THE ROLE OF EPINEPHRINE IN THE SECRETION OF THE ADRENAL CORTEX*

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In 1943 in a review of the factors producing adrenal cortical hypertrophy, Tepperman, Engel, and Long[31] suggested that, "The cortical hormone may inhibit the adrenotropic activity of the anterior lobe, and the adrenotropic secretion may therefore increase if the cortical hormone concentration in the body fluids falls. A fall in the cortical hormone concentration may be brought about by an increase in the rate of inactivation ('utilization') of the hormone."

This suggestion was prompted by the observation of Ingle, Higgins, and Kendall[12] that the administration of cortical hormone to normal rats was followed by atrophy of their adrenal cortices, and that simultaneous administration of adrenotrophic hormone (ACTH) prevented such atrophy. The rapid inhibition of ACTH release by the administration of cortical hormone to normal animals has been adequately demonstrated by Sayers and Sayers.[24] These authors have shown that the depletion of the adrenal ascorbic acid which normally follows exposure of animals to a variety of conditions and agents, and which is initiated by ACTH discharge, does not occur if adrenal cortical steroids are injected prior to the imposition of the previously effective agent.

In consequence they put forward a view almost identical with that expressed above, i.e., that a wide variety of circumstances and agents increase the rate of utilization of cortical hormone by the cells of the tissues. This soon leads to a reduction in the blood level of cortical hormone which acts as an effective stimulus to the release of ACTH, thus assuring a continued high level of adrenal cortical secretion. Conversely, any situation in which the blood level of cortical hormone rises to above normal levels brings about an inhibition of ACTH secretion, thus lessening the secretory rate of the adrenal cortex. In this way continued high and possibly deleterious blood levels of cortical hormone are prevented.

In 1945, Long and Fry[15] and, later, Long[13, 14] pointed out that many of the circumstances associated with the release of ACTH are those which

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for many years have been known to be also associated with an increased degree of activity of the autonomic nervous system and a concomitant release of epinephrine. They reported that the injection or infusion of epinephrine is also followed by a decline in adrenal ascorbic acid and cholesterol which fails to occur in hypophysectomized animals. It was suggested, therefore, that epinephrine is an important factor in the activation of ACTH secretion and may well serve as the trigger mechanism by which adequate amounts of adrenal cortical hormone are rapidly made available for the bodily needs of the moment.

While these studies were in progress, Vogt,[35, 36] using an ingenious technique by which the content of cortical hormone in the venous effluent blood of the gland of the dog could be determined, reported that epinephrine in physiological amounts is a potent stimulus of this secretion. The characteristics of this effect of epinephrine on the cortical secretion were, first, the rapidity with which the increased output of cortical hormone occurred, and secondly, the comparatively long period after cessation of epinephrine infusion that the high output of cortical hormone was maintained. The one significant difference from the results of Long, *et al.*, was the conclusion that this effect of epinephrine was independent of the presence of the pituitary—a conclusion based on one reported experiment on a decapitated dog.[36]

Sayers and Sayers,[25] although they also found that epinephrine injection is followed by adrenal cortical activation, are inclined to the belief that the effect of epinephrine is due to its capacity to increase the utilization of cortical hormone, and is thus only an indirect stimulus to ACTH secretion.

The present paper and that immediately following are concerned with reconciling the two important differences in the results of these three groups of investigators. The questions at issue are these: (a) Does epinephrine produce an augmentation of adrenal cortical secretion by its *direct* effect on the cells of the adrenal cortex, or is this effect mediated by a preliminary stimulation of ACTH secretion from the anterior lobe of the pituitary? (b) Since, in our experience, epinephrine only augments cortical secretion in the presence of the anterior lobe, the second question is concerned with the mechanism by which epinephrine provokes the secretion of ACTH. This might be (i) indirectly through an enhancement of cortical hormone utilization in the tissues, as Sayers and Sayers suggest; or (ii) by a direct effect on the cells of the anterior lobe that are responsible for ACTH formation and release. Data will be presented which appear to answer these two questions, and an outline will be given of the main components of the mechanism through which the autonomic nervous system and the adrenal medulla may increase the rate of liberation of adrenocorticotrophic hormone.

Methods

In this and the following paper two methods have been employed to detect the increased secretory activity of the adrenal cortex of albino rats. The first of these is the reduction in adrenal cholesterol and ascorbic acid that occurs when the blood level of ACTH is increased by natural or artificial means. The specificity of this response of the adrenal cortex to ACTH was first reported from this laboratory by Sayers, *et al.*[26] Since that time the method has been extensively used to detect adrenal cortical activation and has been adapted for use as an assay method by Sayers, Sayers, and Woodbury.[25] Actually, it is a measure of increased blood levels of ACTH, but the physiological coupling of this hormone with adrenal cortical secretion makes it equally applicable for estimation of the latter.

The second method depends on the capacity of the 11-oxy adrenal cortical steroids to cause a decrease in the number of circulating eosinophiles. This was first described by Thorn and his colleagues,[9] and is analogous to the reduction in circulating lymphocytes found by Dougherty and White[6] and of the monocytes described by Thompkins.[33] The eosinopenic response is a sensitive method for the detection of increased blood levels of these cortical hormones. The close relationship which exists between the decline in adrenal ascorbic acid and that of the circulating eosinophiles is described in detail in the paper which follows this.

