Effect of Continuous Injection of Epinephrine on Adrenal Cortex and Anterior Hypophysis.* (20567)

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The role played by epinephrine in exciting the pituitary-adrenocortical axis during stress is not clear. Some investigators hold that this hormone acts only as a non-specific stressor. Others imply, however, that epinephrine is a specific "trigger" substance which activates the hypophysis to secrete corticotropin when the organism is under stress(1). McDermott *et al.*(2) postulate that epinephrine is involved particularly during the early phase of adjustment to stressful conditions. Most of the evidence in support of the latter position has been derived from short-term experiments. Since epinephrine disappears rapidly from the blood stream(3), its concentration cannot be maintained at a constant level if the hormone is administered by intermittent injections. The availability of an apparatus for continuous injection of small animals made possible this study of the effect of the prolonged action of epinephrine on the hypophysis and adrenal cortex. When so administered, one should expect to find histological evidence of accelerated secretory activity in these glands if epinephrine carries out a specific role in stimulating the release of corticotropin by the anterior hypophysis.

Procedure. Male rats of the Sprague-Dawley strain having an initial weight of approximately 300 g were used in these experiments. They were force-fed a medium carbohydrate diet(4) by stomach tube each morning and late afternoon. The technics and diet were modifications of those of Reinecke, Ball and Samuels(5). Each 24-hour dose of epinephrine hydrochloride (Upjohn) was contained in 1 ml of physiological saline with 1 mg of ascorbic acid added as an anti-oxidant (Groups 1-4, Table I). The control rats received an equal amount of saline and ascorbic acid. Several dilutions of epinephrine were made from the original 1:1000 concentration. In order to administer maximal doses of epinephrine over a period of weeks, it was necessary to begin with a moderate dose and to increase dosage as tolerance developed. Doses larger than those used here killed the rats. Each rat was placed in a metabolism cage which restricted its activity so that the animal was unable to reverse its position. A 21-gauge needle, having a barb attached to its shank to prevent withdrawal, was placed subcutaneously. Sterile needles were used for replacement every 48 hours. The solutions were administered to 6 rats simultaneously by a continuous injection machine. Three of the 6 rats received epinephrine and 3 received control injections. The room temperature was 74° to 78°F. At the end of a continuous injection period of 1 to 21 days the rats were anesthetized with cyclopal for autopsy.

Since Finerty, Hess and Binhammer(6) have postulated that accelerated secretion of corticotropin is accompanied by an increase in the phospholipid content of the anterior hypophysis, this material was stained in the pituitary glands of some animals by the method of Baker(7) after fixation in formaldehyde-calcium. Adrenal glands were fixed in Bouin's fluid and stained with the Masson(8) procedure. Others were preserved in 10% neutral formalin buffered to pH 7 by 0.2 M phosphate buffer. Frozen sections of these glands were stained with Sudan black B or by the Schultz(9) procedure for the demonstration of cholesterol and its esters. Paraffin-embedded sections of other adrenal glands similarly fixed were stained for esterase activity by the naphthol AS acetate technic of Gomori(10). The criteria used as evidence of secretion of corticotropin under the influence of epineph-

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TABLE I. Effect of Continuous Injection of Epinephrine on Mean Weight of Body, Thymus and Adrenal Gland.

Group	Treatment	Duration (days)	No. of rats	Body wt (g) Initial	Body wt (g) Final	Thymus wt (mg)	Adrenal (1) wt (mg)
I	Epinephrine 1:5000	7	9	273±11†	264± 8	121±66	30± 4
	1:2500	7					
	1:1000	7					
	Saline	21	9	278± 9	276±16	246±48	30± 2
II	Epinephrine 1:1000	2	3	296±10	295± 4		
	Saline	2	3	300±13	298± 5		
III	Epinephrine 1:5000	7	3	349± 9	313± 5		37±25
	1:2500	14					
	Saline	21	3	345± 9	303±18		29± 2
IV	Epinephrine 1:5000	1–2	4	326± 4	316± 4		
	Saline	1–2	4	333± 4	327± 5		
V	Epinephrine 1:2500	1	9	332±48		375±62	24± 2
	Saline	1	9	333±38		389±63	28± 3

