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EFFECTS OF SINGLE AND REPEATED DOSES OF RESERPINE ON THE SECRETION OF ADRENOCORTICOTROPHIC HORMONE

BY

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The effects of single and repeated doses of reserpine on the secretion of adrenocorticotrophic hormone (ACTH) were studied in rats by determining changes in the adrenal and plasma concentrations of corticosterone, in the adrenal ascorbic acid levels, and in the adrenal gland weights. Treatment with doses of reserpine which induced sedation caused prolonged hypersecretion of ACTH. Such treatment did not impair the release of ACTH in response to a stressful stimulus as recorded by an increase in the plasma corticosterone even when there was no concomitant depletion in the adrenal ascorbic acid. This finding casts doubt on the value of the adrenal ascorbic acid determination as an index of ACTH secretion.

A single injection of reserpine promotes the release of ACTH from the pituitary gland as shown by the findings that both adrenocortical activation (Wells, Briggs & Munson, 1956; Kitay, Holub & Jailer, 1959; Khazan, Sulman & Winnik, 1961; Maickel, Westermann & Brodie, 1961) and depletion of ACTH stores in the hypophysis (Kitay *et al.*, 1959; Saffran & Vogt, 1960; Maickel *et al.*, 1961) have been reported. According to Maickel *et al.* (1961), reserpine produces these effects by depressing inhibitory pathways in the hypothalamus which control the secretion of a humoral factor influencing ACTH discharge. Other workers, however, have reached the conclusion that it is simply a non-specific stressing action of reserpine which accounts for these changes (Saffran & Vogt, 1960; Khazan *et al.*, 1961).

Chronic treatment with reserpine has been shown to produce adrenal hypertrophy as a result of hypersecretion of ACTH (Gaunt, Renzi, Antonchak, Miller & Gilman, 1954; Hertting & Hornykiewicz, 1957). It has also been reported that chronic treatment with reserpine prevents the ether stimulus from lowering the adrenal ascorbic acid. This latter effect has been ascribed either to an inhibitory effect of reserpine on the pituitary (Wells *et al.*, 1956) or to the exhaustion of the ACTH stores in the gland (Kitay *et al.*, 1959).

The present experiments were undertaken to study this problem further by determining in rats the effects of reserpine on ACTH release, as indicated by changes

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in the adrenal and plasma concentrations of corticosterone, changes in the adrenal ascorbic acid concentrations, and changes in the adrenal weights. Corticosterone is the major corticosteroid secreted by the adrenal glands of rats (Bush, 1953).

METHODS

Male Wistar rats (weighing 150 to 210 g) housed at 23° C in single screened cages were used throughout. They were allowed food and water *ad lib.* and were left undisturbed for 24 hr before treatment. After treatment, they were returned to the single cages until the completion of the experiment.

Hypophysectomy. Hypophysectomy was carried out using the parapharyngeal approach, and the rats were used 24 hr later.

Sampling of blood. Blood was collected from the abdominal aorta under light ether anaesthesia. All the samplings were carried out in the animal house within 2 min of opening the cages. The time of collection was fixed between 9.30 a.m. and 11 a.m.

Removal of adrenal glands. Rats were decapitated under light ether anaesthesia within 1 min of opening the cages, and their adrenal glands were removed, cleaned and weighed.

Ether stimulus. The ether stimulus was applied for 60 sec, and, after 30 min (for corticosterone) or 60 min (for ascorbic acid), the rats were sacrificed under light ether anaesthesia. Such a stimulus has been shown by Sayers & Burks (1955) to release ACTH from the pituitary.

Measurement of corticosterone. The plasma corticosterone was determined fluorimetrically using 1.5 ml. samples according to the method of Zenker & Bernstein (1958). The adrenal corticosterone was similarly determined after allowing the weighed glands to stand in absolute ethanol (1 ml.) for 24 hr at 0° C before grinding and adding 4 ml. distilled water. Both determinations were made on the Aminco-Bowman spectrophotofluorimeter.