Chemical. Analyses for ascorbic acid content of the adrenal glands were made by the method of Roe and Kuether.[23] Cholesterol was determined by the procedure of Sperry.[29] Liver and muscle tissues were analyzed for glycogen by a modification of the well-known Pflüger method. Blood samples were collected from the tail before and after the experimental procedures for the estimation of glucose,[18] lactic acid,[3] and plasma amino nitrogen.[10]

Hematological. Direct counts of blood eosinophiles were made on freely flowing samples of tail blood by the method of Randolph,[21] modified to stain only eosinophilic cells. A diluting fluid containing 0.05% phloxine* in 50% propylene glycol* was prepared freshly every few days by a 1 : 1 aqueous dilution of a 0.1% stock solution in 100% propylene glycol which was stored in the refrigerator. Mixing time was standardized to obtain uniform cell distribution in the 1 : 20 dilution of the blood sample. In our experience, drops from the middle portion of the pipette gave close agreement on both sides of two 0.2 mm.-depth Levy counting chambers. Selection of uniform diluting pipettes is recommended for satisfactory results. Variations in resting levels of circulating eosinophiles were encountered in spite of the above precautions. However, our range for the rat has never been as wide as that reported by Recant, Hume, Forsham, and Thorn,[22] and repeated counts every few days revealed similar values for any one animal. Figures obtained in this laboratory by both the Randolph[21] and Forsham, *et al.*[9] techniques ranged between 100-200 cells per cubic mm. for the normal rat.

Anesthesia. Warmed 1% solutions of sodium pentobarbital (Nembutal) in physiological saline were injected intraperitoneally; intact rats received 5 mg./100 g., while hypophysectomized, adrenalectomized, and other operated animals were given 3-4 mg./100 g. Whenever postoperative respiratory difficulty was anticipated, Evipal was

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used as an anesthetic at a dose level of 7.5-10 mg./100 g. For local anesthesia a 1:20 dilution of 20% Novocain was employed.

Drugs. Epinephrine hydrochloride (Adrin, 1:1000, Sharp & Dohme) was used,* diluted with saline as noted in tables. Reduced glutathione (Paragon) in a concentration of 20 mg. % was added to protect the epinephrine from oxidation during administration. Intravenous, intraperitoneal, and intramuscular (gastrocnemius) infusions were made through a 26-gauge needle connected to a perfusion pump adjusted to deliver 0.5 ml. of fluid per 100 g. of body weight per hour.

Intraocular injections of epinephrine were done under light Nembutal anesthesia. The sclerocorneal junction of the rat's eye was nicked with a pointed scalpel blade, some aqueous humor was allowed to escape, and then 0.05 ml. of a 1:250,000 dilution of Adrin (0.2 micrograms) were slowly introduced with a fine gauge needle.

Adrenal cortical hormone used was either Wilson's extract or Upjohn's Lipo-Adrenal and was administered as noted in the tables. The desoxycorticosterone acetate injected was either Cortate (Schering)† containing 5 mg. in 1 ml. sesame oil, or a suspension of crystalline D.C.A.† prepared by dissolving 20 mg. in 1 ml. of warm absolute alcohol and by diluting this with 9 ml. of physiological saline. All adrenal cortical steroids were injected subcutaneously, in divided dosages if the volume was large.

Lilly's insulin (Iletin), U-40, was diluted 1:40 with saline so that 0.1 U. (0.1 ml.) per 100 gm. was injected intraperitoneally. For the intravenous route 0.05 U./100 g. were introduced into the femoral vein, which had been exposed under local Novocain anesthesia.

Ergotamine tartrate (Gynergen, Sandoz) containing 0.5 mg./ml. was injected subcutaneously in doses of 0.5 mg./100 g.

Preparation of animals. Sprague-Dawley male rats ranging from 200-300 g. were used. They were housed in a constant temperature room at 78-80° F. The diet was Laboratory Chow, except in the case of the rats with diencephalic lesions and those with transection of the spinal cord, where a high-fat diet was fed.‡ In the experiments described in this paper, all rats were fasted for 24 hours except where indicated in the tables. The studies in the second paper were made on non-fasted animals, primarily because repeated fasting brought about untoward loss of weight in the spinal and diencephalic animals. In every case, eosinophile counts were made at the same hour on each experimental day, and food was withheld during the observation period.

Hypophysectomy, adrenalectomy, adrenal enucleation *in situ,* orchidectomy, and splenectomy were all performed by standard procedures.[8] Adrenalectomized rats were maintained on physiological saline as drinking fluid, while adrenal demedullated animals were given saline to drink for the first postoperative week. Four to five weeks were allowed for cortical regeneration[11] before the latter animals were tested, and none was used after ten weeks.[1]

Spinal animals were prepared by transecting the cord at the level of the third thoracic (or, in a few instances, at the first lumbar) vertebra under Evipal anesthesia. By keeping the rats in a warm room (80° F.) and by establishing a routine of nursing care which included manual expression of the urine from the bladder twice daily until

* Crystalline L-epinephrine bitartrate (obtained from the Sterling-Winthrop Research Institute as a gift to Dr. Desmond Bonnycastle), when infused intramuscularly in normal rats at a rate of 2 micrograms per 100 g. per hour, reduced the adrenal ascorbic acid concentration by 45%. However, L-arterenol bitartrate (Winthrop-Stearns) injected subcutaneously in normal rats in doses of 5 or 20 micrograms/100 g. had no significant effect on the adrenal ascorbic acid. The relative hypoglycemic effects of the two drugs were of the order reported elsewhere.

† Gift of the Schering Corporation.

‡ Lard, 34%, casein, 25.5%; sucrose, 25.5%; liver powder, yeast, and salt mixture, 5% of each by weight.

automaticity was effected, about 50% of these animals could be brought to a good state of health and nutrition. None was used which had not regained or surpassed his preoperative weight.

"Diencephalic" animals were prepared by placing electrolytic lesions in the thalamus and hypothalamus with the aid of the Horsley-Clarke stereotaxic instrument. Out of a number of rats operated upon and studied, six were selected for inclusion in the present report; they were in good health, gave no evidence of obesity or disturbances of temperature regulation, and were similar to the spinal and adrenal demedullated rats in that they consistently failed to secrete endogenous epinephrine.

