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FUNCTIONAL CHANGES PRODUCED IN THE ADRENAL CORTEX OF THE RAT BY ADMINISTRATION OR BY RELEASE OF CORTICOTROPHIN

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This work forms part of an investigation which seeks to determine whether changes in function accompany the morphological changes produced in the adrenal cortex by the administration of drugs. The majority of drugs exert their action through a release of corticotrophin (ACTH), and this paper will therefore deal with the effect of various forms of administration of ACTH on the appearance and secretory performance of the adrenal cortex. Procedures known to cause release of endogenous ACTH will also be dealt with. Only corticosterone secretion is measured in these experiments. It is the only glucocorticoid known to be secreted by the rat adrenal.

The purpose of the work was to assess cortical activity by the most direct means possible, that is by estimating the hormone secreted into the blood. In large animals this can be achieved while the animal is resting and unstressed as was shown by Hume & Nelson (1954), who implanted a permanent cannula into the left adrenal vein of the dog. To obtain adrenal vein blood in small animals, a major operation under surgical anaesthesia is required, and adrenocortical secretion, far from representing resting levels, is greatly accelerated in these experimental conditions. Some preliminary experiments were therefore carried out in order to assess more precisely the extent to which the surgical procedures stimulated cortical secretion.

METHODS

Operations. Male rats of about 250–400 g body weight were anaesthetized with urethane (1·75 g/kg subcutaneously) or pentobarbitone (45 mg/kg intraperitoneally). One femoral vein was cannulated to permit intravenous injections, the left renal hilum was tied, the left renal vein exposed and tributaries other than the adrenal vein were tied. After the injection of heparin (1000 i.u./kg) and the occlusion, by a ligature or by a fine clip, of the renal vein at its entry into the vena cava, a cannula was rapidly introduced into the renal vein near its origin and the escaping blood collected into ice-cooled siliconed centrifuge tubes. The clip was used when more than one sample was required and collection had to be interrupted.

Estimation of corticosterone. The plasma prepared from adrenal blood was extracted, the extract purified, chromatographed on paper and the corticosterone region located on the paper by U.V. absorption. The steroid was eluted from the paper and estimated colorimetrically by its reaction with blue tetrazolium, absorption at 520 mμ being measured in a Unicam spectrophotometer. This instrument gave better results than the Spekker absorptiometer used previously; cells of 1 cm light path were used, and 4 ml. ethanol was added to the reaction mixture after incubation. Further dilution of the solutions was not required with amounts of steroids up to at least 25 μg per sample. For all other details of procedure see Vogt (1955).

Injection schedules. All injections which preceded the operation were given subcutaneously. For the short-term treatment with ACTH the injections were spread over a period of 36 hr, 4 doses of long-action ACTH (Cortrophin Z, Organon Laboratories, called 'ACTHZ') being injected twelve-hourly together with doses of soluble ACTH; two additional doses of soluble ACTH were given 4 and 8 hr after the second of the twelve-hourly injections. ACTH-Armour was used for the early, ACTH-Organon for the later, experiments. The hormones were of porcine origin and contained between 1·2 and 2·5 i.u./mg. In most experiments, each dose of soluble ACTH was 4 i.u., each dose of 'ACTHZ' 5 i.u., so that the total given amounted to 44 i.u. Two rats had a total of only 16 i.u. (12 i.u. soluble and 4 i.u. long-action ACTH) and two a total of 54 i.u. (24 i.u. soluble and 30 i.u. long-action ACTH, 6 doses having been given instead of the usual 4). Adrenal blood was collected between 1½ and 4 hr after the last injection. The *long-term* treatment consisted of daily injections of 1 i.u. 'ACTHZ' for periods ranging from 7 to 14 days, or of 2 daily injections of 1 i.u. 'ACTHZ' for 4 days. The interval between blood collection and the last injection was 24 hr when 1 i.u./day had been given, and 13–15 hr after 2 i.u./day.

