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Effect of prolonged treatment with tetracosactrin on hypothalamo-pituitary-adrenal function in the rat

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Summary

1. The functional integrity of the hypothalamo-pituitary adrenal (HPA) system was assessed in rats after prolonged treatment with tetracosactrin.

2. Adrenal size and sensitivity to corticotrophin were increased.

3. Both the circadian rhythm and the stress-induced increase in HPA activity were inhibited. The circadian rhythm recovered before the response to stress.

4. Tetracosactrin given after betamethasone treatment delayed the return of a normal HPA response to stress.

5. Suppression of HPA activity appeared to be the result of a direct action of tetracosactrin and not entirely due to the elevations in the plasma corticosterone concentration which it caused.

Introduction

Suppression of hypothalamo-pituitary-adrenal (HPA) activity is a well known hazard in patients given long term treatment with corticosteroids, but the effects on HPA function of similar treatment with corticotrophin (ACTH) are still controversial. It has been claimed that, in addition to having some clinical advantages over the corticosteroids (West, 1962), corticotrophin also causes very little suppression of HPA function (Bacon, Daly, Myles & Savage, 1968 ; Carter & James, 1970) but this is not a universal view (Bierich, Kersten & Maruektad, 1959 ; Hayes, 1956 ; Levell, Stitch, & Noronha, 1970 ; Reed, Clayman & Palmer, 1964).

Our experiments were done, therefore, to investigate HPA function after prolonged corticotrophin treatment using the rat as a laboratory model. Tetracosactrin (synthetic ACTH) was used rather than natural corticotrophin to minimize the risks of antibody formation (El-Shaboury, 1968 ; Landon, Friedman & Greenwood, 1967).

Methods

Male Sprague-Dawley rats (Fisons Pharmaceuticals Ltd.) weighing 175–275 g were used for all the experiments. The conditions under which the animals were kept have been described previously (Hodges & Mitchley, 1970a, b).

Tetracosactrin (Cortrosyn-depot, Organon). This was diluted with 0·9% sodium chloride solution to concentrations of 5 μg/ml or 100 μg/ml and was given subcutaneously to the rats in a volume of 0·1 ml/100 g. Control rats were injected with the same volume of 0·9% sodium chloride solution.

Corticosterone (Organon). This was made into a fine suspension in 0·9% sodium chloride solution in a concentration of 2 mg/ml and was injected subcutaneously in

a volume of 0·5 ml/100 g. Control rats were injected with the same volume of 0·9% sodium chloride solution.

Tetracosactrin was injected once and corticosterone twice daily. The injections were given for two periods of 5 days with a 2 day interval during which the animals were untreated.

Betamethasone disodium phosphate (Betnesol, Glaxo). This was given to rats for 2 weeks in a concentration of 2 μg/ml in the drinking water. On each of the first 3 days after the treatment the rats were injected with Cortrosyn-depot in a dose of 10 μg/100 g or 0·9% sodium chloride solution.

At the end of the treatment the activity of the HPA system was assessed by measuring adrenal sensitivity to ACTH, the circadian rhythm in the plasma corticosterone concentration, and the rise in the plasma corticosterone concentration in response to stress as described previously (Hodges & Mitchley, 1970a, b). For adrenal sensitivity tests a short acting preparation of tetracosactrin (Cortrosyn, Organon) was used whereas the long acting preparation (Cortrosyn-depot) was always used for treatment.

Results

The effect of tetracosactrin on the weights of the adrenal glands is shown in Table 1. After 0·5 μg/100 g for 2 weeks the adrenal glands were larger than in the controls but not significantly so ($P>0·2$). The higher dose of tetracosactrin ((10 μg/100 g)/day) caused adrenal enlargement which was highly significant ($P<0·001$)

TABLE 1. *Adrenal weights in rats treated with tetracosactrin for 2 weeks*

Tetracosactrin (cortrosyn-depot) ((μg/100 g)/24 h)	Days after end of treatment	Adrenal weight (mg/100 g) Treated	Control
0·5	1	17·4±2·0 (18)*	
	2	16·1±1·5 (18)	14·4±1·4 (30)
	4	15·6±1·1 (18)	
10	1	32·1±5·6 (18)	
	2	30·9±5·6 (18)	
	3	29·2±3·2 (18)	17·4±1·0 (48)
	4	27·2±2·7 (18)	
	6	24·6±2·0 (18)	

* Numbers of animals in parentheses.