Pituitary transplants were made into the anterior chamber of one eye of hypophysectomized rats (Evipal anesthesia) on the second day following the hypophysectomy. Pregnant female rats near term were used as donors. After slitting the sclero-corneal junction of the hypophysectomized rat's eye, one-half of the anterior pituitary tissue of the donor rat was introduced into the anterior chamber with the aid of a trocar. Aseptic technique was used throughout the operation.* One month later when the grafts were well established, experimentation was begun.

Stimulation of the central end of the sciatic nerve and exposure to cold have been described in earlier reports.[13, 14] Hemorrhage experiments were conducted as described by Sayers, *et al.*[27]

Bioassay of pituitary grafts. After the grafted eyes were removed from the hypophysectomized animals, the transplants were dissected out. Two grafts were extracted separately for adrenocorticotrophic assay. Saline to which had been added two drops of N/10 NaOH was used for extraction. Adrenocorticotrophic activity was tested according to the method of Sayers, Sayers, and Woodbury.[28] Parallel blood eosinophile tests were made. The remainder of the dissected grafts were pooled and extracted with saline for test of any gonadotrophic activity present. Assays were run on groups of immature rats and mice according to the technique of Evans, Varney, and Koch.[7]

Results

Effect of exogenous epinephrine. In their preliminary report, Long and Fry[15] stated that the subcutaneous or intravenous injection of epinephrine into normal rats brought about a decline in adrenal ascorbic acid and cholesterol. Similar injections into hypophysectomized rats were without effect on the levels of these adrenal constituents (Table 1). Since the time of their report, many more experiments have been carried out in which not only was the epinephrine administered by other routes, but the minimum quantity required to produce a fall in adrenal ascorbic acid and cholesterol has been roughly established.

The effects of epinephrine administered by the intravenous route to rats anesthetized with Nembutal are seen in Table 2. It will be observed that the intravenous injection of 0.9% NaCl over a period of sixty minutes is in itself sufficient to cause some fall in adrenal ascorbic acid but not in adrenal cholesterol. Such changes in adrenal ascorbic acid are also found after mild procedures such as incision of the skin and the insertion of a needle into the femoral vein. They would appear to be due to the reflex

* Dr. H. S. N. Greene carried out the implantations for us, according to the technique which he has recently described in detail (Yale J. Biol., 1950, *22,* 611-620).

secretion of epinephrine from the animal's own adrenal medulla. Indeed, in these experiments the infusion of a total of two micrograms of epinephrine in a corresponding quantity of normal saline produced no greater

TABLE 1

EFFECT OF THE SUBCUTANEOUS INJECTION OF EPINEPHRINE ON THE ADRENAL CHOLESTEROL AND ASCORBIC ACID OF NORMAL AND HYPOPHYSECTOMIZED RATS

Injection mg./100 g.	*Duration of experiment hours*	*No. of rats*	*Adrenal*	
			Cholesterol g./100 g.	*Ascorbic acid mg./100 g.*
		Normal rats		
none	...	23	4.05 ± 0.14	423 ± 13
0.02 x 1	1	6	3.80 ± 0.33	226 ± 24†
0.02 x 1	2	6	2.88 ± 0.28	233 ± 24
0.02 x 1	4	8	3.76 ± 0.19	252 ± 16
0.02 x 4	4	11	2.26 ± 0.22	216 ± 9
		*Hypophysectomized rats**		
none	...	13	5.60 ± 0.43	418 ± 26
0.02 x 4	4	9	5.34 ± 0.37	411 ± 13

* Three days postoperative.

† Data reported in the following paper show that the response within one hour following any subcutaneous injection is mainly a response to endogenous epinephrine from the animal's own adrenals.

TABLE 2

EFFECT OF INTRAVENOUS INFUSION* OF EPINEPHRINE ON THE ADRENAL CHOLESTEROL AND ASCORBIC ACID OF NORMAL AND HYPOPHYSECTOMIZED RATS (NEMBUTAL ANESTHESIA)

No. of rats	*Infusion*		*Total μg./100 g.*	*Time hrs.*	*Adrenal*	
					Cholesterol g. %	*Ascorbic acid mg. %*
23	Normal	None	..	..	4.05 ± 0.14	423 ± 13
6	"	Saline*	..	1	....	256 ± 25
6	"	"	..	2	4.01 ± 0.41	... ..
6	"	Epinephrine (1/250,000)	2	1	....	221 ± 20
6	"	" (1/25,000)	40	2	2.31 ± 0.18	104 ± 17
13	Hypox.	None	..	..	5.60 ± 0.43	418 ± 26
3	"	Epinephrine (1/25,000)	40	2	5.35	342

* Rate: 0.5 ml. fluid per 100 g. body weight per hour.

fall in adrenal ascorbic acid than the saline alone. On the other hand, the infusion of a total of 40 micrograms of epinephrine over a two-hour period caused a marked fall not only in adrenal ascorbic acid but also in adrenal cholesterol, the latter being an effect not obtained with saline alone.

In hypophysectomized rats even the intravenous injection of forty micrograms of epinephrine was without any such effect on the adrenal ascorbic acid or cholesterol, although the release of additional endogenous epinephrine and other factors associated with the operative technique were presumably present.