RESULTS

Intravenous infusions of ACTH

In order to see whether the collection of adrenal vein blood from a rat challenged the adrenal cortex to maximal activity or left room for further stimulation, two consecutive 15-minute samples of adrenal blood were collected, one being taken before, the second during and immediately after an infusion of ACTH. The results were checked by experiments in which two consecutive samples of adrenal blood were taken, but no ACTH was administered. In all experiments the femoral blood pressure was recorded, and small quantities of rat blood were infused whenever the blood pressure showed signs of declining. Tables 1 and 2 summarize the results. Table 1 shows that, when no ACTH was given, the secretion rate of corticosterone was steady during the collection of the two samples. Table 2 indicates that the effect of infusing ACTH was not consistent. When the mean of all the first samples (28·1 ± 2·6) is compared with the mean of all the second samples (33·6 ± 2·4), a small increase is seen which is not significant. If, however, the rats are grouped according to the size of their initial secretion, it is obvious that the rats (Nos. 1–5) with an initial secretion below the mean of the whole group responded to ACTH with a significant increase in corticosterone production, whereas the remaining rats, in which secretion was high initially, showed but small fluctuations in either direction. It is of interest that the highest figures obtained after an infusion of ACTH did not appreciably exceed the highest spontaneous secretion rate (see rat No. 10).

It follows from these observations that the secretion rate as measured in these experiments is either maximal or not far from maximal; obviously the anterior pituitary of rats subjected to cannulation of the renal vein produces enough ACTH to ensure that the adrenal cortex works to full or nearly full capacity, so that very little further stimulation is exerted by exogenous ACTH. In spite of the fact that exogenous ACTH thus had little effect on

TABLE 1. Corticosterone content of two consecutive 15 min samples (S_1 and S_2) of adrenal vein blood collected from the same rat

Expt. no.	Body weight (g)	Plasma collected (g) S_1	Plasma collected (g) S_2	Weight of left adrenal (mg/kg body wt.)	Corticosterone (μg/g gland/min) S_1	Corticosterone (μg/g gland/min) S_2	Change (%)
1	242	2·2	2·1	80	22·1	21·1	−4·5
2	294	1·5	1·4	71	33·2	33·7	+1·5
3	280	1·7	1·7	73	30·5	32·5	+6·7
						Mean	+1·2

Experiments in pentobarbitone anaesthesia; rat blood infused whenever required to keep blood pressure steady.

TABLE 2. Effect of intravenous ACTH on corticosterone secretion of rat adrenals

Expt. no.	Body weight (g)	Plasma collected (g) S_1	Plasma collected (g) S_2	Weight of left adrenal (mg/kg body wt.)	Corticosterone (μg/g gland/min) S_1	Corticosterone (μg/g gland/min) S_2	Change (%)
1	310	1·6	3·0	72	15·6	20·3	+30·1
2	350	1·9	4·3	82	18·7	25·6	+36·9
3	370	1·6	1·5	52	20·1	30·7	+52·8
4	327	1·4	2·8	74	21·5	32·9	+53·1
5	370	1·8	2·3	54	25·0	42·5	+70·0
				Means (1–5)	20·2±1·8	30·4*±4·3	+48·7
6	345	1·9	5·0	67	29·2	25·5	−12·7
7	370	2·5	3·6	53	32·3	33·2	+3·0
8	310	1·1	2·7	74	38·0	43·5	+14·5
9	373	1·4	2·4	64	38·3	41·9	+9·4
10	314	3·3	5·5	56	41·1	39·6	−3·6
				Means (6–10)	35·8±2·3	36·7±3·5	+2·1
				Means (1–10)	28·1±2·6	33·6±2·4	

S_1, control sample of adrenal blood (15 min); S_2, sample collected during and after an infusion of approximately 0·25 i.u. ACTH (given over 8–14 min). Experiments in pentobarbitone anaesthesia; rat blood infused whenever required to keep blood pressure steady. Figures after the means represent S.E. of the mean.

* Significantly greater than S_1 ($P<0·05$).

cortical secretion, the infusions usually caused a large increase in adrenal blood flow. (Compare plasma volumes in Tables 1 and 2.) These increases occurred although the control of the blood pressure by replacement of the lost blood was never quite adequate, so that the blood pressure was somewhat lower during collection of the second sample. This point is illustrated in Fig. 1 which reproduces the blood-pressure record of Expt. 7 (Table 2).

These observations would suggest that the vasodilatation caused by administration of ACTH is not linked to the increase in (at least the final stages of) synthetic activity but is an independent effect of ACTH on cortical tissue.