Measurement of ascorbic acid. The ascorbic acid concentration in the adrenal gland was determined by the method of Roe (1954).

Reserpine solution. Ampoules of Serpasil (Ciba) containing 2.5 mg/ml. were used. Weaker solutions were made by diluting this liquid with distilled water. Reserpine was given intraperitoneally, control animals receiving the corresponding volume of the vehicle (Ciba).

Corticosterone. Corticosterone (Free Alcohol) was generously supplied by Dr Tindall, of Organon Laboratories, London.

RESULTS

Single dose of reserpine (2.5 mg/kg)

Effect on corticosterone. After reserpine, the plasma corticosterone steadily increased to the high level of about 28 μg/100 ml. in 1 hr. This level was maintained for the next 15 hr and then decreased, but even at 24 hr it was significantly higher than the resting value obtained from 34 observations, namely, 6.2 ± 0.3 μg/100 ml. (see Fig. 1). After corresponding injections of vehicle, the plasma corticosterone also rose to about 28 μg/100 ml. in 1 hr, but returned to the resting value by 4 hr. The resting adrenal corticosterone value obtained from 41 observations was 13.5 ± 0.1 μg/g gland (see Fig. 1). However, the increase in the adrenal corticosterone after reserpine was less marked than that in the plasma, and standard errors of each value were large. After corresponding injections of vehicle, the adrenal corticosterone was slightly raised during the first hour and then returned to the resting level. The adrenal glands increased in weight by about 25%, and this was accompanied by a large loss in body weight (Table 1).

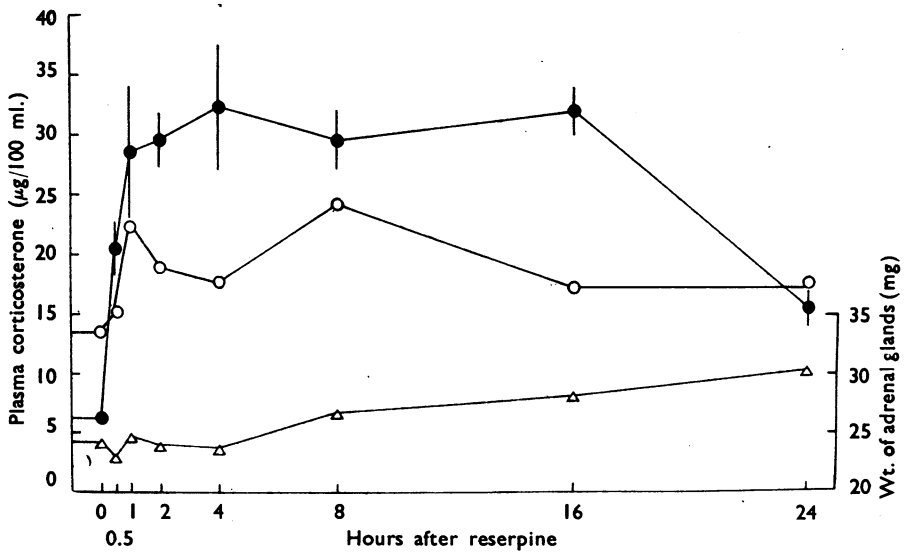

Fig. 1. Effect of reserpine (2.5 mg/kg) on the plasma (μg/100 ml., ●——●) and adrenal (μg/g, ○——○) corticosterone and on the weight of the adrenal glands (mg., △——△) of rats. Vertical lines represent the standard errors of the plasma corticosterone values. Resting values represent the mean of at least 34 animals. Each point on the curves is the mean of 6 to 20 animals.

TABLE 1

EFFECT OF DAILY DOSES OF RESERPINE ON BODY WEIGHT AND ADRENAL WEIGHT, MEASURED 24 HR AFTER THE LAST INJECTION

* Mean of 41 animals. † Mean of 40 animals.