In an endeavor to prevent the release of endogenous epinephrine, further groups of normal and hypophysectomized rats were infused either intraperitoneally or intramuscularly (under Nembutal anesthesia) with solutions of epinephrine in saline. Control groups were infused with saline alone. Intraperitoneal injections of epinephrine (Table 3) in a total amount of 0.5-2.0 micrograms in one hour brought about significant falls

TABLE 3

EFFECT OF INTRAPERITONEAL INFUSION* OF EPINEPHRINE ON THE ADRENAL CHOLESTEROL AND ASCORBIC ACID OF THE NORMAL RAT (NEMBUTAL ANESTHESIA)

No. of rats	*Infusion*	*Total μg./100 g.*	*Adrenal*	
			Cholesterol g. %	*Ascorbic acid mg. %*
7	Saline*	....	4.00 ± 0.10	419 ± 18
9	Epinephrine (1/250,000)	2.00	3.78 ± 0.21	228 ± 12
5	" (1/500,000)	1.00	....	283 ± 34
8	" (1/1,000,000)	0.50	....	277 ± 34
4	" (1/5,000,000)	0.10	....	414 ± 19
11	" (1/10,000,000)	0.05	3.40 ± 0.20	326 ± 26

* Rate: 0.5 ml. per 100 g. body weight per hour. Duration of all infusions 1 hour.

in adrenal ascorbic acid but did not influence the cholesterol content of the gland. Amounts of epinephrine less than this, or the infusion of saline alone, was not followed by any consistent change in the ascorbic acid level.

The effects of intramuscular infusion are recorded in Tables 4 and 5. In these experiments only the changes in adrenal ascorbic acid were followed. It will be observed that the intramuscular infusion of saline alone over a period of one hour did not cause any fall in the ascorbic acid level. On the other hand, the infusion over a period of one hour of between 0.25 and 0.50 micrograms of epinephrine was associated with a highly significant decline in ascorbic acid. Amounts less than this did not cause any significant change in the level.

The time of infusion is of importance. In Table 4 it will be seen that whereas the infusion of 0.50 micrograms of epinephrine in a period of one hour was followed by a significant decrease, the infusion of the same quantity over a period of 15 minutes was without effect.

The absence of any such effects in hypophysectomized rats is recorded in Table 5. In these experiments the left adrenal was removed first, since (as

will be shown later) this procedure has no effect on the ascorbic acid content of the second gland in hypophysectomized animals. The epinephrine solution was then infused into the femoral vein for one hour, and the second gland removed. In no instance was there any significant change in the

TABLE 4

EFFECT OF INTRAMUSCULAR INFUSION* OF EPINEPHRINE ON BLOOD GLUCOSE AND ADRENAL ASCORBIC ACID OF THE NORMAL RAT (NEMBUTAL ANESTHESIA)

No. of rats	*Procedure* *Infusion*	*Total µg./100 g.*	*Time min.*	*Blood glucose mg. %*	*Adrenal ascorbic acid mg. %*
50	None	....	..	76 ± 2	422 ± 26
8	Saline	....	60	73 ± 1	452 ± 18
4	Epinephrine (1/250,000)	0.500	15	106 ± 12	407 ± 15
4	" "	1.000	30	98 ± 3	292 ± 7
6	" "	2.000	60	90 ± 5	294 ± 12
4	" (1/1,000,000)	0.125	15	68 ± 6	378 ± 9
4	" "	0.250	30	65 ± 8	308 ± 35
6	" "	0.500	60	.....	228 ± 16
7	" (1/1,750,000)	0.290	60	.....	293 ± 16
7	" (1/2,500,000)	0.200	60	.....	391 ± 18
2	" (1/5,000,000)	0.100	60	.....	383

* Rate: 0.5 ml. fluid per 100 g. body weight per hour.

TABLE 5

EFFECT OF INTRAMUSCULAR INFUSION* OF EPINEPHRINE ON THE ADRENAL ASCORBIC ACID OF NORMAL AND HYPOPHYSECTOMIZED RATS (NEMBUTAL ANESTHESIA)

No. of rats		*Infusion*	*Adrenal ascorbic acid* *Total µg./100 g.*	*Right mg. %*	*Left mg. %*	*Decrease %*
8	Normal	Saline	..	.....	452 ± 18	...
6	"	Epinephrine (1/250,000)	2	.....	294 ± 12	34.5
18	Hypox.	None	..	388 ± 13	377 ± 11	3.0
22	" †	Epinephrine (1/250,000)	2	386 ± 11	380 ± 10	1.3
8	" Incomplete	" "	2	398 ± 28	254 ± 18	36.2

* Rate: 0.5 ml. fluid per 100 g. body weight per hour.
† Experiment one day following 3-7 days' postoperative treatment either with ACTH or whole anterior pituitary.

ascorbic acid content of the second gland. Furthermore, it made no difference in the results whether the adrenal glands had been allowed to undergo atrophy following hypophysectomy, or whether they had been maintained by injections of ACTH, or by daily implants of fresh rat anterior lobe tissue.

It would appear, therefore, from these experiments that epinephrine administered either subcutaneously or intramuscularly does not act directly upon the adrenal cortex to produce an increased secretion, but can do so only by its capacity to stimulate the secretion of ACTH from the anterior lobe of the pituitary.

Endogenous epinephrine. The site of action of epinephrine released by the adrenals *in situ* was studied in experiments similar to those in which

TABLE 6

EFFECTS OF COLD, HEMORRHAGE, OR PAIN ON ADRENAL CHOLESTEROL AND ASCORBIC ACID OF NORMAL AND HYPOPHYSECTOMIZED RATS

No. of rats	*Procedure*	*Adrenal*		*No. of rats*	*Adrenal*	
		Cholesterol g. %	*Ascorbic acid mg. %*		*Cholesterol g. %*	*Ascorbic acid mg. %*
		Normal rats			*Hypox. rats**	
23	Controls	4.05 ± 0.14	423 ± 13	13	5.60 ± 0.43	418 ± 26
	Cold room, 0°-4° C.					
10	1 hr.	3.69 ± 0.74	233 ± 20	4	5.90 ± 0.32	379 ± 12
11	2 hr.	2.70 ± 0.19	290 ± 17	..	....	... ..
17	4 hr.	2.40 ± 0.17	... ..	8	5.72 ± 0.42	379 ± 18
18	6 hr.	2.07 ± 0.20	420 ± 35	..	....	... ..
9	24 hr.	2.19 ± 0.42	... ..	..	....	... ..
	Sensory nerve stimulation†					
6	End of stimulation	3.96 ± 0.38	270 ± 3	2	5.95	412 ..
6	30 min. after stimulation	4.16 ± 0.21	268 ± 4	..	....	... ..
6	60 min. after stimulation	3.08 ± 0.33	237 ± 18	..	....	... ..
	Hemorrhage‡					
6	1 hr.	....	166 ± 6	6	....	398 ± 24
6	3 hr.	....	251 ± 32	6	....	385 ± 23
6	6 hr.	....	292 ± 20	6	....	378 ± 26

* Three days postoperative. Fasting period shortened from 24 to 18 hours.