TABLE 2. *Adrenal sensitivity to corticotrophin in rats which had been pre-treated with tetracosactrin for 2 weeks*

Tetracosactrin (cortrosyn-depot) ((μg/100 g)/24 h)	Days after end of treatment	Plasma corticosterone increment (μg/100 ml) 30 min after subcutaneous tetracosactrin (0·25 μg/100 g) Treated	Control
0·5	1	41·9±1·4 (6)	
	2	39·7±1·7 (6)	33·7±4·0 (12)
	4	47·6±2·1 (6)	
10	1	62·2±3·1 (6)	
	2	54·7±2·1 (8)	
	3	51·9±2·8 (8)	33·6±2·0 (24)
	4	62·6±3·2 (9)	
	6	67·7±3·8 (8)	
	8	64·7±3·0 (8)	

and which persisted for at least 8 days after the end of the treatment. Adrenal sensitivity to the test dose of ACTH (0·25 μg/100 g s.c.) was increased by prolonged treatment with both doses of tetracosactrin (Table 2) and even 8 days later, when there was some regression in the weight of the adrenal glands, the sensitivity was still enhanced.

Although the adrenal sensitivity to ACTH was not impaired by prolonged treatment with the smaller dose of tetracosactrin, and there was some circadian rise in the plasma corticosterone concentration (Fig. 1a), there was no rise in the concentration of corticosterone in the plasma in response to ether immediately after the treatment (Fig. 1b), but it was almost normal on the second day. Both the circadian rhythm and the stress-induced rise in the plasma corticosterone concentration were abolished completely by the larger dose of tetracosactrin (Fig. 2a, b). The