Dose mg/kg	Length of treatment (days)	No. of rats	Mean daily change in body weight (g)	Mean adrenal weight (±s.e.) (mg)
0	10	16	+3·6	24·1±0·3*
0·1	10	16	+3·6	24·2±0·8
0·5	5	26	−1·3	30·0±1·0
0·5	9	14	−2·6	30·0±1·3
2·5	1	13	−17·1†	30·0±0·9
2·5	4	20	−12·0	32·8±0·9
(Vehicle)	10	16	+4·1	23·6±1·7

To explore the role played by the pituitary in producing these responses, the corticosterone levels were next measured in hypophysectomized rats.

Reserpine after hypophysectomy. The plasma and adrenal corticosterone values of hypophysectomized rats were 4.4±0.4 μg/100 ml. and 9.8±0.07 μg/g respectively. After reserpine, these were 5.0±0.3 μg/100 ml. and 9.9±0.5 μg/g. This shows that reserpine induces adrenal corticoid output only when the pituitary-adrenal axis is intact.

Ether stimulus after reserpine. The decrease in corticosterone level 16 hr after reserpine may be due to a reduction in its stimulating action on the hypophysis or to an exhaustion of ACTH in the gland (Kitay *et al.*, 1959 ; Maickel *et al.*, 1961). This was tested by applying the ether stimulus to the animals, 24 hr after reserpine.

The plasma corticosterone was significantly raised, the level reached being similar to that found after the ether stimulus had been applied to rats not previously injected with reserpine (Fig. 2). Thus, 24 hr after a single dose of reserpine, there is no impairment in the ACTH release, as recorded by an increase in the plasma corticosterone level.

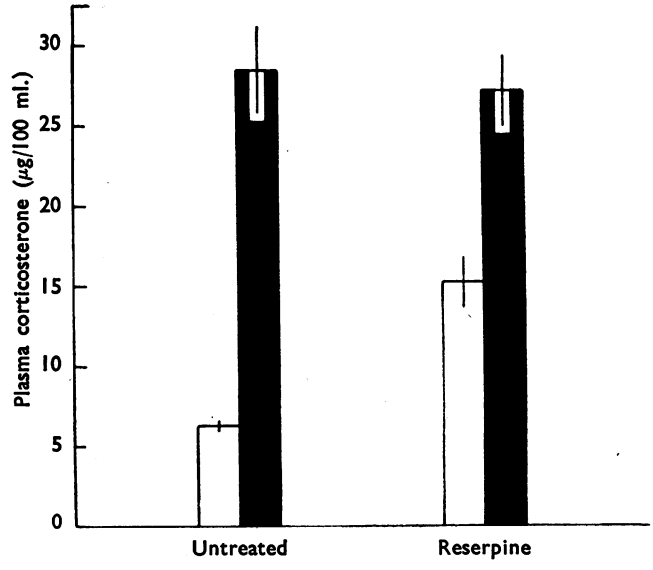

Fig. 2. Plasma corticosterone of untreated rats and rats receiving reserpine (2.5 mg/kg) 24 hr previously, before (open columns) and after (filled columns) the ether stimulus. Each value represents the mean (±s.e.) of 6 to 11 animals.

Repeated doses of reserpine

First experiment. Rats received 0.1 mg/kg of reserpine daily for 10 days ; 24 hr after the last dose their plasma and adrenal corticosterone concentrations were found to be similar to those of rats receiving 10 doses of vehicle and to those of rats handled daily but not injected (Table 2). Other groups of rats which had been similarly treated were subjected to the ether stimulus at this time before being

TABLE 2.