† Central end of sciatic nerve stimulated for 15 seconds every minute for fifteen minutes. Nembutal anesthesia.

‡ Rats bled 2% of body weight during one hour. No anesthesia.

epinephrine was given by injection. Long and Fry,[15] and Long[13,14] have pointed out that the cholesterol and ascorbic acid of the adrenal gland decrease in amount following hemorrhage, scalding, cold exposure, or electrical stimulation of the central end of a mixed nerve, and they have noted that each of these conditions is known to bring about a release of epinephrine from the adrenal medulla. In order to determine whether this epinephrine may stimulate the adrenal cortex directly, or whether its action requires the intermediation of the anterior lobe of the pituitary, the experiments summarized in Table 6 were carried out on normal rats and rats

with the hypophysis removed. Normal animals exposed to a temperature of 0-4° C. showed a decrease in both the cholesterol and ascorbic acid content of the adrenals, the ascorbic acid falling the more quickly of the two (minimal level in one hour) and returning to the control level as the exposure was continued for 6 hours. The cholesterol fell more slowly (minimal level in 6 hours), and was only slightly restored even though the exposure was continued for 24 hours. These changes are dependent upon the anterior lobe, since in the experiments performed upon hypophysectomized rats, no significant change occurred in either the adrenal cholesterol or ascorbic acid content. Results of the same nature were obtained when hypophysectomized rats were subjected to stimulation of the central end of the sciatic nerve (under anesthesia), or to hemorrhage.

TABLE 7

EFFECT OF LAPAROTOMY AND REMOVAL OF ONE ADRENAL ON THE ASCORBIC ACID CONTENT OF THE SECOND ADRENAL* (NEMBUTAL ANESTHESIA)

No. of rats		*Adrenal ascorbic acid* *Right* mg. %	*Left* mg. %	*Decrease* %
12	Normal	406 ± 11	229 ± 6	43.6
18	Hypox.†	388 ± 13	377 ± 11	2.8

* Left gland removed one hour after right gland.

† Fasting period shortened from 24 to 18 hours.

The experiments recorded in Table 7 show that, in normal rats, the removal of one adrenal gland produces within one hour a marked decrease in the ascorbic acid content of the second gland. This fall does not occur in the absence of the pituitary, which suggests that it is brought about, as is the adrenal response to cold exposure, hemorrhage, or painful stimulation, through the release of epinephrine acting either directly or indirectly upon the anterior lobe. It seems clear from the results in the hypophysectomized animals that the action cannot be directly upon the adrenal cortex, and observations suggesting that the effect of epinephrine is a direct one upon the anterior hypophysis have already been published in preliminary form.[16] They will be further discussed in the second paper.

To the conditions already described, where the adrenal cortex becomes active presumably through the intermediation of epinephrine, there may be added another, viz., insulin hypoglycemia, already studied histologically by Marthe Vogt.[37] Utilizing normal rats and rats with denervated adrenals, Vogt found that under certain circumstances insulin brought about a loss both of adrenal cortical lipid and of medullary epinephrine. Rats with denervated adrenals did not show the loss of epinephrine, and many of

them also failed to undergo a loss of cortical lipid. Since some did lose cortical lipid, however, Vogt concluded that a secretion of epinephrine ". . . contributes to the loss of cortical lipids seen after insulin injection, but is not indispensable for it" (p. 403).

The data of Table 8 confirm Vogt's conclusion that insulin may activate the adrenal cortex via the adrenal medulla. Whether administered intraperitoneally (0.10 U./100 g.) or intravenously (0.05 U./100 g.), insulin caused a significant fall in adrenal ascorbic acid. In either case a hypoglycemia was found, with blood sugar levels of 25-30 mg. % at the end of

TABLE 8

EFFECT OF PRETREATMENT WITH GLUCOSE OR ADRENAL CORTICAL HORMONE ON THE ONE-HOUR RESPONSE OF THE NORMAL RAT ADRENAL TO INSULIN

No. of rats	*Injection*	*Units per 100 g.*	*Adrenal ascorbic acid mg. %*	*Blood glucose*		*Plasma amino N, 1 hour mg. %*
				Intial	*1 hour*	
				mg. %		
6	Saline	.....	381 ± 20	.....	73 ± 5	6.9 ± 0.3
7	Saline-Insulin	0.10 I.P.	207 ± 9	.....	28 ± 2	4.8 ± 0.2
7	Insulin	0.05 I.V.	273 ± 24	78 ± 3	26 ± 4	5.8 ± 0.2
6	Glucose*	.....	362 ± 14	.....	145 ± 4	5.5 ± 0.3
6	Glucose*-Insulin	0.10 I.P.	356 ± 30	.....	64 ± 10	5.4 ± 0.3
9	Insulin—Pretreated with cortical hormone†	0.10 I.P.	397 ± 19	.....	30 ± 2	4.4 ± 0.2

* Subcutaneous injection of 8 ml. of 10% glucose 15 minutes before the insulin.
† Rats received 2 ml. Upjohn's Lipo-Adrenal Extract 3 hours before the insulin.

one hour. In another group of experiments, glucose solution was injected before the insulin in order to prevent the hypoglycemia; under these conditions the fall in adrenal ascorbic acid did not occur, which shows that insulin as such is not an integral part of the activating mechanism. Since hypoglycemia brings into play most of the sympathetic nervous system, its influence upon the adrenal cortex is easily explained in the terms outlined above. Glucose may be said to block the mechanism in that it abolishes the hypoglycemia, which in this instance serves as the call for epinephrine secretion.