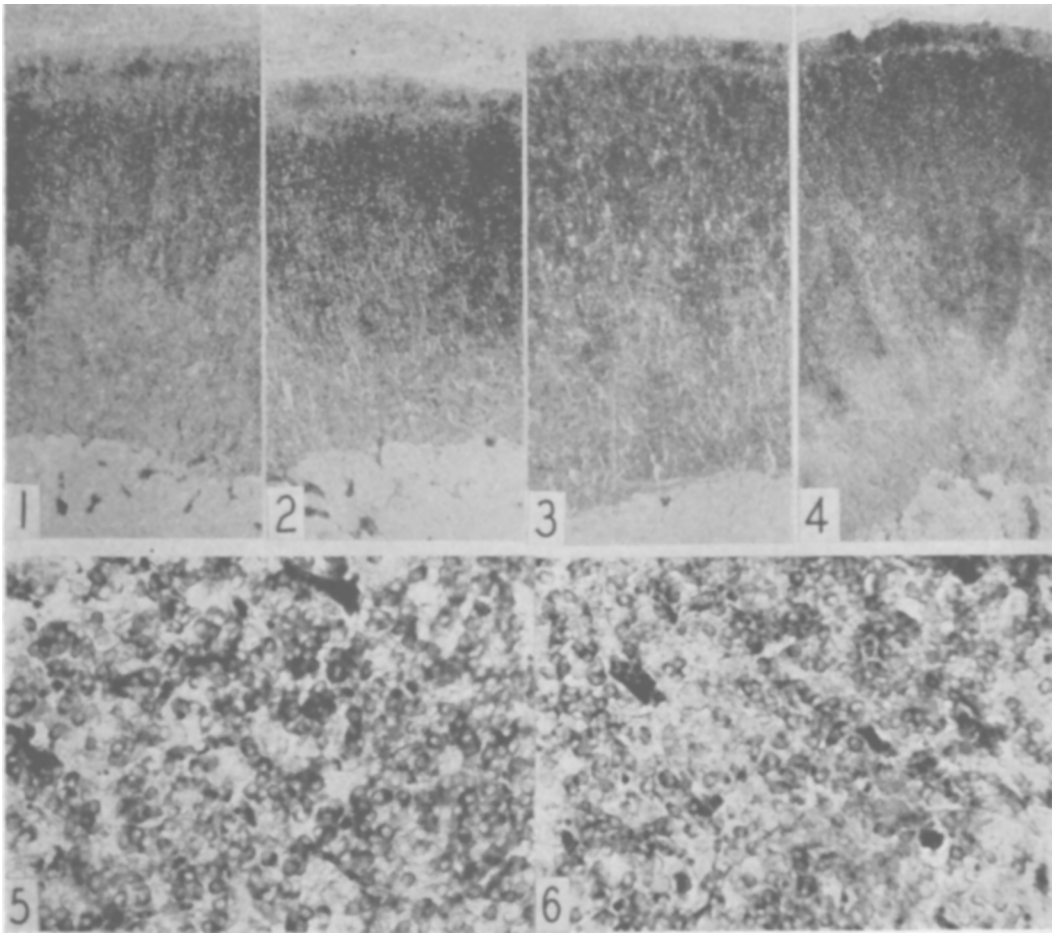
† Stand. dev. $= \frac{\sqrt{\Sigma d^2}}{N}$.

rine were: a) the phospholipid content of the anterior hypophysis, b) weight of the adrenal glands, c) size and mitotic activity of parenchymal cells in the zona fasciculata, and d) depletion of sudanophilic lipid and e) of cholesterol from the adrenal cortex. Since administration of exogenous corticotropin to rats elicits all of these changes in the adrenal cortex(11), epinephrine might induce similar effects if it is capable of causing the hypophysis to liberate significant quantities of corticotropin.

Observations. All of the rats exhibited transitory glycosuria which was maintained only when the dose of epinephrine was increased over that given at the beginning of treatment.

Fig. 5 and 6 show that the injection of epinephrine for 21 days did not increase the total amount of phospholipid in the anterior hypophysis or the number of cells which contained large amounts of phospholipid. As judged by all of the criteria of secretory activity used in this study, epinephrine failed to stimulate the adrenal cortex consistently. The total weight of the gland was not increased (Groups 1 and 5, Table I). An increase in mean weight of the adrenal glands is indicated in Group 3 but of the 3 rats receiving epinephrine, the adrenal glands of one were massive in size whereas those of the other two rats were lighter than the adrenal glands of their controls. Excluding this exceptional rat which exhibited signs of epinephrine toxicity and was near death prior to autopsy, there was no hypertrophy or hyperplasia of the parenchymal cells in the zona fasciculata of the cortex in any experiment. Significant depletion of sudanophilic lipid did not occur (Fig. 1 to 4). The intensity of color induced in the adrenal cortex by the Schultz procedure was somewhat less in only a few rats injected with epinephrine and ascorbic acid for 21 days. Esterase activity is evident in all zones of the cortex in control animals, being most intense in the zona glomerulosa and zona fasciculata. It was not altered in either zone by epinephrine.