EFFECT OF RESERPINE (0.1 MG/KG FOR 10 DAYS) ON THE PLASMA (μG/100 ML.) AND ADRENAL (μG/G) CORTICOSTERONE, BEFORE AND AFTER THE ETHER STIMULUS. THE EFFECTS OF RESERPINE VEHICLE AND OF SIMPLY HANDLING THE RATS ARE ALSO SHOWN. EACH VALUE REPRESENTS THE MEAN (±S.E.) OBTAINED FROM 7 TO 8 ANIMALS

	Corticosterone concentration			
	Plasma		Adrenal glands	
Treatment	Before	After	Before	After
Reserpine	8·8±1·0	34·2±3·0	12·3±1·2	27·2±2·7
Vehicle	9·3±1·5	30·2±1·8	10·3±1·3	27·9±5·5
Handled daily	6·6±0·5	26·8±3·7	10·8±0·5	14·4±2·2

sacrificed. The plasma corticosterone was similarly raised in the three groups, but the increase in the adrenal corticosterone of the animals receiving injections was significantly greater than that in the rats handled daily (Table 2).

During this treatment no signs of the reserpine syndrome (sedation, blepharospasm, diarrhoea) were observed in these animals. Reserpine at this dose level neither affected the normal rate of growth nor the adrenal weight (Table 1). The last findings contrast with those of Gaunt *et al.* (1954), but these authors used the same dose for 14 days. The results indicate that this dose of reserpine neither induces ACTH hypersecretion nor does it impair the response of the pituitary-adrenal system to stress.

Second experiment. Rats received 0.5 mg/kg of reserpine daily for 5 days; 24 hr after the last dose their plasma corticosterone concentrations were significantly higher than those of rats which were handled daily but not injected (Table 3). The

TABLE 3

EFFECT OF RESERPINE (0.5 MG/KG FOR 5 DAYS) ON THE PLASMA (μG/100 ML.) AND ADRENAL (μG/G) CORTICOSTERONE, BEFORE AND AFTER THE ETHER STIMULUS. THE EFFECT OF HANDLING THE RATS DAILY IS ALSO SHOWN. EACH VALUE REPRESENTS THE MEAN (±S.E.) OBTAINED FROM 6 TO 8 ANIMALS

	Corticosterone concentration			
	Plasma		Adrenal gland	
Treatment	Before	After	Before	After
Reserpine	16·0±4·2	49·3±6·1	12·4±1·0	19·7±1·8
Handled daily	6·4±0·7	27·2±1·8	11·3±0·5	15·1±1·0

adrenal corticosterone was not increased, nor was there any change in the adrenal ascorbic acid (Table 4). Other groups of rats which had been similarly treated were subjected to the ether stimulus at this time before being sacrificed. The plasma corticosterone was further increased and greatly exceeded that of rats in the control group (that is, those handled daily). There was also an increase in the adrenal corticosterone (Table 3). The adrenal ascorbic acid depletion was less pronounced than in the control group (Table 4). During this treatment the animals showed signs of the reserpine syndrome. There was a loss of body weight and adrenal weight increased by about 25% (Table 1).

TABLE 4

EFFECT OF DAILY DOSES OF RESERPINE ON THE MEAN (±S.E.) ADRENAL ASCORBIC ACID CONCENTRATIONS (MG/100 G), BEFORE AND AFTER THE ETHER STIMULUS

Dose (mg/kg)	Length of treatment (days)	No. of rats	Mean adrenal ascorbic acid		Depletion (%)
			Before	After	
0	10	9	434±13	282±7	35
0·5	5	7	464±11	346±8	25
Vehicle only	5	3	471±17	294±5	38
0·5	9	6	368±13	340±13	8
Vehicle only	9	3	424±10	281±12	34
2·5	4	10	310±9	294±11	5
Vehicle only	4	6	416±12	276±9	34

Third experiment. The small dose of reserpine (0.5 mg/kg) was next given to rats for a longer period, namely, 9 days. Twenty-four hours after the last dose their plasma corticosterone (14.8 ± 3.7 μg/100 ml.) was significantly higher than the resting value. At this time the adrenal ascorbic acid concentration was significantly lower than that of the control group (Table 4). Other groups of rats which had been similarly treated were then subjected to the ether stimulus, but no further fall in the adrenal ascorbic acid concentration was observed (Table 4).