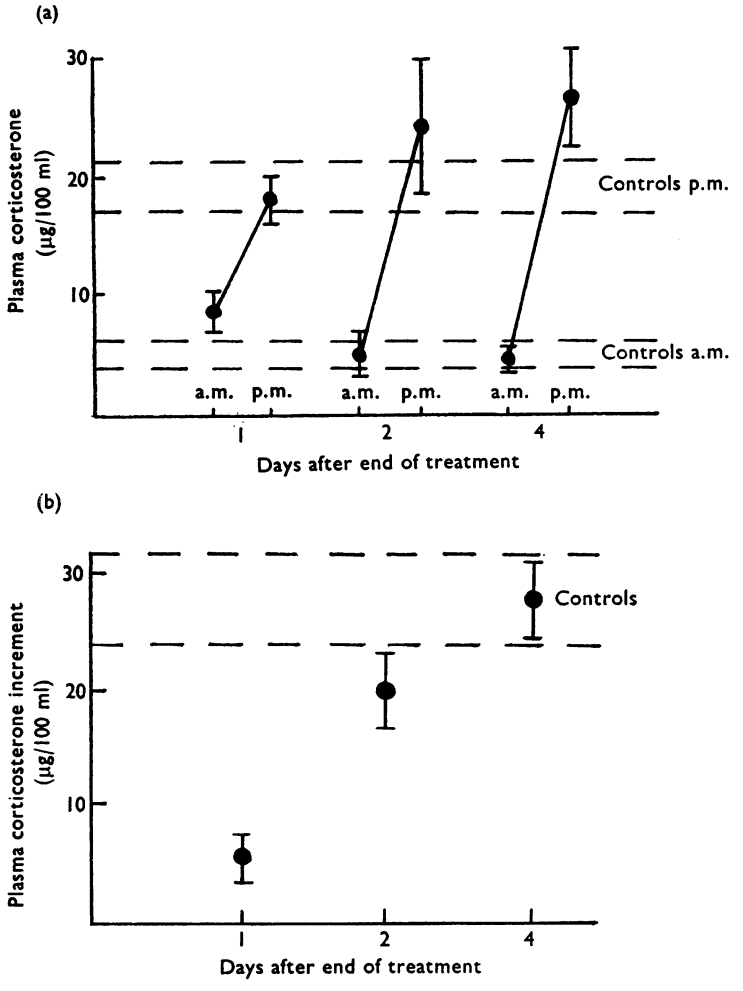

FIG. 1. Circadian rhythm and stress-induced increments in the plasma corticosterone concentration in rats at various times after treatment for 2 weeks with tetracosactrin (0·5 μg/100 g subcutaneously daily). Figure 1a shows the plasma corticosterone concentration at 09.00–10.00 h and 17.00–18.00 h and Fig. 1b shows increments in plasma corticosterone 30 min after the stress of exposure to ether vapour for 1 minute. Each point is the mean of six to eight determinations and is shown with its S.E. The paired horizontal lines indicate control values ±S.E. (eighteen observations).

circadian rhythm returned on the third day after the end of the treatment when its excursion was exaggerated. A response to stress, however, was not present until later when it also was enhanced.

In untreated rats one injection of the lower dose of tetracosactrin caused only a small rise in the plasma corticosterone concentration and it returned to normal within 4 hours. However, after the higher dose the plasma corticosterone concentration was elevated for about 12 h (Fig. 3).

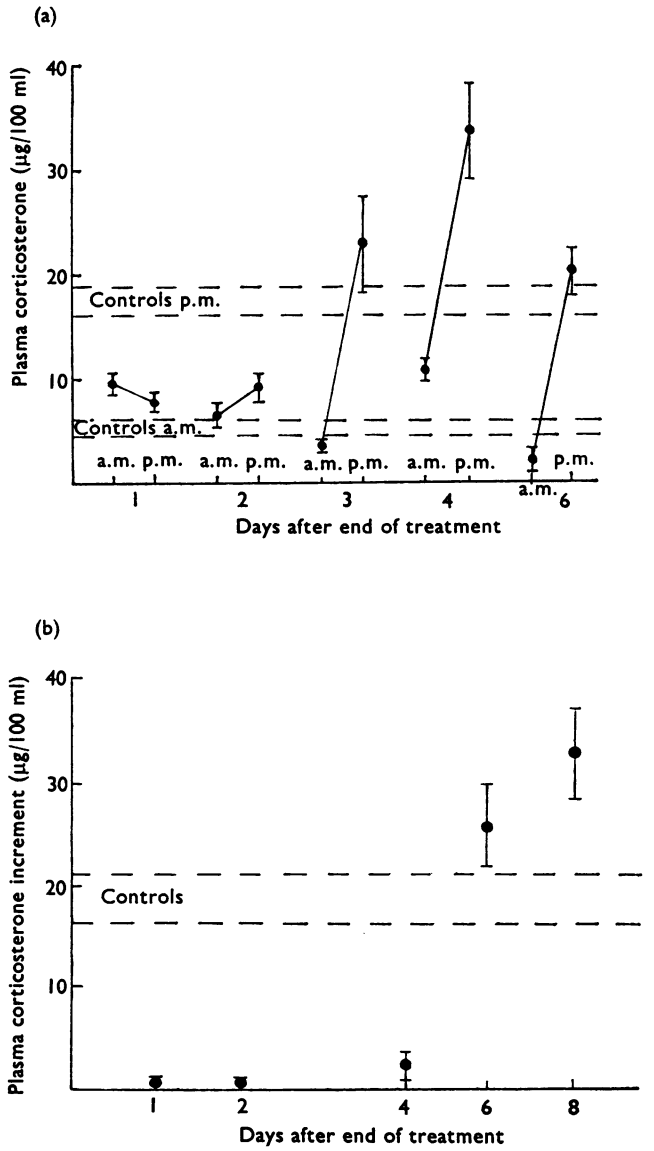

FIG. 2. Circadian rhythm (Fig. 2a) and stress-induced increments (Fig. 2b) in the plasma corticosterone concentration in rats at various times after treatment for 2 weeks with tetrocasactrin (10 μg/100 g subcutaneously daily). The results are expressed as in Fig. 1.