Inhibition of ACTH secretion. The account just given, however, is not the whole picture of the mechanism of ACTH secretion, as the last line of Table 8 demonstrates. Here the rats were treated with cortical extract, and then given insulin three hours later. As in the group pretreated with glucose, activation of the adrenal cortex was prevented by the cortical hormone. But there was an important difference between the two types of

pretreatment in that, whereas the glucose abolished the insulin hypoglycemia, the cortical extract did not do so. At the end of one hour the blood sugar level was 30 ± 2 mg. %. This hypoglycemia undoubtedly called forth a discharge of epinephrine; nevertheless, ACTH was not secreted, which means that the blocking action of the cortical hormone must have been different from that of the glucose.

The concept that cortical steroids may suppress ACTH release was adopted by Sayers and Sayers[24] to explain their finding that pretreatment with cortical extract prevents adrenal activation during cold exposure, heat, histamine, epinephrine, and the injection of typhoid organisms. We have, in part, confirmed their observations (see below), and in addition have attempted to learn the site of this blocking action—whether directly upon the anterior pituitary, or through some metabolic change associated

TABLE 9

EFFECT OF GLUCOSE PRETREATMENT ON THE ADRENAL ASCORBIC ACID OF NORMAL RATS EXPOSED TO COLD (1° C.) FOR ONE HOUR

No. of rats		*Adrenal ascorbic acid mg. %*	*Blood glucose† mg. %*
6	Fasted	234 ± 23	68 ± 3
7	Glucose injection*	234 ± 17	136 ± 11
6	Glucose—Room temp.	362 ± 14	145 ± 4

* Subcutaneous injection of 8 ml. of 10% glucose 15 minutes before exposure to cold.
† Samples taken at the end of cold exposure.

with the activity of cortical hormones or of epinephrine or of both. The well-known influence of the adrenal cortex upon carbohydrate metabolism (specifically, upon gluconeogenesis), suggested itself as the type of metabolic reaction which might be involved. If a need for carbohydrate were the critical factor, cortical steroids might increase the supply of carbohydrate and thereby inhibit the endocrine mechanisms concerned with gluconeogenesis. Experiments designed to test this hypothesis, however, failed to support it. As noted above, insulin hypoglycemia did not lead to increased adrenal cortical secretion when cortical extract had been given beforehand, in spite of the animal's need for extra glucose, as manifested in blood sugar levels of 30 ± 2 mg. %. Moreover, the administration of glucose to rats exposed to cold for one hour (Table 9) did not prevent cortical activation even though the animals had more than enough glucose for their metabolic needs and experienced slight hyperglycemia (136 ± 11 mg. %). In experiments where both cortical extract and epinephrine were given, the epinephrine retained its metabolic actions, including its effect upon muscle glycogen and blood lactic acid (Table 10). Nevertheless, the

adrenal ascorbic acid of the group treated with cortical steroids fell only 72 ± 16 mg. % in response to epinephrine, while that of the rats not pretreated fell 218 ± 16 mg. %. All of these experiments suggest a direct inhibition or blocking of the anterior pituitary by the cortical hormones.

Our experiments did not confirm those of Sayers in one respect, that is, that desoxycorticosterone acetate also exerts a blocking action. Cheng and

TABLE 10

THE INFLUENCE OF ADRENAL CORTICAL HORMONE PRETREATMENT UPON THE METABOLIC EFFECTS OF EPINEPHRINE IN THE NORMAL RAT

	*Epinephrine**	*Epinephrine plus cortical extract*†*
Number of rats	9	10
Muscle glycogen, mg. %		
Initial	549 ± 11	568 ± 20
Loss	156 ± 24	177 ± 17
Liver glycogen, mg. %		
Final	215 ± 4	512 ± 7
Blood glucose, mg. %		
Initial	66 ± 3	68 ± 3
Gain	34 ± 8	63 ± 9
Blood lactic acid, mg. %		
Initial	13 ± 1	13 ± 2
Gain	13 ± 2	11 ± 2
Adrenal ascorbic acid, mg. %		
Initial	422 ± 19	450 ± 9
Loss	218 ± 16	72 ± 16

* All rats were fasted 24 hours before epinephrine (0.02 mg./100 g.) was injected subcutaneously. They were sacrificed one hour later.

† Two ml. of Upjohn's Lipo-Adrenal Extract was injected 1½ to 3 hours before epinephrine injection.

Sayers[4] have shown that large doses of D.C.A. implanted as pellets lead to an increased insulin sensitivity in demedullated rats. This hypersensitivity they attributed to cortical insufficiency, an expression of an inhibition of the anterior pituitary. Their report, however, did not mention any direct measurement of adrenal function (such as determination of adrenal ascorbic acid or cholesterol, or changes in the various white blood cell counts), Nor did they consider the possibility that some complication, possibly a toxic effect of the large doses of D.C.A. or a diminished food intake, may have contributed to their results. In our experience, as shown in Table 11, whether given one or three hours before cold exposure, neither D.C.A. in

oil nor D.C.A. in suspension prevented the fall in ascorbic acid. By contrast, the lipoadrenal extract completely prevented the change in ascorbic acid when given three hours prior to cold exposure, and allowed only an insignificant fall when given one hour before the rats were placed in the cold room. These data indicate that, at least in these doses in acute experiments, the synthetic compound D.C.A. does not block ACTH secretion. In view of the rather striking actions of D.C.A. upon electrolyte metabolism, these data further suggest that electrolyte changes are not an important part of the mechanism stimulating ACTH output.