Fourth experiment. Kitay *et al.* (1959), Saffran & Vogt (1960), and Maickel *et al.* (1961) have reported that large doses of reserpine deplete the ACTH stores in the pituitary, and it was of interest to determine the effects of repeated large doses both on the adrenal ascorbic acid concentration and on the plasma corticosterone, before and after the ether stimulus. Rats were therefore given 2.5 mg/kg of reserpine

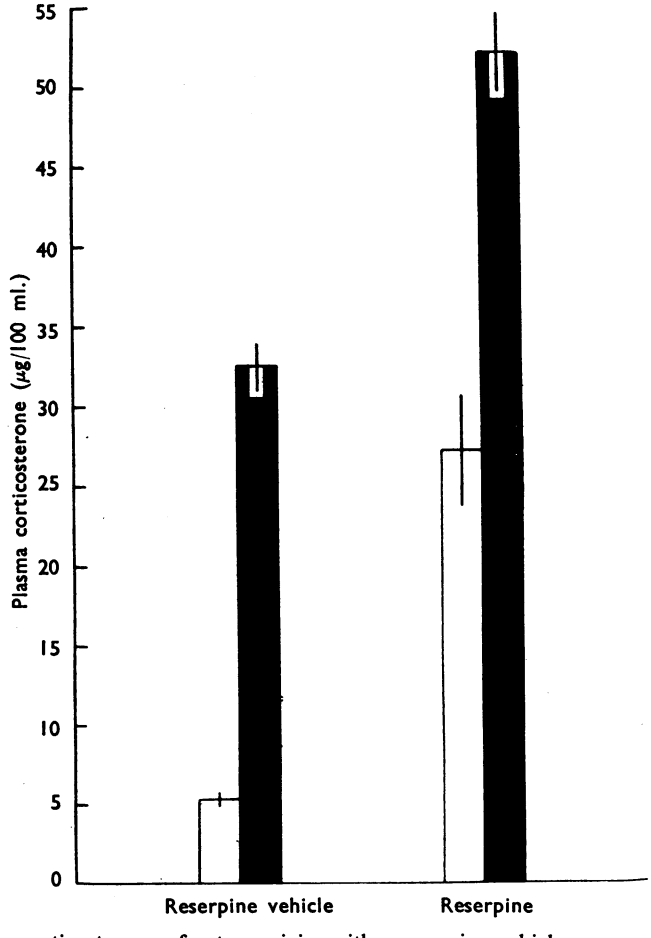

Fig. 3. Plasma corticosterone of rats receiving either reserpine vehicle or reserpine (2.5 mg/kg) for 4 days before (open columns) and after (filled columns) the ether stimulus. Each value represents the mean (±s.e.) of 7 to 10 animals.

daily for 4 days and used 24 hr after the last injection, by which time there was a marked loss in body weight (Table 1). The adrenal glands of these animals increased in weight by about 35% during this treatment (Table 1). Their plasma corticosterone (27.3 ± 3.4 μg/100 ml.) was significantly increased and the adrenal ascorbic acid level (310 ± 9 mg/100 g) was markedly lower than the control value. After the ether stimulus, however, there was a significant increase in the plasma corticosterone (Fig. 3) but no further depletion in the adrenal ascorbic acid (Table 4).

DISCUSSION

The present results show that reserpine, in a single dose of 2.5 mg/kg, raises the plasma and adrenal corticosterone and causes an increase in the weight of the adrenal glands. Since these effects do not occur in hypophysectomized rats, it can be assumed that they are a consequence of ACTH discharge from the pituitary. The plasma corticosterone rises to a high value 1 hr after reserpine and even after 24 hr is still above the resting level; furthermore, the weight of the adrenal glands at this time has increased by about 25%. These changes are indicative of persistent and prolonged ACTH release, but this effect is unlikely to be the result of circulating reserpine, since Shore *et al.* (1957) have shown that reserpine is no longer detectable after a few hours. Recently, Maickel *et al.* (1961) reported that the plasma corticosterone had returned to the resting value 20 hr after reserpine. However, these authors used a different dose of reserpine and their injections were made intravenously; furthermore, their resting value (15 μg/100 ml. plasma) was considerably higher than that found in the present study.