In order to determine whether the effects of prolonged treatment with tetracosactrin on the HPA system were due to the tetracosactrin itself or secondary to the high plasma corticosterone concentrations, rats were injected with corticosterone to mimic the blood corticoid changes which occurred during tetracosactrin treatment. The plasma corticosterone concentrations at various times after injection of the steroid itself are also shown in Fig. 3. The time relationships of the changes in plasma corticosterone after ACTH and exogenous steroid were obviously different but the areas under the curves were similar. The effect of the corticosterone on body growth was also similar to that of the tetracosactrin (Fig. 4). Although

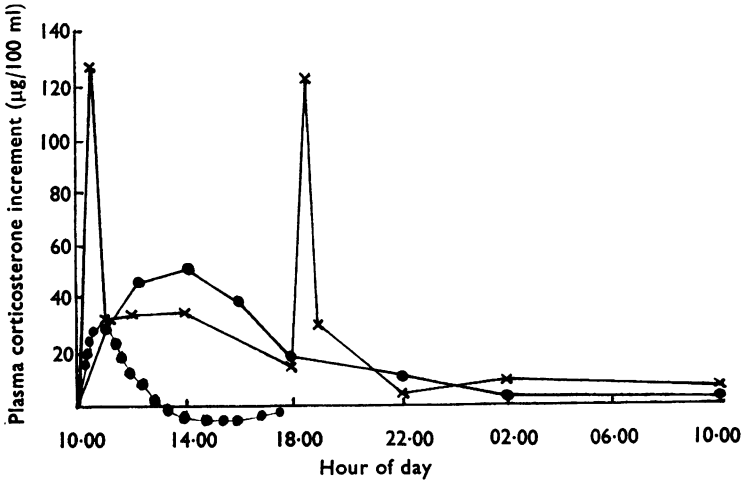

FIG. 3. Increments in the plasma corticosterone concentration at various times after one injection of tetracosactrin (0·5 μg/100 g or 10 μg/100 g) (●——●) or two of corticosterone (1 mg/100 g) (×——×). Tetracosactrin was injected at 10.00 h and corticosterone at 10.00 h and 18.00 hours. Each point is the mean of six determinations. Standard errors, which varied from 1 to 6 μg/100 ml, are not shown.