Discussion. The auto-oxidation of epinephrine while in the hypodermic syringes presents a problem which may not have been solved completely in these experiments. It was prevented in the 21-day injection experiments by the addition of ascorbic acid to the fluid being injected. Bacchus *et al.* have reported that treatment of rats with ascorbic acid prevents the eosinopenia and depletion of adrenal cholesterol which occurs following the administration of epinephrine(12) but does not block the eosinopenic response which follows treatment with corticotropin(13). They concluded that ascorbic acid either antagonizes epinephrine directly or inhibits the release of



Adrenal cortices shown in Fig. 1 to 4 were fixed in 10% formalin and stained with Sudan black B (×60). Anterior hypophyses illustrated in Fig. 5 and 6 were stained with Baker's acid hematein (×210).

FIG. 1. Control for Fig. 2.
FIG. 2. After inj. of epinephrine (1:5000) for 48 hr without ascorbic acid.
FIG. 3. Control for Fig. 4.
FIG. 4. After inj. of epinephrine (1:5000, one wk; 1:2500, one wk; 1:1000, one wk) with ascorbic acid.
FIG. 5. Control for Fig. 6.
FIG. 6. After inj. of epinephrine as for Fig. 4.

corticotropin by the hypophysis. These observations indicate that ascorbic acid may have been responsible for the absence of pituitary-adrenal response to epinephrine in Groups 1 through 4. However, the quantity of ascorbic acid administered by Bacchus *et al.*(13) was enormous as compared with that used in our experiments. They injected 88 mg/100 g body weight intraperitoneally as compared with 1 mg per rat in our study. It seems improbable that this small amount of ascorbic acid could interfere with the action of epinephrine. Epinephrine did elicit the usual pharmacologic signs of its action in these experiments and in high dosage was definitely toxic.

In order to eliminate the use of ascorbic acid, epinephrine was injected continuously without ascorbic acid (Group 5, Table I) for 24 hours since during this period there was little evidence of oxidation of epinephrine as indicated by coloration of the solution. Likewise, in this experiment there was no histological evidence of adrenocortical stimulation by epinephrine.

As determined by chemical analysis, it is generally recognized that a fall in concentration of cholesterol occurs in the adrenal cortex immediately after administration of epinephrine for short periods of time(1). This effect is mediated by the increased secretion of corticotropin by the anterior hypophysis. The decrease in cholesterol which follows the injection of corticotropin involves the esterified but not the free form and may occur without any alteration in the amount of free fat in the adrenal cortex(14). The Schultz reaction applied to tissue sections is only a crude quantitative index of concentration and does not differentiate esterified from free cholesterol. In our experiments, a reduction in the former may have been masked by the free cholesterol present in the gland. Nevertheless, exogenous corticotropin induces a striking reduction in the intensity with which the Schultz procedure stains the adrenal cortex. The negative observations herein reported are important because they show that injection of epinephrine fails to duplicate the histological alterations induced in the adrenal cortex by injection of corticotropin(11) even though corticotropin presumably mediates the action of epinephrine on the adrenal cortex.

Several studies in man have failed to reveal significant stimulation of the adrenal cortex by the administration of epinephrine. Nelson *et al.*(15) found no rise in the concentration of 17-hydroxy adrenal steroids in the blood after intravenous injection of epinephrine. Jeffries *et al.*(16) did not observe an increase in urinary 17-ketosteroid excretion. Thorn *et al.* (17) reported that intravenous injection of epinephrine in quantities sufficient to cause eosinopenia and striking systemic reactions to the drug, failed to increase the output of 17-hydroxy adrenal steroids. Our observations concur with these investigations in man and lead to the conclusion that epinephrine is not capable of stimulating the hypophysis to secrete corticotropin at a rapid rate.

Summary. Under the conditions of our experiments, the continuous subcutaneous injection of massive doses of epinephrine into rats for 1 to 21 days, failed to elicit histological evidence of pituitary-adrenocortical stimulation.

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