These experiments also suggest that a certain length of time may be needed for the blocking action to occur, since the blocking was greater when the cortical steroids were given four hours than when injected only one

TABLE 11

EFFECT OF PRETREATMENT WITH D.C.A. OR LIPO-ADRENAL EXTRACT ON ADRENAL ASCORBIC ACID OF RATS EXPOSED TO COLD (0°-4° C.) FOR ONE HOUR

No. of rats	*Procedure*	*Time between injection and exposure hrs.*	*Adrenal ascorbic acid mg. %*
18	Controls	..	427 ± 8
6	Cold exposure	..	237 ± 13
4	" " + Lipo ext. 2 ml.	1	385 ± 20
3	" " " " " "	3	431
5	" " " D.C.A. in oil 4 mg.	1	226 ± 26
6	" " " " " " "	3	319 ± 32
3	" " " " suspension "	1	250
3	" " " " " "	3	209

hour before cold exposure. Furthermore, the blocking action of a given amount of cortical extract may be greater when an animal is subjected to a relatively light stress than when the stress is more severe (unpublished data). There appear to be definite quantitative relationships between the severity of a stress, the amount of cortical extract needed to suppress ACTH secretion, and the time intervals during which the stress and the injected steroids are acting.[25]

The specific nature of the blocking of ACTH release by injected cortical extract is also apparent in experiments where other types of blocking agents have been found to be without effect upon the hypophysis, as Tepperman and Tepperman[32] have noted. The data of Table 12 confirm their report, showing that ergotamine tartrate, an autonomic blocking agent, does not alter the adrenal cortical response to epinephrine; rather, ergotamine activates the cortex when given by itself. Results similar to these have also been obtained with other blocking agents, including dibenamine and tetra-

ethylammonium chloride[3, 30] (Fry, unpublished), and it appears likely that drugs of this type have as one of their actions a preliminary excitation of the central nervous system and adrenal medulla.[19]

ACTH secretion in the absence of epinephrine. Vogt's[37] studies following denervation of the adrenals have already been referred to, with her conclusion that the medulla is involved in but not indispensable to cortical activation. Her data were sometimes equivocal on this point, possibly because it is difficult to denervate the adrenal without injuring its blood supply. Attempts to repeat this work have not given entirely satisfactory results in our hands. Since the point is an important one, however, another type of experiment was performed for the same purpose, that is, to attempt

TABLE 12

FAILURE OF ERGOTAMINE TARTRATE TO PREVENT THE EFFECT OF EPINEPHRINE ON ADRENAL CHOLESTEROL AND ASCORBIC ACID OF THE NORMAL RAT

No. of rats	*Injection*	*Adrenal*	
		Cholesterol g. %	*Ascorbic acid mg. %*
11	Epinephrine*	2.26 ± 0.22	216 ± 9
6	Ergotamine†-Epinephrine*	2.94 ± 0.32	248 ± 10
7	Ergotamine†	....	267 ± 26

* Subcutaneous injection of 0.02 mg./100 g. body weight each hour for 4 hours.
† Subcutaneous injection of 0.6 mg./100 g. body weight 15 minutes before the first injection of epinephrine.

to assess the importance of epinephrine in stimulating a secretion of ACTH. To this end the adrenal medullae were removed from a series of rats to deprive them of their endogenous epinephrine. Following demedullation, a definite sequence of changes occurs in the adrenal remnants, resulting in regeneration of cortical tissue capable of maintaining the rat without evidence of cortical deficiency.[11] The ascorbic acid concentration remains low (Table 13, line 2), but it is decreased still further when ACTH is given (Fry, unpublished data).

Experiments carried out upon these animals showed that the adrenal medulla is sometimes required, sometimes not required for increased cortical activity. Stimulation of an afferent nerve trunk for 15 minutes in demedullated rats did not alter the adrenal ascorbic acid concentration (Table 13), whereas in normal rats, a marked fall had been found under similar conditions (Table 6). This difference in response suggests that the cortical stimulation seen in the normal animals was brought about through a reflex secretion of epinephrine, and emphasizes the fact that the mechanism is a rapidly acting one since it can produce the change within a relatively short time. On the other hand, the cortex can still be stimulated

in the absence of the medulla (although, as will be shown in the later paper, the stimulation is delayed). Thus, fasted, demedullated rats showed a very real degree of cortical activation when exposed to cold for 4 hours, or 3-4 hours after removal within one hour of blood amounting to two per cent of their body weight. Under these latter conditions epinephrine is not required and the secretion of increased amounts of ACTH can occur in the absence of the adrenal medulla.

It will be apparent that there must be at least two mechanisms influencing the anterior lobe in its adrenotrophic function. One is a quickly acting mechanism operating through the adrenal medulla and release of epinephrine; the other is slower and remains after the medulla has been removed. The nature of these two mechanisms and their interaction are described in detail in the paper which follows.