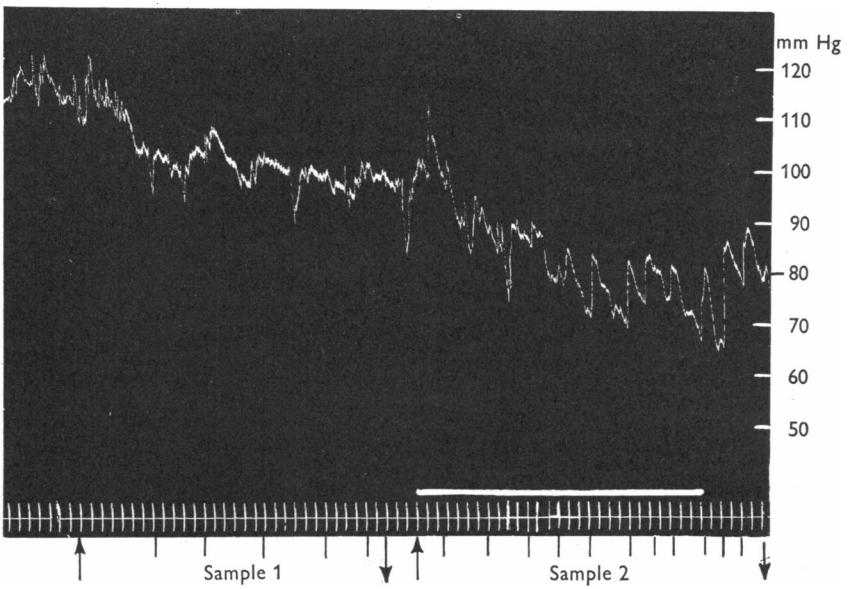

Fig. 1. Femoral blood pressure of rat 7 (Table 2); effect of ACTH. There was some decline in blood pressure during collection of the second blood sample, but the adrenal flow was increased (see Table 2). White line, infusion of 0·25 i.u. ACTH into the femoral vein; ↑, beginning, ↓, end of collection of blood samples; |, intravenous injections of 0·5–0·6 ml. rat blood; time marker, 30 sec.

Subcutaneous administration of ACTH for various periods preceding the sampling of adrenal vein blood

When a single dose of ACTH is injected into a rat, the adrenal lipids (a large fraction of which consists of cholesterol) are first reduced and later increased in quantity (Sayers, Sayers, Liang & Long, 1946). When the doses are large or given repeatedly, the adrenal size increases, but the size attained is not correlated with the loss or storage of lipids. It was of interest therefore to compare the functional capacity of enlarged adrenals both in the early phase when the lipid content was lower than normal and in the later phase when it was larger.

Experiments were carried out, in which approximately the same dose of ACTH was given over a period of about 2 weeks to one group of rats, and within 36 hr to a second group (Table 3, *a* and *c*). All the glands of the first group were engorged with lipids, whereas those of the second group showed loss of lipids. Corticosterone secretion per gram of gland was normal in the first group and, owing to the adrenal hypertrophy, secretion per whole

gland was significantly raised. Such adrenals therefore endow their owners with a potentially higher corticoid secretion during a severe stress. The second group of rats appeared to show decreased secretion per gram of gland and normal secretion per whole gland. Since adrenal enlargement was smaller than in the first group, it was decided to try and confirm this trend on groups treated for the same period but with larger doses of ACTH (Table 3, *d* and *e*). The results of each subgroup (*d* and *e*) should be compared with their own controls, done at approximately the same time with the same batch of rats and the same anaesthetic.

TABLE 3. Action of ACTH injected subcutaneously on corticosterone secretion of rat adrenals

No. of rats	Body weight (g) Initial	Final	No. of injections	Weight of left adrenal (mg/kg body weight)	Corticosterone (μg/g gland/min)	(μg/gland/hr/kg body wt.)	Histology
	(*a*) 1 i.u. long-action ACTH† per day. (Blood collection in urethane anaesthesia)						
5	320	314	11–14	86***±5	29·2±3·5	152**±19	Engorged with lipids
11	(Controls)	332	—	63±2	25·4±1·5	96±9	—
	(*b*) 1 i.u. long-action ACTH† twice daily during 4 days. (Blood collection in pentobarbitone anaesthesia)						
7	297	298	8	88***±7	28·3±3·3	145*±8	Lipids abundant
26	(Controls)	326	—	66±2	29·1±1·6	115±7	—
	(*c*) 12 i.u. soluble ACTH and 4 i.u. long-action ACTH† injected within a period of 36 hr. (Blood collection in urethane anaesthesia)						
2	322	317	6‡	75±2	20·0±0·05	92±4	Loss of lipids
	(*d*) 24 i.u. soluble ACTH and 20 i.u. long-action ACTH† injected within a period of 36 hr. (Blood collection in urethane anaesthesia)						
6	353	335	6‡	105***±3	16·8***±2·2	106±14	—
11	(Controls for *c* and *d*)	332	—	63±2	25·4±1·5	96±9	—
	(*e*) 24 i.u. soluble ACTH and 20 (5 rats) or 30 (2 rats) i.u. long-action ACTH† injected within a period of 36 hr. (Blood collection in urethane anaesthesia)						
7	307	301	6–8‡	117***±6	21·6***±1·4	150***±9	Severe loss of lipids
6	(Controls)	316	—	59±3	29·6±1·1	104±5	—

