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# THE EFFECT OF DESOXYCORTICOSTERONE ACETATE ON THE RELEASE OF ADRENOCORTICOTROPHIN BY THE PITUITARY GLAND

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The results of experiments performed in many laboratories indicate that the adrenocorticotrophic activity of the pituitary gland is controlled, to some extent, by the level of adrenocortical hormones in the blood. Many workers have shown that the chronic administration of adrenocortical extracts to rats results in atrophy of the adrenal cortex. Sayers and Sayers (1947) and Long (1947) demonstrated, in rats, that the fall in adrenal ascorbic acid concentration, which normally follows the application of various types of stress stimuli, could be prevented by previous treatment of the animals with adrenocortical hormones.

Sayers and Sayers (1947) found that several crystalline cortical steroids, including desoxycorticosterone, were effective in inhibiting pituitary adrenocorticotrophic activity. However, Moya and Selye (1948), Hall, Finerty, Hall, and Hess (1951), and Gershberg, Fry, Brobeck, and Long (1950) did not find that DOCA prevents the release of ACTH in response to stress.

The experiments which form the subject of this paper were carried out to re-examine the effect of DOCA on the stress-induced secretion of ACTH and to determine whether this steroid possesses any pituitary inhibitory effect. Changes in the concentration of ascorbic acid in the adrenal glands were used as indices of increased adrenocortical, and hence of adrenocorticotrophic, activity. The form of stress employed was unilateral adrenalectomy under ether anaesthesia.

## MATERIALS AND METHODS

The experiments were performed on male albino Wistar rats, weighing from 130 to 250 g. and maintained on a diet of cubes, cabbage, and water.

Twenty-four hours before being used the rats were transferred to a room in which the temperature was thermostatically controlled at 70° F. and where the experiments were performed. During the course of the experiments the animals were given access to food and water.

*DOCA.*—Deoxycortone acetate, *B.P.* (Organon), was used. Stock solutions were prepared containing 5, 10, 15, and 20 mg. DOCA per ml. in arachis oil. The DOCA tended to crystallize out from the solutions of highest concentration, when the solutions were allowed to stand, but the steroid redissolved readily on heating. The injections of DOCA were administered in volumes of 1 ml. per 100 g. body weight of the animals. The solutions were warmed to 37–40° C. before injection to facilitate administration and to keep the highest concentration of DOCA in solution.

*Removal and Analysis of Adrenal Glands*

The removal of adrenal glands from dead or anaesthetized animals was carried out by the dorsal approach. Excised adrenal glands were dissected free from fat and connective tissue with a fine pair of scissors. Care was taken to remove all traces of extra-adrenal tissue without damaging the capsule. The glands were weighed as quickly as possible to minimize the loss of moisture, and placed in test-tubes containing a little acid-washed sand and 4% trichloracetic acid solution. They were minced with the aid of a glass rod and the sand, and the tubes were stored in a refrigerator overnight. The ascorbic acid contents of the tubes were determined on the following day by the method of Roe and Kuether (1943). The results were expressed as mg. ascorbic acid per 100 g. adrenal tissue.

## RESULTS

### *The Effect of Unilateral Adrenalectomy on the Ascorbic Acid Concentration in the Intact Adrenal Glands of Normal Rats*

One hundred and eight animals were used in this experiment. Control animals were killed by a blow on the head and their adrenal glands were removed as quickly as possible. Test animals were anaesthetized with ether and their left adrenal glands were removed and discarded. The skin incisions were closed and the animals were allowed to recover from the operations. The rats were killed after various time intervals after unilateral adrenalectomy. The right adrenals were removed,

TABLE I

ASCORBIC ACID CONCENTRATIONS IN THE REMAINING ADRENAL GLANDS OF RATS AT VARIOUS TIME INTERVALS AFTER UNILATERAL ADRENALECTOMY

Controls A: adrenals removed from freshly killed rats.
Controls B: adrenals removed immediately after ether anaesthesia.

| No. of Rats | Time Interval in Hours Between Unilateral Adrenalectomy and Sacrifice | Mean Ascorbic Acid Concentration mg./100 g. Adrenal Tissue ± Standard Error |
|---|---|---|
| 24 | Controls A | 438±5 |
| 12 | Controls B | 367±8 |
| 12 | ½ | 290±15 |
| 12 | 1 | 264±12 |
| 12 | 2 | 251±8 |
| 12 | 4 | 261±16 |
| 12 | 8 | 363±18 |
| 12 | 16 | 475±17 |

and their ascorbic acid contents were determined. The members of one group of animals were anaesthetized with ether and their right adrenal glands, removed under anaesthesia, were assayed for ascorbic contents.