When stress, such as an ether stimulus, was applied to the animals 24 hr after a single dose of reserpine, a significant rise in the plasma corticosterone was obtained, the value reached being of a similar order to that found when untreated rats were given the ether stimulus. Thus, whether or not ACTH depletion occurs as a result of reserpine treatment, there is still enough to be released in response to an acute stimulus. This result is in contrast with that obtained by Maickel *et al.* (1961), who found that a stressful stimulus did not raise the plasma corticosterone in rats given a single dose of reserpine 20 hr previously.

The present results also show that reserpine causes ACTH release only when given in doses which induce sedation and loss in body weight. For example, 0.1 mg/kg for 10 days produced no overt sedation or loss in body weight and there was no increase in the basal plasma corticosterone level or adrenal gland weight. On the other hand, 0.5 mg/kg for 5 or 9 days and 2.5 mg/kg for 4 days induced marked sedation and loss in body weight, together with a raised plasma corticosterone and an enlarged adrenal gland. A similar enlargement of the adrenal gland has been reported by Gaunt *et al.* (1954), Hertting *et al.* (1957), and Kitay *et al.* (1959), and contrasts with the result of Wells *et al.* (1956). When the ether stimulus was applied to animals receiving the higher daily doses of reserpine there was a marked rise in the plasma corticosterone (to about 50 μg/100 ml.), indicating that enough ACTH was released to increase the output of adrenal corticoid. This result shows that, whatever depletion of ACTH stores in the pituitary has occurred as a result of prolonged reserpine treatment, it is possible to obtain the release of additional ACTH in response to an acute stimulus such as ether.

There is disagreement in the literature concerning the effect of a single dose of reserpine on the level of ascorbic acid in the adrenal gland. Although all workers agree that depletion occurs, recovery time differs. Kitay *et al.* (1959), for example, found a 35% depletion in the adrenal ascorbic acid even 24 hr after a single dose of reserpine, whereas Maickel *et al.* (1961) reported that the ascorbic acid level had almost returned to the basal value by this time. This has not been studied in the present experiments, in which only the effect of repeated doses of reserpine on the adrenal ascorbic acid has been followed. The concentration of adrenal ascorbic acid did not differ from the resting value when measured 24 hr after the last of five doses of 0.5 mg/kg, and the ether stimulus then produced a 25% depletion. Doses of 2.5 mg/kg themselves produced about 25% depletion of adrenal ascorbic acid and the ether stimulus was then ineffective. Wells *et al.* (1956) and Kitay *et al.* (1959) have already reported that reserpine treatment prevents the ether stimulus from lowering the adrenal ascorbic acid, but these authors did not estimate plasma corticosterone values.

It has been found in the present study that changes can occur in the plasma corticosterone level unaccompanied by modification of the adrenal ascorbic acid concentration. This casts doubt on the value of adrenal ascorbic acid determinations as an index of ACTH secretion, and is in agreement with conclusions of some previous workers (Slusher & Roberts, 1957 ; Schönbaum, Casselman & Large, 1959). Recently, Rerup & Hedner (1961) compared the depletion of adrenal ascorbic acid, after ACTH injection, with the rise in plasma corticosterone, using the same animals, and found that the plasma corticosterone method was more sensitive and more specific. It now seems to be a more reliable method, too, and indicates that reserpine treatment, whilst itself inducing prolonged hypersecretion of ACTH, does not impair the discharge of further ACTH in response to acute stress.