All observations are given as mean ±S.E. of the mean.

* Significant ($P<0·05$); ** significant ($P<0·02$); *** significant ($P<0·01$); † 'Cortrophin Z' Organon; ‡ injection schedule see under 'Methods'.

In each instance there is a significant decrease in corticosterone secretion per unit weight of tissue. The total secretion per gland is unchanged in group *d* and increased in group *e*. Lipid loss was severer than with the smaller doses of ACTH.

The results demonstrate that, in the early phase of the adrenal response to ACTH, hypertrophy of the gland leads to reduced functional capacity per gram of tissue. This is probably due to the fact that, under the stimulus of

ACTH, synthesis of the enzymes required for the production of cortical hormones does not keep pace with the formation of the highly vascular new tissue mass. Whether such an adrenal produces more hormone under conditions of stress than its normal counterpart will depend on how rapidly the new tissue becomes fully functional. This process was obviously in progress in some rats of group *d*, and in all rats of group *e*.

Kass, Hechter, Macchi & Mou (1954) have reported changes in the proportion of cortical steroids in rabbits given prolonged treatment with ACTH. Under the influence of this stimulus, rabbit adrenals which do not normally secrete cortisol, secrete this compound as the main adrenal steroid. No such effect was seen in the rats. The cortisol region of the chromatogram was examined in many rats which had been given ACTH for one or two weeks, but cortisol was not found, nor did the sodium fluorescence test (Bush, 1953*a*) reveal any other $\alpha\beta$-unsaturated keto-steroids not normally present in adrenal effluent. There must be fundamental differences in the hydroxylating enzyme systems from adrenals of rats and rabbits; Hofman (1957) made the interesting suggestion that the absence of *in vivo* 17-hydroxylation in the rat is not due to lack of 17-hydroxylase but to 'over-abundance of 11 β-hydroxylase'; once a compound is hydroxylated in the 11β-position, it is no longer attacked by 17-hydroxylase.

The last experiments have shown that an adrenal endowed with increased total secretory capacity can be produced by 36 hr intensive treatment with ACTH. In previous work (Vogt, 1957), much larger increases in hormone secretion during stress had been obtained when amphenone (1, 2-bis(*p*-aminophenyl)-2-methylpropan-1-one) was fed to rats in a daily dose of 0·2 g/kg for a period of as little as 4 days. This drug, which had been investigated by Hertz and his collaborators (see Hertz, Tullner, Schricker, Dhyse & Hallman, 1955) was shown by these workers to cause an excessive deposition of cholesterol in the adrenal cortex. The question arose whether a release of ACTH by amphenone would be sufficient to account for these rapid increases in cholesterol deposition and secretory power. Since very large doses of ACTH given over 36 hr did not produce comparable effects, smaller doses (1 i.u. per injection) of long-action ACTH were injected twice daily during 4 days, on the assumption that this dosage would be more likely to imitate the endogenous production of ACTH induced by amphenone.

Of the seven rats used for this experiment, three were given, in addition to the ACTH, a daily injection of arachis oil. This might have acted both as a stress and as a source of unsaturated fatty acids and thus have favoured cholesterol synthesis. The effects on adrenal size and performance and on cholesterol deposition were, however, identical with those seen in the rats given ACTH only, so that for the present purpose the rats (Table 3*b*) are treated as one group. The effects observed were an increase of 26 % in corticosterone produc-

tion per whole gland, and an adrenal hypertrophy of 30%, very feeble effects when compared with the rise in corticosterone secretion of 114% and in adrenal weight of 60% obtained after 4 days of amphenone. In contrast to the loss of lipid when ACTH had been given in very large doses, accumulation of lipids followed this milder treatment; in this respect, too, the ACTH produced but an attenuated replica of the effect of amphenone.