The results obtained are shown in Table I and Fig. 1.

The values obtained for the concentration of ascorbic acid in the adrenal glands of control animals were very constant. In general, the left adrenal gland of the male albino rat was found to be heavier than the right and contained more ascorbic acid, but, when the results were expressed as concentrations of ascorbic acid per 100 g. adrenal tissue, there was good agreement between the values for the right and left glands in the same animal.

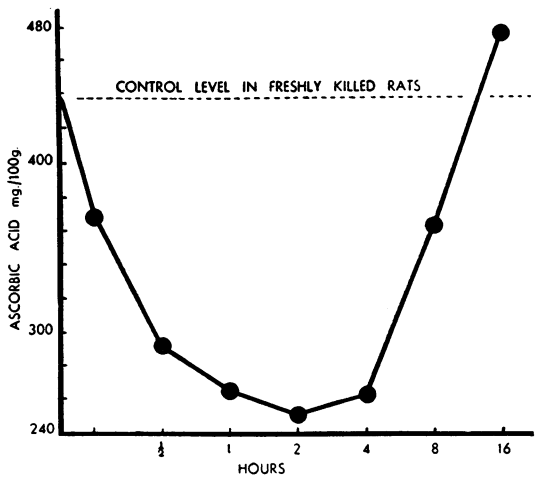


FIG. 1.—Ascorbic acid concentrations in the remaining adrenal glands of rats at various time intervals after unilateral adrenalectomy. Ordinate: Adrenal ascorbic acid concentrations. Abscissa: Time interval between unilateral adrenalectomy and sacrifice.

Unilateral adrenalectomy under ether anaesthesia resulted in a very rapid depletion of the ascorbic acid concentration in the intact adrenal glands. The concentration of adrenal ascorbic acid fell quickly and reached its minimum value of approximately 58% of the normal level, one to two hours after the performance of the operation. This low adrenal ascorbic acid concentration was maintained for four hours. After eight hours the level of ascorbic acid had commenced to return to normal. After sixteen hours the mean concentration was slightly higher than normal.

The time taken to anaesthetize a rat and to remove one of its adrenal glands was approximately four minutes. The fall in adrenal ascorbic acid concentration was found to take place very rapidly. Four minutes from the commencement of anaesthesia there had occurred a significant ($P<0.01$) fall in adrenal ascorbic acid concentration to approximately 84% of the control level. It was for this reason that in the next experiment the adrenal glands first removed from the animals were discarded, and normal unoperated rats were used as controls.

*The Effect of Unilateral Adrenalectomy on the Ascorbic Acid Concentration in the Intact Adrenal Glands of Rats Pre-treated with Desoxycorticosterone Acetate*

The experiment was carried out on 600 rats. The animals were divided into two main groups. The members of the first group received intraperitoneal or subcutaneous injections of various doses of DOCA, and at specific time intervals afterwards half the number in the group were anaesthetized with ether and their left adrenal glands were removed and discarded. One hour later these unilaterally adrenalectomized animals were killed. The right adrenal glands were removed and transferred to trichloracetic acid solution for the determination of their ascorbic acid contents. The unoperated animals in the group were killed at the same time after receiving the injections of DOCA. Their right adrenals were removed and estimated for ascorbic acid contents. The rats in the second group received injections of arachis oil instead of DOCA. Subsequently they were subjected to the same treatment as the members of the first group. A number of control animals were killed without any previous treatment.

Solutions of DOCA in arachis oil were injected intraperitoneally in doses of 5, 10, and 15 mg., and subcutaneously in doses of 5, 10, and 20 mg. DOCA per 100 g. body weight. Both the DOCA solutions and arachis oil were given in volumes of 1 ml. per 100 g. Preliminary experiments had

## TABLE II

### ASCORBIC ACID CONCENTRATIONS IN THE INTACT ADRENAL GLANDS OF RATS FOLLOWING UNILATERAL ADRENALECTOMY PERFORMED AFTER THE INJECTION OF DOCA

Test animals were unilaterally adrenalectomized at various time intervals (indicated in the Table) after the injection of DOCA, and the concentrations of ascorbic acid in their intact adrenals were determined one hour later