TABLE 13
EFFECT OF ADRENAL DEMEDULLATION ON RESPONSE OF ADRENAL CHOLESTEROL AND ASCORBIC ACID TO COLD, HEMORRHAGE, AND PAIN

No. of rats		*Body weight g.*	*Adrenal*		
			Weight g./100 g. B.W.	*Cholesterol g. %*	*Ascorbic acid mg. %*
23	Normal controls	235 ± 4	14.0 ± 0.1	4.05 ± 0.14	423 ± 13
17	Adrenal demedullated controls	212 ± 7	12.1 ± 0.6	2.48 ± 0.15	237 ± 16
3	Sciatic stimulation†	225 ± 13	9.4 ± 0.3	2.26 ± 0.35	241 ± 48
6	Cold (4° C., 4 hrs.)	188 ± 2	11.9 ± 0.7	1.50 ± 0.18	129 ± 12
7	Hemorrhage*	235 ± 7	15.0 ± 0.7	1.52 ± 0.23	98 ± 11

* Blood loss in one hour equivalent to 2 per cent of body weight. Adrenals removed 3-4 hours later.
† Central end of sciatic nerve stimulated for 15 seconds every minute for 15 minutes. Adrenals removed immediately following stimulation.

Discussion

It is now possible to account for some of the apparently contradictory results earlier obtained in studying pituitary-adrenal relationships. Since there appear to be at least two mechanisms by which ACTH secretion may be stimulated, it is not surprising that experiments designed to study only one sometimes gave equivocal results. We have encountered this difficulty often, as did Vogt[37] in her study of the action of insulin upon the adrenal cortex. It seems likely that the cortical activation which she noted in 3-4½-hour experiments upon animals with denervated adrenals may have been brought about through the slower of the two types of mechanisms, the "metabolic phase" of McDermott, *et al.*[17] Long's question[14] as to whether activation of the adrenal cortex "depends on" a release of epinephrine may be answered in similar terms. As the data of Table 13 indicate, under

certain conditions epinephrine is required, especially when rapid cortical activation is to be brought about in response to mild stimuli. If the disturbance to which the animal is subjected is severe or prolonged, however, cortical activation occurs without epinephrine. Another question which may be answered concerns the sensitivity to stress of animals with demedullated or denervated adrenal glands.[14] Such animals, though more sensitive than normal rats,[38] are much more resistant than adrenalectomized or hypophysectomized animals, because secretion of ACTH and cortical hormones can still occur even when epinephrine secretion has been prevented.

Another source of confusion has been a failure to realize that the timing of experiments is a critical matter. McDermott, *et al.*,[17] have found that responses to endogenous epinephrine are best studied within one hour, since by the end of four hours, the "metabolic phase" may intervene to obscure the original response. The latter reaction may have been a factor in Vogt's insulin experiments, which were continued for as long as four and one-half hours.[37] Many of the earlier experiments done in various laboratories were carried out under this handicap, although when reviewed with this point in mind, they may acquire a meaning at first not apparent. Finally, the ease with which animals release their own epinephrine has caused a certain amount of difficulty. It is true that by careful handling of the animals, warming of solutions to be injected, and appropriate use of anesthesia when indicated, the effects of fright and pain can be minimized.[22] But care and experience are required to accomplish this result; and where the usual laboratory routines are followed, autonomic activity almost certainly occurs in the early stages of most experiments. A striking example of the effect of endogenous epinephrine is provided by the experiments where laparotomy was done for removal of one adrenal, followed by removal of the second gland one hour later (Table 7). During and after removal of the first adrenal, the spinal cord must have received from the abdomen and viscera a bombardment of sensory impulses which reflexly stimulated the medulla of the remaining gland, and thus activated the anterior pituitary and the adrenal cortex.

It may be well to point out the unusual sensitivity of this mechanism, and the small quantity of epinephrine necessary to set it in action. Given intramuscularly, a quantity as small as 0.04 μg./kg./min. (0.290 μg./100 g./hr.) was effective when administered for 60 minutes, while 0.08 μg./kg./min. was adequate when given for half that time, or when infused intraperitoneally for one hour. Results following intravenous infusion were probably like these, but they are more difficult to interpret because control studies with intravenous infusion of saline showed that endogenous epinephrine was being released. The experiments using intraperitoneal or intramuscular infusions were more conclusive because saline given by these routes was without effect. The small doses of epinephrine used here may be compared with those used by Cori, Cori, and Buchwald,[5] who found that in

anesthetized rabbits 0.5 μg./kg./min. had only a slight effect on blood pressure; in the absence of anesthesia, 0.05 μg./kg./min. had no hyperglycemic action, and 0.1 μg./kg./min., infused for two hours, produced mild hyperglycemia. The doses which we have used compare favorably with these, and appear to be well within the physiological range. The data of Table 4 (above) indicate that epinephrine may stimulate the anterior pituitary when infused in amounts too small to cause a rise in blood sugar. Unpublished data show that not only blood sugar, but also serum potassium concentration, lactic acid, and amino acids as well as muscle glycogen may remain unaffected by small doses of epinephrine which do, nevertheless, increase ACTH secretion (Fry). On the other hand, as noted earlier (Table 10), after the anterior lobe has been blocked by the administration of cortical steroids, larger doses of epinephrine fail to stimulate the gland although they have their usual metabolic effects. These experiments seem to indicate that the metabolic effects of epinephrine are not correlated with its action upon the anterior pituitary gland.

That epinephrine is an agent capable of exciting the adrenal cortex is now generally accepted,[15, 29, 25, 34, 37] and does not need further emphasis. How this excitation is brought about has been a matter of debate.[20] Unlike the earlier experiments of Vogt,[36] our data seem to show without exception that ACTH is the intermediary of the epinephrine activity, and that the site of action of the epinephrine is the anterior lobe. This finding is in accord with the data of Pincus and Hechter, who were unable to detect any increased output of steroids from perfused beef adrenals when epinephrine was added to the perfusate.[20] This conclusion also agrees with that of McDermott, Fry, Brobeck, and Long,[16] who by applying epinephrine directly to anterior lobe tissue were able to demonstrate a direct activation. Their data indicate that epinephrine cannot be said to have this action through any influence that it may have upon the rate of peripheral utilization of cortical hormone, as proposed by Sayers.[25] This subject will be considered further in the following paper.

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