Secretion of demedullated adrenals; effect of adrenaline infusions

The acceleration of cortical secretion by infusions of adrenaline in the rat is considered to be mediated by a release of ACTH. Under the conditions of renal vein cannulation there is bound to be stimulation of the adrenal medulla, and therefore any effect of additional, infused adrenaline would of necessity be masked. Experiments to test whether infusions of adrenaline would give the same results as infusions of ACTH, i.e. mild stimulation in instances of low initial hormone secretion, had thus to be carried out on demedullated or denervated adrenals. Demedullation was chosen, and carried out by enucleation of the glands which were then allowed to regenerate from a thin lining of capsular cells; thus information was simultaneously obtained on the functional capacity of adrenal regenerates.

The time allowed for regeneration of the cortex was 3 months or more, except for three rats examined after a lapse of 4–6 weeks. The results were the same for all time intervals. A number of the bilaterally operated rats proved unsuitable, because the right adrenal had grown much larger than the left adrenal from which the adrenal vein blood was being collected. In the last two animals, therefore, the right gland was extirpated and only the left gland allowed to regenerate. Figures obtained from very small glands were discarded, since estimates of weight of cortical tissue and of secretion of hormone were subject to large errors; the smallest gland the yield of which is included in the calculations weighed 9·4 mg. In any case, comparison of secretion from the regenerated glands with that of normal glands on a weight basis is subject to small errors caused by the lack of medullary tissue on the one hand and some degree of scar formation on the other.

In spite of a large scatter of adrenal weights, secretion per gram of demedullated gland lay nearly within the normal range. There was no correlation, not even a negative one, between adrenal weight and rate of secretion per gram of tissue. Owing to the fact that most adrenals were smaller than normal, mean secretion per whole gland was only 44% of the control value, but there was overlap between operated and normal animals (Table 4). As was to be expected, total secretion usually increased with the size of the gland, but there was no strict parallelism between the two sets of figures. Histologically, adrenal lipids showed a normal distribution; medullary tissue was never found.

Infusion of adrenaline (approximately 5 μg given over 8 min into the femoral

TABLE 4. Effect of demedullation and of adrenaline infusions on corticosterone secretion by rat adrenals

	No. of rats	Body wt. (g)	Weight of left adrenal (mg)	Weight of left adrenal (mg/kg body wt.)	Corticosterone secreted (μg/g gland/min) S_1	Corticosterone secreted (μg/g gland/min) S_2	Corticosterone secreted (μg/g gland/min) Change (%)	Corticosterone secreted (μg/gland/kg body wt./hr)
Means								
Demedullated	10	413	14·9	36	30·8	30·5	−1	64
Controls	26	326	21·3	66	29·1	—	—	115
Ranges								
Demedullated	10	390–450	9·4–22·8	21–57	20·0–45·0	20·5–41·0	−22 to +39	37–93
Controls	26	262–374	16·2–28·6	43–82	20·1–42·2	—	—	63–215

Blood collection in pentobarbitone anaesthesia. S_1, control sample of adrenal blood (15 min); S_2, sample collected during the last 4 min of, and for 11 min after, an infusion of approximately 5 μg adrenaline; infusions into the femoral vein in eight, into the carotid artery in two rats. Rat blood infused whenever required to keep the blood pressure steady.

vein of eight, and into the carotid artery of two rats) produced no change in the mean rate of the secretion (S_1 and S_2, Table 4).

In contrast to the experiments in which ACTH was infused, only two of the six glands with secretion rates below the mean increased their corticosterone production after the infusion. It is likely that, owing to the smaller mass of cortical tissue, secretion from most of these glands was maximally stimulated from the start. With a view to increasing the concentration of adrenaline in the brain and the pituitary, in two of the rats the adrenaline was injected into the carotid artery. Corticosterone secretion was not affected.

DISCUSSION

The observation that ACTH given intravenously during the collection of adrenal vein blood did not consistently increase corticosterone secretion agrees with Bush's (1953*b*) findings. The fact that only rats with a low initial secretion showed a (moderate) acceleration of secretion indicates that pituitary stimulation by the stress of the experiment was maximal in some, and not very far from maximal in the remaining rats. Stimulation by ACTH from a genuine 'resting' level increases secretion by a much greater factor than that of 1·5 observed in the rats subjected to renal vein cannulation. Thus secretion as measured in such experiments corresponds to maximal or near-maximal secretory capacity of the adrenal cortex.