Control rats were killed without previous surgical interference, and their adrenal ascorbic acid concentrations were determined. The figures in brackets in the last column refer to the number of rats in the group. — = controls killed; i.p. = intraperitoneally; s.c. = subcutaneously

| Dose of DOCA mg./100 g. Body Weight | Time Interval in Hours Between Injection and Operation | Mean Ascorbic Acid Concentrations mg./100 g. Adrenal Tissue ± Standard Error |
|---|---|---|
| 0 i.p. | — | 444±17 (6) |
| 0 ,, | 1 | 273±25 (6) |
| 5 ,, | — | 442±31 (6) |
| 5 ,, | 1 | 357±21 (6) |
| 10 ,, | — | 415±13 (6) |
| 10 ,, | 1 | 374±34 (6) |
| 15 ,, | — | 355±15 (6) |
| 15 ,, | 1 | 374±25 (6) |
| 0 s.c. | — | 373±34 (6) |
| 0 ,, | 1 | 259± 9 (6) |
| 5 ,, | — | 448± 8 (8) |
| 5 ,, | 1 | 279±10 (8) |
| 10 ,, | — | 404±16 (8) |
| 10 ,, | 1 | 271± 8 (8) |
| 20 ,, | — | 391±13 (8) |
| 20 ,, | 1 | 258±16 (8) |
| 0 i.p. | — | 419± 7 (6) |
| 0 ,, | 2 | 216± 9 (6) |
| 5 ,, | — | 398±18 (6) |
| 5 ,, | 2 | 351±33 (6) |
| 10 ,, | — | 432±18 (6) |
| 10 ,, | 2 | 354±21 (10) |
| 15 ,, | — | 387±23 (6) |
| 15 ,, | 2 | 365±27 (6) |
| 0 s.c. | — | 368±15 (6) |
| 0 ,, | 2 | 239±11 (6) |
| 5 ,, | — | 401±10 (8) |
| 5 ,, | 2 | 253±16 (8) |
| 10 ,, | — | 357±12 (8) |
| 10 ,, | 2 | 266± 9 (8) |
| 20 ,, | — | 383±15 (8) |
| 20 ,, | 2 | 273±16 (8) |
| 0 i.p. | — | 457± 8 (6) |
| 0 ,, | 4 | 269±15 (6) |
| 5 ,, | — | 438±11 (6) |
| 5 ,, | 4 | 315±13 (6) |
| 10 ,, | — | 432±15 (6) |
| 10 ,, | 4 | 409±24 (10) |
| 15 ,, | — | 424±16 (6) |
| 15 ,, | 4 | 433±18 (6) |
| 0 s.c. | — | 396±10 (6) |
| 0 ,, | 4 | 274±11 (6) |
| 5 ,, | — | 404±10 (8) |
| 5 ,, | 4 | 375±25 (8) |
| 10 ,, | — | 423±19 (8) |
| 10 ,, | 4 | 382±12 (8) |
| 20 ,, | — | 407±17 (8) |
| 20 ,, | 4 | 361±12 (8) |
| 0 i.p. | — | 421±10 (6) |
| 0 ,, | 8 | 264±10 (6) |
| 5 ,, | — | — |
| 5 ,, | 8 | 262±11 (6) |
| 10 ,, | — | — |
| 10 ,, | 8 | 284±21 (6) |
| 15 ,, | — | 425±16 (6) |
| 15 ,, | 8 | 360±15 (6) |
| 0 s.c. | — | 429±25 (6) |
| 0 ,, | 8 | 275±14 (6) |
| 5 ,, | — | 429± 8 (8) |
| 5 ,, | 8 | 323±13 (8) |
| 10 ,, | — | 424±10 (8) |
| 10 ,, | 8 | 373±24 (8) |
| 20 ,, | — | 424±10 (6) |
| 20 ,, | 8 | 378±30 (6) |
| 0 i.p. | — | 453±21 (6) |
| 0 ,, | 16 | 260±11 (6) |
| 5 ,, | — | — |