Finally, the low resting plasma corticosterone concentration in the present experiments (6.2 ± 0.3 μg/ml.) may account for the observed longer duration of reserpine action on ACTH release than that obtained by Maickel *et al.* (1961). Our animals were kept quiet in single screened cages for at least 24 hr and sacrificed only at a specified time of the day in order to avoid diurnal variations in adrenal activity (McCarthy, Corley & Zarrow, 1960). The low resting level of plasma corticosterone and the different strain of rats may account for some of the differences between previous results and those reported in this paper.

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REFERENCES

BUSH, I. E. (1953). Species difference in adrenocortical secretion. *J. Endocrin.*, **9**, 95–100.

GAUNT, R., RENZI, A. A., ANTONCHAK, N., MILLER, G. J. & GILMAN, M. (1954). Endocrine aspects of the pharmacology of reserpine. *Ann. N.Y. Acad. Sci.*, **59**, 22–35.

HERTTING, G. & HORNYKIEWICZ, O. (1957). Beeinflussung der durch Reserpin hervorgerufenen Nebennierenrindenhypertrophie durch Cortison. *Acta Endocrinol.*, **26**, 204–208.

KHAZAN, N., SULMAN, F. G. & WINNIK, H. Z. (1961). Activity of pituitary-adrenal cortex axis during acute and chronic reserpine treatment. *Proc. Soc. exp. Biol. N.Y.*, **106**, 579–581.

KITAY, J. I., HOLUB, D. A. & JAILER, J. W. (1959). "Inhibition" of pituitary ACTH release after administration of reserpine or epinephrine. *Endocrinology*, **65**, 548–554.

McCARTHY, J. L., CORLEY, R. C. & ZARROW, M. X. (1960). Diurnal rhythm in plasma corticosterone and lack of diurnal rhythm in plasma compound, F-like material in the rat. *Proc. Soc. exp. Biol. N.Y.*, **104**, 787–789.

MAICKEL, R. P., WESTERMANN, E. O. & BRODIE, B. B. (1961). Effects of reserpine and cold-exposure on pituitary-adrenocortical function in rats. *J. Pharmacol. exp. Ther.*, **134**, 167–175.

RERUP, C. & HEDNER, P. (1961). On the assay of corticotrophin: comparison of the plasma corticosteroid and adrenal ascorbic acid response. *Acta Endocrinol.*, **38**, 220–235.

ROE, J. H. (1954). Chemical determination of ascorbic, dehydroascorbic, and diketogulonic acids. *Methods of Biochemical Analysis*, **1**, 115–139. Interscience Publ., N.Y.

SAFFRAN, M. & VOGT, M. (1960). Depletion of pituitary corticotrophin by reserpine and by a nitrogen mustard. *Brit. J. Pharmacol.*, **15**, 165–169.

SAYERS, G. & BURKS, R. (1955). Blood A.C.T.H. during ether anaesthesia. *Fed. Proc.*, **14**, 131.

SCHÖNBAUM, E., CASSELMAN, W. G. B. & LARGE, R. E. (1959). Studies on the time course of the response of the adrenal cortex to histamine and cold. *Can. J. Biochem. Physiol.*, **37**, 399–404.

SHORE, P. A., PLETSCHER, A., TOMICH, E. G., CARLSSON, A., KUNTZMAN, R. & BRODIE, B. B. (1957). Role of serotonin in reserpine action. *Ann. N.Y. Acad. Sci.*, **66**, 609–617.

SLUSHER, M. A. & ROBERTS, S. (1957). Fate of adrenal ascorbic acid: relationship to corticosteroid secretion. *Endocrinology*, **61**, 98–105.

WELLS, H., BRIGGS, F. N. & MUNSON, P. L. (1956). The inhibitory effect of reserpine on ACTH secretion in response to stressful stimuli. *Endocrinology*, **59**, 571–579.

ZENKER, N. & BERNSTEIN, D. E. (1958). The estimation of small amounts of corticosterone in rat plasma. *J. biol. Chem.*, **231**, 695–701.