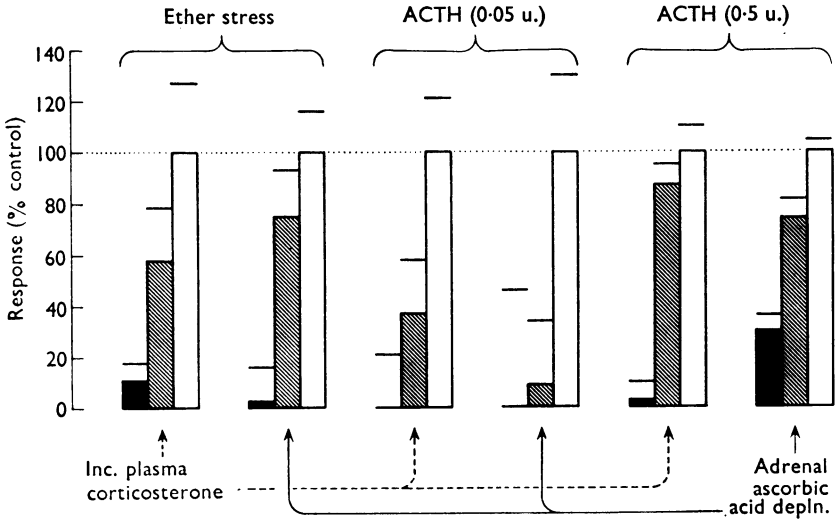

FIG. 2. Increments in plasma corticosterone concentration and depletions of adrenal ascorbic acid in rats which, 48 hr after termination of prolonged cortisol treatment, were subjected to the stress of ether anaesthesia or injected subcutaneously with corticotrophin. Each result represents the mean of twelve determinations and is expressed as a % of the response in twelve saline injected controls. The horizontal bars above the columns represent the standard errors. ■, 2·0 mg cortisol subcutaneously; ▨, 0·2 mg cortisol subcutaneously; □, saline control.

TABLE 2. *Plasma corticosterone concentrations (mean ±S.E., n=12) in rats at various time intervals after cessation of cortisol treatment*

Cortisol mg/day	Plasma corticosterone (μg/100 ml.) Days after last injection				
	2	4	8	16	32
2·0	7±0·6	14±0·7	12±0·7	14±4	18±3
0·2	13±1	17±2	17±2	18±2	20±3
Saline controls	13±2	22±3	18±2	18±2	23±3

Discussion

It was surprising that a daily dose of 0·2 mg cortisol/100 g body weight produced only slight impairment of adrenal function, no significant reduction in plasma corticosterone concentration, and no adrenal atrophy, because, in man, a similar dose in terms of body weight is known to produce considerable inhibition of HPA function. In the rat, however, the effects produced by a tenfold increase in cortisol dosage were considerable, and the results of our experiments are in line with the clinical finding of impaired HPA function after long term steroid therapy. However, the slowness of the return to normal of the resting plasma corticosterone concentration is not in accord with findings in man (Roe, Mitchell & Pennington, 1966).

The reduction in the functional integrity of the HPA system was accompanied by a failure of growth and a marked weight loss. After steroid treatment had been stopped, the growth rate of the rats was accelerated and tended to catch up with that of the controls. Similar observations have been made in children (Friedman & Strang, 1966) in whom the previously impaired growth rate was accelerated when ACTH was substituted for prednisone treatment. The cause of the reduced growth rate is not completely explained. It may be due to inhibition of the release of growth hormone, and Hartog, Gaafer & Fraser (1964) found some reduction in serum growth hormone concentrations in patients treated with steroids. However, Morris, Jorgensen, Elrick & Goldsmith (1968) showed that large doses of human growth hormone given to children on steroid treatment failed to prevent the retardation of growth. The weight loss may also have been partly due to the negative nitrogen balance caused by the steroid treatment (Bellamy, 1964 ; Bellamy & Leonard, 1964).

Our findings demonstrate the complete failure of functional activity of the HPA system after cortisol treatment, and its gradual recovery after withdrawal of the steroid. The question remains whether the reduced activity of the system is due to a reduced ability of the hypothalamo-pituitary complex to secrete corticotrophin, or a failure of the adrenal gland to respond normally to the hormone. The rise in plasma corticosterone and the depletion in ascorbic acid which normally occur in response to stress did not take place at the end of the treatment, nor did the changes in response to exogenous ACTH. At this stage hypothalamo-pituitary impairment and adrenal failure could co-exist. The possibility that both pituitary and adrenal

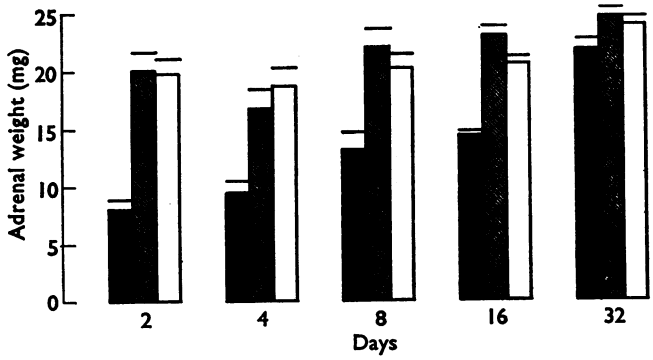

FIG. 3. Adrenal weights at various time intervals after the termination of prolonged cortisol treatment. Each column represents the mean of twelve determinations. The horizontal bars above the columns indicate the standard errors. ■, 2·0 mg cortisol subcutaneously; ▨, 0·2 mg cortisol subcutaneously; □, saline control.