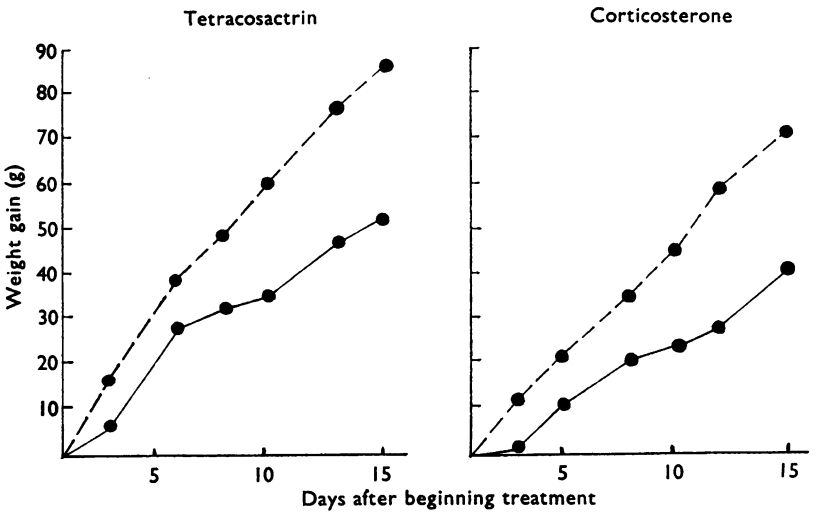

FIG. 4. Growth rates in rats treated for 2 weeks with corticosterone (1 mg/100 g s.c. twice) or tetracosaction (10 μg/100 g s.c. once a day). Each point is the mean weight of at least thirty animals. (●– – –●), Controls; (●——●), treated.

treatment with corticosterone for 2 weeks caused no significant adrenal atrophy, both the circadian rhythm and the stress-induced rise in the plasma corticosterone concentrations were abolished on the first day after the treatment as they were in the rats treated with the higher dose of tetracosactrin. The circadian rhythm returned on the fourth day and the response to stress on the sixth day after stopping the treatment (Fig. 5a, b). Thus, the time courses of recovery of HPA function after corticosterone and after ACTH were similar.

In order to determine whether ACTH could affect the recovery of normal HPA function after prolonged corticosteroid treatment a group of rats was given betamethasone (2 μg/ml) in the drinking water for 2 weeks. At the end of the treatment tetracosactrin (10 μg/100 g s.c.) was injected once on each of 3 consecutive days and the plasma corticosterone concentrations were measured 4 h after the injections. The results which are illustrated in Fig. 6 show that the rises in plasma corticosterone concentration after the first, second or even the third injection of ACTH were much smaller in the betamethasone treated rats than in the controls.

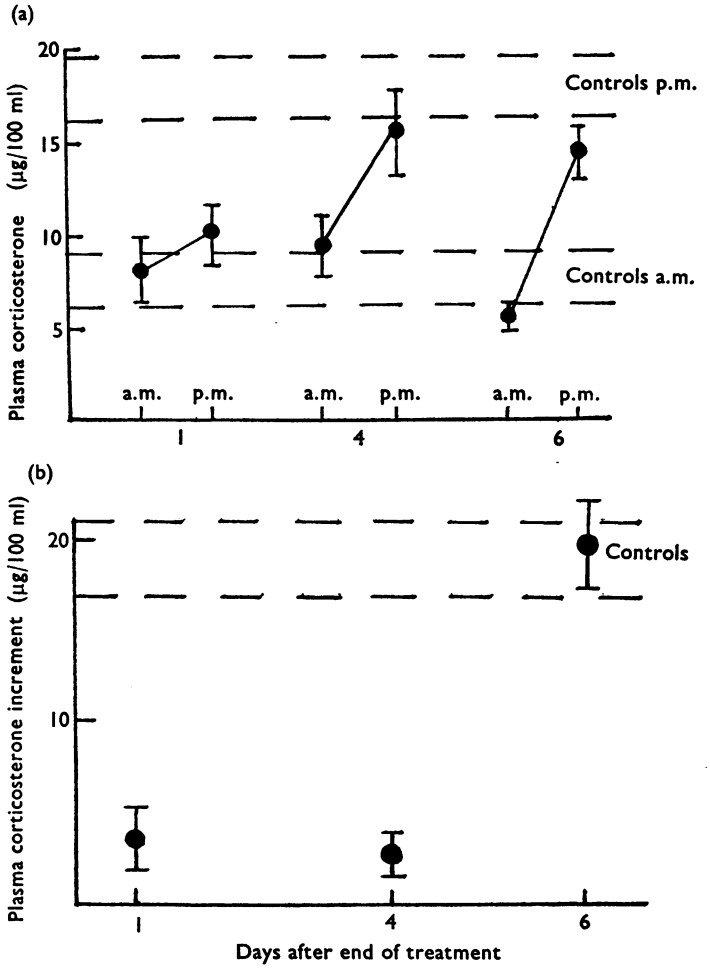

FIG. 5. Circadian rhythm (a) and stress-induced increments (b) in the plasma corticosterone concentration in rats at various times after treatment for 2 weeks with corticosterone (1 mg/100 g s.c. twice daily). The results are expressed as in Fig. 1.