TABLE II—*continued*

| Dose of DOCA mg./100 g. Body Weight | Time Interval in Hours Between Injection Between Operation | Mean Ascorbic Acid Concentrations mg./100 g. Adrenal Tissue ± Standard Error |
|---|---|---|
| 5 i.p. | 16 | 277±16 (6) |
| 10 ,, | — | — |
| 10 ,, | 16 | 287±17 (6) |
| 15 ,, | — | 430±16 (6) |
| 15 ,, | 16 | 284±20 (6) |
| 0 s.c. | — | 460±15 (6) |
| 0 ,, | 16 | 288± 5 (6) |
| 5 ,, | — | 429±19 (8) |
| 5 ,, | 16 | 307±14 (8) |
| 10 ,, | — | 420±10 (8) |
| 10 ,, | 16 | 402± 9 (8) |
| 20 ,, | — | 475±10 (6) |
| 20 ,, | 16 | 453±20 (6) |
| 0 s.c. | — | 516± 7 (6) |
| 0 ,, | 32 | 273±13 (6) |
| 5 ,, | — | 462±20 (6) |
| 5 ,, | 32 | 315±26 (6) |
| 10 ,, | — | 468±16 (6) |
| 10 ,, | 32 | 384±40 (6) |
| 20 ,, | — | 479±16 (6) |
| 20 ,, | 32 | 475±12 (6) |

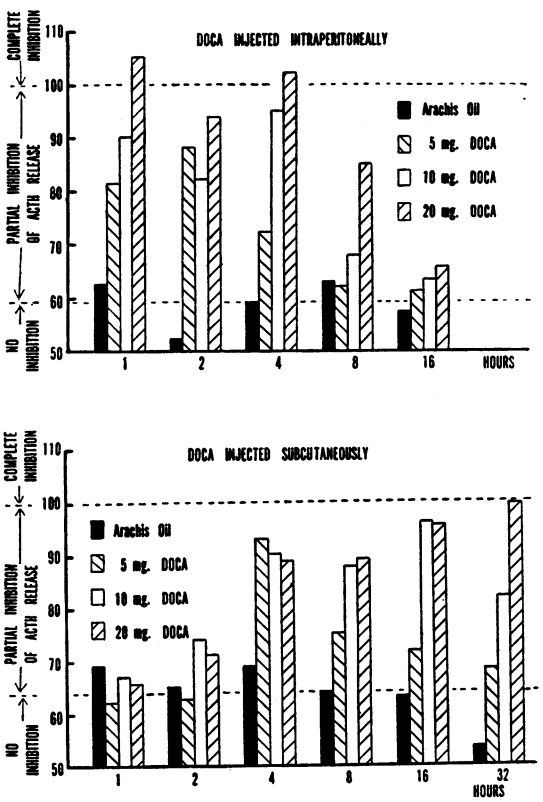


FIG. 2.—The effect of DOCA on the adrenal ascorbic acid depletion caused by unilateral adrenalectomy. Ordinate: Adrenal ascorbic acid concentration, one hour after the operation, expressed as a percentage of the control level in unoperated rats. The figures below the columns represent the time interval in hours between the injections and the performance of unilateral adrenalectomy.

shown that DOCA administered intraperitoneally in doses of 20 mg. or more per 100 g. constituted a severe form of stress and resulted in prolonged adrenal ascorbic acid depletion. For this reason, 15 mg. per 100 g. was the largest dose of DOCA administered by the intraperitoneal route. The results obtained are summarized in Table II and Fig. 2.

Intraperitoneal injections of arachis oil in the volumes used and of DOCA in doses equivalent to 5 and 10 mg. per 100 g. body weight produced no marked changes in adrenal ascorbic acid concentration. The administration of 15 mg. DOCA by the intraperitoneal route produced a definite fall in the concentration of ascorbic acid in the adrenal glands, but the levels had returned to normal four hours after the DOCA had been injected. The subcutaneous injection of arachis oil and the DOCA solutions caused no marked adrenal ascorbic acid depletion.

Unilateral adrenalectomy under ether anaesthesia produced an adrenal ascorbic acid depletion in rats which had been pre-treated with arachis oil only similar to that observed in the previous experiment on normal rats (black columns of Fig. 2). Pre-treatment of the rats with desoxycorticosterone acetate reduced the changes in adrenal ascorbic acid concentration which normally followed unilateral adrenalectomy.

The intraperitoneal administration of 5 mg. DOCA partially prevented the fall in adrenal ascorbic acid which resulted from the stress of the operation for a period of one to four hours after the DOCA had been injected. The 5 mg. dose of desoxycorticosterone acetate, administered subcutaneously, partially prevented the adrenal ascorbic acid depletion, caused by the operation, four to 32 hours after it had been injected.

Partial prevention of the fall in the concentration of ascorbic acid by 10 mg. DOCA was most marked four hours after it had been injected intraperitoneally and 16 hours after its subcutaneous administration.