This secretory capacity can be increased by measures which cause adrenal hypertrophy, provided this hypertrophy does not at the same time effect changes in the tissue which reduce its secretory capacity per unit of tissue mass. Increases in total secretory capacity by the production of more, fully functional, tissue were obtained in the present work by daily administration of moderate doses of ACTH for not less than 4 days. Still greater increases, reported elsewhere, occur when amphenone is fed to rats. In both instances lipid stores rise with the increase in glandular weight. Attempts at producing, with various dosages of ACTH, effects comparable in size and speed with those elicited by amphenone were unsuccessful.

These findings do not prove that the effects of amphenone cannot be due exclusively to the release of ACTH, particularly in view of the fact that there may be differences between pig hormone and rat hormone. However, they do at least suggest that amphenone may have additional modes of action. There may be a direct influence of amphenone on the metabolism of the adrenal cortex, or the action on the anterior pituitary may be complex and involve the secretion of growth hormone.

Decrease in total secretory capacity was seen in undersized glands which had regenerated from the capsules after adrenal enucleation; in such adrenals performance per gram of gland was unchanged. A fall in secretory capacity

per gram of gland was obtained by causing precipitous hypertrophy of the cortex with huge doses of ACTH administered during the course of 36 hr. The concentration of lipids was reduced by this treatment; the total secretory potential was never reduced, and often raised, so that such glands did not bestow a disadvantage on their bearer. An extreme example of decreased secretory capacity per gram of gland, in which total secretion per adrenal was reduced also, has been reported to result from treatment with hexoestrol (Vogt, 1955). Here loss of lipid was much more severe than after intensive treatment with ACTH.

This survey suggests that large stores of lipids characterize fully functional adrenal tissue, whereas lipid depletion, if severe, may indicate a reduction of the output obtainable on maximal stimulation. This does not imply that lipid content bears any relation to the amount of corticoids actually secreted in the living animal, as this is rarely maximal: in fact, there is evidence that any acceleration of secretion is accompanied by an initial decrease in lipid stores.

SUMMARY

1. When ACTH is infused intravenously into rats from which adrenal vein blood is being collected from the cannulated left renal vein, little change is effected in the secretion of corticosterone. Adrenals with a low initial secretion show a rise averaging 50%, whereas adrenals with high initial secretion do not change their output. It follows that the secretion measured under these conditions represents the maximal or near-maximal effort of which the tissue is capable. All the observations referred to below apply exclusively to the accelerated secretion brought about by the operative stress.

2. Prolonged treatment with moderate doses of ACTH (2 i.u. daily for at least 4 days, or 1 i.u. daily for at least 11 days) produces no change in the corticosterone secretion per gram of tissue. It does, however, by causing enlargement of the gland, increase the hormone production per gland.

3. Large doses of ACTH given within a short time (16–54 i.u. in 36 hr) cause a fall in secretory capacity per gram of gland. There is either no change or some increase in secretion per whole gland. The precipitously formed new tissue is obviously not immediately fully functional.

4. Adrenals regenerated from their capsule after enucleation exhibit normal corticosterone output per gram of tissue, but secretion per gland is reduced as a result of the small size of the adrenals.

5. When adrenaline was infused intravenously into rats bearing regenerated adrenals, secretion was not affected.

6. When the rate of corticosterone secretion per gram of gland is compared with the lipid stores of the tissue, lipid-rich glands are found to have a high, and lipid-depleted glands a low secretory potential in conditions of maximal stimulation.

We are grateful to Organon Laboratories Ltd., for a generous supply of soluble ACTH and of long-action 'Cortrophin Z', obtained through the courtesy of Dr J. Dekanski, and to Armour and Company who supplied a batch of the soluble ACTH obtained from the Medical Research Council. Our thanks are also due to the Medical Research Council for a grant (to M. V.) defraying part of the expenses of this work. The work was done during the tenure by M. H. of an I.C.I. Fellowship.

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Note added in proof. According to a recent paper by Hoet, Renold, Hertz & Thorn (1957), the formula of amphenone is now considered to be 3,3-di(*p*-aminophenyl)butan-2-one dihydrochloride.

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