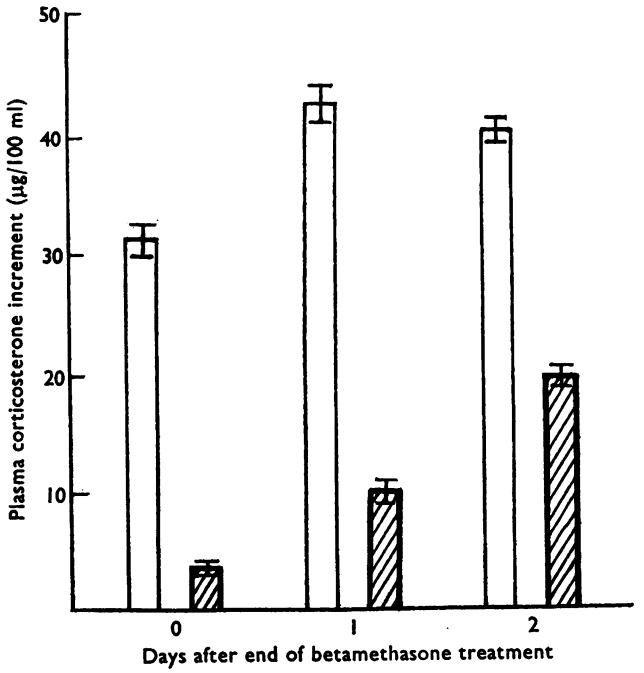

FIG. 6. Increments in the concentration of corticosterone in the plasma 4 h after the injection of tetracosactrin (Cortrosyn-depot) (10 μg/100 g s.c.) once on each of 3 successive days. (Open columns, controls ; shaded columns, immediately after treatment for 2 weeks with betamethasone (2 μg/ml) in the drinking water. Each column represents the mean of six determinations and is shown with its S.E.

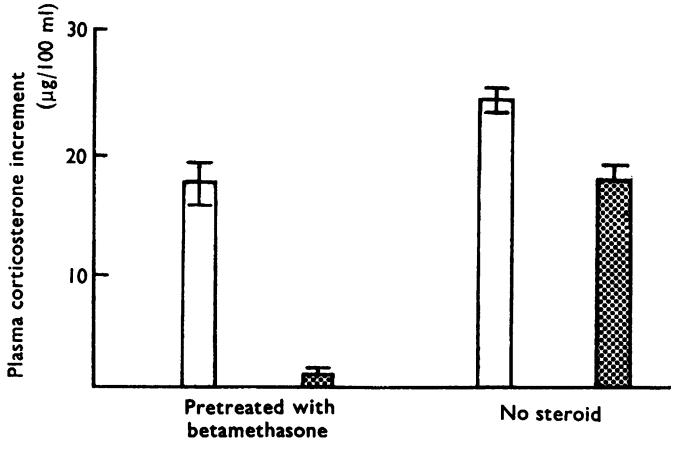

FIG. 7. Increments in the concentration of corticosterone in the plasma 30 min after exposure to ether stress in rats treated with betamethasone (2 μg/ml in the drinking water for 2 weeks, left hand columns) and in rats which did not receive betamethasone (right hand columns). Shaded columns refer to rats which were given tetracosactrin (Cortrosyn-depot 10 μg/100 g, s.c.) on 3 consecutive days after the end of betamethasone treatment and before the stress and the unshaded columns to animals injected with normal saline instead. Each column represents the mean of six determinations and is shown with its S.E.