Fifteen mg. DOCA, given intraperitoneally, completely prevented the adrenal ascorbic acid depletion due to unilateral adrenalectomy one, two, and four hours after its injection. This dose itself produced a fall in adrenal ascorbic acid concentration, and therefore its effect in preventing adrenal ascorbic acid depletion was most obvious four hours after it had been injected, when the control adrenal ascorbic acid level had returned to normal.

Twenty mg. DOCA, injected subcutaneously, completely prevented any stress-induced change in adrenal ascorbic acid concentration 16 and 32 hours after its administration.

About one-third of the total number of the rats which received the greatest doses of DOCA by the intraperitoneal route collapsed after the administration of the steroid. The animals passed into a coma half to one hour after the DOCA had been injected. In the comatose animals, respiration was hardly detectable, the heart rate was slow, and the skin of the feet and face was cyanosed. Only a few of the comatose rats showed no reflex responses to pinching the feet or tail. The majority still reacted to these stimuli, and showed exaggerated responses to the sound stimulus caused by opening and shutting their cage doors.

Subsequent experiments showed that, if these " anaesthetized " rats were allowed to recover, they regained consciousness six to eight hours later and resumed apparently normal activity. It was found that there existed no correlation between the animals which exhibited the most obvious symptoms of DOCA intoxication and those which showed most marked adrenal ascorbic acid depletion. None of the rats which were given subcutaneous injections of DOCA exhibited this phenomenon. The " anaesthetic " effect of large doses of steroids was first noticed by Selye (1941). More recently Cosmos, Duell, and Gaunt (1950) have studied similar effects produced by the administration of desoxycorticosterone glycoside.

## Discussion

There is little doubt that the secretion of ACTH is partly controlled by a peripheral humoral mechanism, i.e., by the level of cortical hormones in the blood. However, there is much confusion in the literature concerning the ability of desoxycorticosterone acetate to depress the increased secretion of ACTH which normally follows stress.

On the one hand, a considerable amount of evidence exists that DOCA can block effectively the output of ACTH. Selye and Dosne (1942) found that the previous administration of DOCA to rats partially prevented the increase in adrenal weight caused by stress. Woodbury, Cheng, Sayers, and Goodman (1950) found, in rats, that the adrenal atrophy caused by DOCA could be prevented by the simultaneous administration of ACTH. They considered that some of the toxic effects of DOCA might be due to the inhibition of pituitary adrenocorticotrophic activity, resulting in adrenocortical insufficiency. Cheng and Sayers (1949) showed that large doses of DOCA, implanted as pellets, caused increased sensitivity to insulin in adrenal-demedullated rats. They attri-

buted this hypersensitivity to adrenocortical insufficiency caused by the inhibition of the adrenocorticotrophic activity of the pituitary gland. Sayers and Sayers (1947) reported that the adrenal ascorbic acid depletion caused by cold (4° C. for one hour) could be prevented by previous treatment of the animals with only 200 μg. desoxycorticosterone (per 100 g. body weight), injected subcutaneously in oil, immediately before the application of the stress.

On the other hand, many workers have been unable to demonstrate the pituitary inhibitory effect of DOCA. Moya and Selye (1948) found that " anaesthetic " doses of DOCA (12–14 mg. per 80–100 g. rat), administered intraperitoneally in vegetable oil, failed to prevent the fall in adrenal ascorbic acid concentration caused by exposure of the rats to cold. They suggested that, in severe stress, the secretion of adrenocorticotrophic hormone is controlled by a mechanism which the " peripheral-humoral " theory fails to explain. Hall, Finerty, Hall, and Hess (1951) found that desoxycorticosterone acetate administered acutely (24 mg. per 80–100 g. rat intraperitoneally in vegetable oil) or chronically (50 mg. per 80–100 g. rat subcutaneously as pellets) caused no adrenal ascorbic acid depletion. They reported that these large doses of DOCA failed to prevent the fall in adrenal ascorbic acid concentration caused by the stress of scalding. Gershberg *et al.* (1950) found that desoxycorticosterone acetate (4 mg. per 100 g. body weight), administered subcutaneously as an oily solution or an aqueous suspension, was ineffective in preventing the adrenal ascorbic acid depletion caused by the exposure of rats to cold.