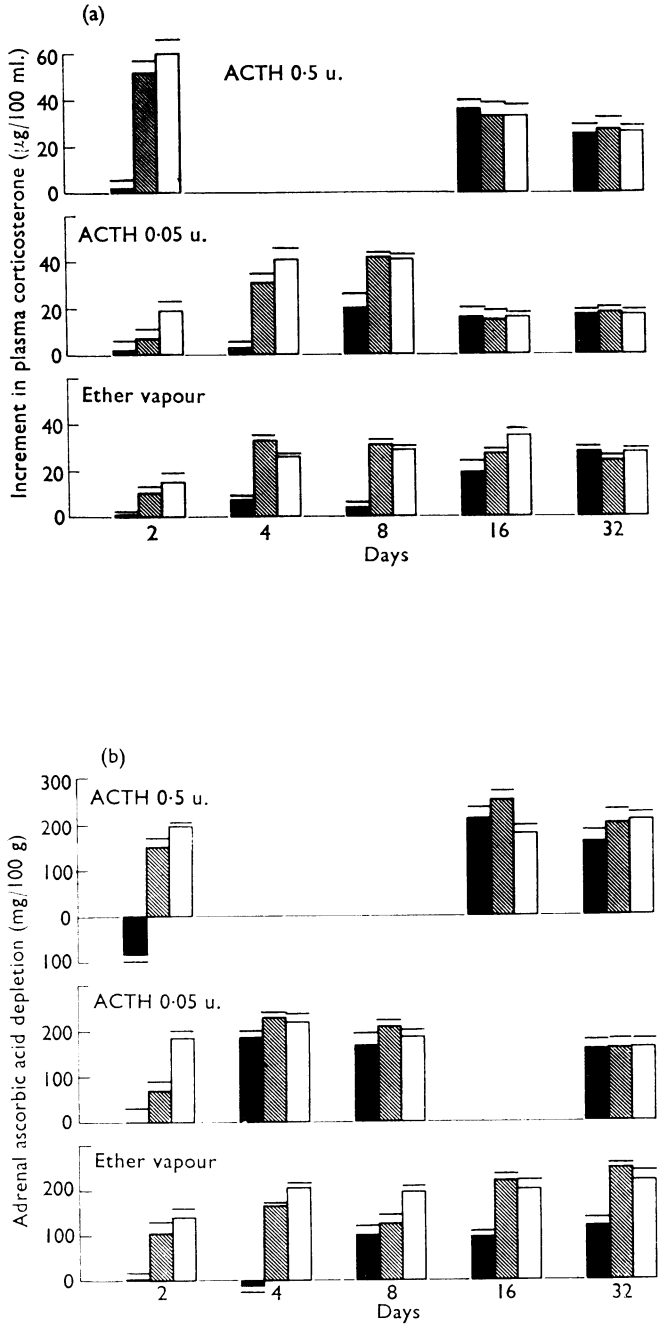

FIG. 4. Increments in plasma corticosterone concentration (a) and adrenal ascorbic acid depletion (b) in rats which, at various times after termination of prolonged cortisol treatment, were subjected to the stress of ether anaesthesia or injected subcutaneously with corticotrophin. Each result represents the mean of twelve determinations. The horizontal bars above the columns represent the standard errors. ■, 2·0 mg cortisol subcutaneously; ▨, 0·2 mg cortisol subcutaneously; □, saline control.

function were impaired initially was suggested by the fact that adrenal sensitivity and reserve returned to normal more rapidly than the functional activity of the entire system. The deficit of hypothalamo-pituitary function was unmasked because adrenal function recovered more rapidly.

These observations may well be relevant to the clinical problems associated with the withdrawal of steroids from patients. Our findings indicate that adrenal function may be normal when pituitary function is impaired. In such a situation, attempts to restore the functional integrity of the hypothalamo-pituitary adrenal system by corticotrophin injections are valueless. A normal adrenal response to exogenous ACTH does not indicate an adequate pituitary adrenocorticotrophic response to stress. A patient responding normally to injected ACTH may be incapable of mobilizing sufficient endogenous corticotrophin even for normal daily requirements. In fact, corticotrophin injections may be contraindicated since pituitary function could be impaired further (Carreon, Canary & Kyle, 1959 ; Plager & Cushman, 1962 ; Bacon, Daly, Myles & Savage, 1968). The solution of problems associated with the withdrawal of steroids from patients may be facilitated by further work on laboratory animals.

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