Four days after the betamethasone treatment adrenal sensitivity to ACTH was back to normal and the responses to a test dose of tetracosactrin (0·25 μg/100 g) were the same in the rats given betamethasone alone as in those given betamethasone followed by tetracosactrin. The responses to stress of the rats in this group were also studied and are shown in Fig. 7. The stress-induced increments in the concentration of corticosterone in the plasma were only slightly reduced by betamethasone alone or tetracosactrin alone but completely abolished by betamethasone and tetracosactrin together in spite of the fact that the adrenal sensitivity to ACTH was normal in the rats pretreated with betamethasone and tetracosactrin.

Discussion

Our previous work (Hodges & Mitchley, 1970b) has shown that prolonged treatment of rats with betamethasone results in inhibition of endogenous ACTH release but the effect is masked by insensitivity of the adrenal cortices to stimulation by ACTH. From the work described here it is evident that prolonged treatment with tetracosactrin also results in a failure to mobilize endogenous ACTH. This is more easily demonstrated because the adrenal sensitivity to corticotrophic stimulation is increased. Our results are in agreement with observations both in man and laboratory animals (Hayes, 1956; Motta, Mangili & Martini, 1965; Plager & Cushman, 1962; Stark, Fachet & Mihály, 1963; Sussman, Librik & Clayton, 1965).

Both the circadian and the stress-induced corticotrophin secretion were inhibited after tetracosactrin. The circadian rhythm returned to normal sooner than the response to stress. A similar dissociation was seen in rats after betamethasone treatment (Hodges & Mitchley, 1970a). This suggests that corticotrophin resembles the corticosteroids by acting on a site in the central nervous system higher than a common pathway for ACTH release (Hodges, 1970).

Inhibition of the release of endogenous ACTH by exogenous ACTH may be either a primary effect of the trophic hormone or secondary to the increased output of corticosteroids. The inhibition of HPA function after prolonged corticosterone treatment recovered in the same way as it did after tetracosactrin treatment. The findings are compatible with the idea that the effects of tetracosactrin are the result of the elevated plasma corticosteroid concentrations. However, a small dose of tetracosactrin, which produced only slight and transient rises in plasma corticosterone, also caused an inhibition of the stress-induced ACTH release. Furthermore, tetracosactrin given after betamethasone increased the delay in recovery of function of the HPA axis without producing any marked elevation in plasma corticosterone. Hence the inhibition of endogenous ACTH secretion is probably due to the hormone itself.

Our results, which suggest that the inhibition of HPA function occurring after the administration of ACTH can be profound, are in contrast with many clinical reports (Carter & James, 1970; Grant, 1970; Holub, Wallace & Jailer, 1960; Nelson, Mackay, Sheridan & Weaver, 1966). Our doses were large but, in terms of body weight, not very different from those sometimes used in man (Dudley-Hart, Taylor, Huskisson & Shenfield, 1971). Our work also indicates that ACTH given after long term corticosteroid administration retards rather than hastens the recovery of normal function of the HPA system. Hence the administration of ACTH as an 'aid' to the withdrawal of corticosteroids may be harmful. There is clinical

evidence in support of this view (Bacon, Beardwell, Myles, Daly & Savage, 1966; Carreon, Canary, Meyer & Kyle, 1960; Ferriman & Page, 1960), but it is not uncommon practice to use ACTH during the withdrawal of corticosteroids (Thompson, 1970; Today's Drugs, 1969a, b). The finding that the HPA system may be unable to respond to stress long after a normal circadian rhythm in pituitary adrenocortical activity has returned may also be of clinical relevance. The circadian rhythm which is present in man soon after the end of either corticosteroid or corticotrophin treatment (Bacon *et al.*, 1968; Graber, Ney, Nicholson, Island & Liddle, 1965; Westerhof, van Ditmars, der Kinderen, Thijssen & Schwarz, 1970) does not necessarily suggest the existence of normal HPA function as is often thought. In certain circumstances it may provide some information about the integrity of the HPA axis (Krieger, Glick, Silverberg & Krieger, 1968) but the ability of the system to respond to stress should also be assessed.

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