The present work showed that previous treatment of rats with large doses of desoxycorticosterone acetate could prevent the fall in ascorbic acid concentration which occurs in the intact adrenal glands after unilateral adrenalectomy. The effect depended upon the dose of DOCA administered, its route of administration, and the time interval between the injection of the steroid and the application of the stress. In these experiments the degree of inhibition of pituitary adrenocorticotrophic activity produced by the DOCA was taken to be inversely proportional to the adrenal ascorbic acid depletion which occurred one hour after unilateral adrenalectomy. Where complete pituitary inhibition was obtained, ascorbic acid levels in the intact adrenal glands were not affected by the anaesthetic and the operation, and were equal to the concentrations found in the adrenals of DOCA-injected, unoperated, control animals. Where no inhibition of adrenocorticotrophic activity occurred the removal of one adrenal gland resulted in the usual considerable fall of ascorbic acid concentration in the other. It was considered that partial pituitary inhibition had been produced when intermediary adrenal ascorbic acid levels were obtained.

These experiments confirmed the observations of Sayers and Sayers (1947) that DOCA can cause inhibition of the adrenocorticotrophic activity of the pituitary gland. However, it was found that the doses of DOCA required to prevent the increased secretion of ACTH, in response to the stress of ether anaesthesia and unilateral adrenalectomy, were fifty to one hundred times as great as those which Sayers and Sayers (1947) found necessary to prevent the effect of cold. The results are in complete contrast to the findings of Moya and Selye (1948), Hall *et al.* (1951), and Gershberg *et al.* (1950). It is probable that Gershberg *et al.* (1950) were unsuccessful in demonstrating the pituitary inhibitory effect of DOCA, since they subjected their rats to stress too soon after the subcutaneous administration of the steroid. It is more difficult to explain the discrepancies which exist between the results obtained in the present work and those of Hall *et al.* (1951) and Moya and Selye (1948).

It is doubtful whether these results augment the evidence that the rate of secretion of ACTH by the adenohypophysis is controlled by a peripheral-humoral mechanism. They have shown that pituitary adrenocorticotrophic activity can be prevented by the previous treatment of rats with DOCA, but the required doses of the steroid were necessarily so great that the physiological significance of the results is obscure. Sayers and Sayers (1947) were successful in obtaining inhibition of pituitary adrenocorticotrophic activity with small doses of cortical steroids, and their results are more in accord with the existence of a peripheral-humoral mechanism for the control of the secretion of ACTH.

One reason for carrying out these experiments was to examine the possibility of modifying the Sayers' adrenal ascorbic acid depletion technique for the bioassay of ACTH (Sayers, Sayers, and Woodbury, 1948) by substituting rats, in which pituitary adrenocorticotrophic activity had been " blocked " with DOCA, for hypophysectomized animals. It was found that injected ACTH still caused adrenal ascorbic acid depletion in rats pretreated with DOCA. Preliminary attempts to make this finding the basis of a bioassay method for ACTH were described recently by Buttle and Hodges (1952) and Reiss, Halkerston, Badrick, and Halkerston (1951).

Since ACTH was still effective in producing adrenal ascorbic acid depletion, it was considered that DOCA had not prevented the stress-induced fall in adrenal ascorbic acid concentration by a direct action on the adrenal cortex. Similar evidence that adrenocortical hormones inhibit pituitary adrenocorticotrophic activity directly, rather than by interfering with the release of steroids from the adrenal cortex, was obtained by Ingle and Kendall (1937) and Sayers and Sayers (1947).


## SUMMARY

1. Unilateral adrenalectomy under ether anaesthesia caused marked ascorbic acid depletion in the intact adrenal glands of normal rats. Maximal effects were produced one to two hours after the performance of the operation.

2. Intraperitoneal injections of desoxycorticosterone acetate in arachis oil caused no fall in the adrenal ascorbic acid concentration in rats, when the doses of DOCA employed were not greater than 10 mg. per 100 g. body weight. Larger doses of DOCA administered by the intraperitoneal route caused adrenal ascorbic acid depletion. Subcutaneous injections of DOCA in arachis oil in doses of up to 20 mg. per 100 g. caused no fall in adrenal ascorbic acid concentration.

3. Pre-treatment of rats with large doses of DOCA tended to prevent the fall in adrenal ascorbic acid caused by unilateral adrenalectomy. The adrenal ascorbic acid depletion, which normally followed the operation, was completely prevented by (*a*) the intraperitoneal administration, one to four hours previously, of 15 mg. DOCA, and (*b*) the subcutaneous administration, 16 to 32 hours previously, of 20 mg. DOCA per 100 g. body weight.


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