servations that fibrinolysin(6) and plasma kinin releasers(8) are both present in plasma and capable of catalyzing the hydrolysis of BAEE.

Summary. The hydrolysis of the synthetic substrate BAEE (Benzoyl-l-arginine ethyl ester) by proteolytic enzymes present in plasma is increased by heparin and its synthetic analogue, Mepesulfate. This action of heparin and Mepesulfate is not produced by the calcium binding anticoagulant, potassium oxalate. The hydrolysis of BAEE by plasma is also catalyzed by glass contact resulting in values which are higher and statistically different from those obtained when the reaction is carried out in polyethylene tubes. The implications of these results and possible mechanisms for these findings are considered.

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Vasopressin and ACTH Release. (27523)

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The hypothesis that vasopressin is the neurohumor from the hypothalamus which stimulates the release of ACTH has had strong support, both in anatomical and physiological studies(1,2,3,4). However, a serious criticism of the physiological evidence has been that very large amounts of vasopressin have been necessary to demonstrate ACTH release. McCann(5) has reported 0.6 U of vasopressin as the effective intravenous dose for depletion of adrenal ascorbic acid in rats with lesions in the median eminence. Casentini *et al.*(4) found that 12 mU produced a comparable depletion in adrenal ascorbic acid when given into the carotid artery of rats blocked with 9-alpha fluorohydrocortisone. These amounts of vasopressin are several thousand times the amount which produces antidiuresis in the rat; according to Dicker (6), the rat is sensitive to the antidiuretic effect of 3.5 μU/100 g body weight. Kwaan and Bartelstone(7) have set up experiments which approach more closely physiological conditions. They found that 2 mU of vasopressin injected into the IIId ventricle of the dog produced an increased secretion of adrenal steroids.

Using a new method for testing ACTH release, we have found that 1.0 μU of vasopressin injected into the arterial blood supply of an ACTH-secreting pituitary tumor grafted in the hind leg of a hypophysectomized rat causes release of measurable amounts of ACTH.

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Methods. Vasopressin solution. A stock solution of vasopressin from a commercial preparation of pituitrin (Pitressin, Parke-Davis) was made up in 0.1% acetic acid solution to contain 200 mU/ml. This was stored in 5 ml vials in the refrigerator at 4°C. Immediately before a test, the vasopressin solutions for that day are made from a fresh vial of the stock solution. This is diluted with saline and solutions of 1.0 μU/ml, 10 μU/ml, 50 μU/ml and 100 μU/ml are prepared. The test animals are injected with 0.1 ml volume of these solutions.

Animal preparation. An autonomous rat pituitary tumor, referred to as mammotropic pituitary tumor (MtT-F4) was furnished us by Dr. Jacob Furth, who had induced the primary tumor by implantation of a single pellet of stilbestrol (7-8 mg) into a rat of the Fischer strain(8). This tumor elaborates growth hormone, prolactin and ACTH in large amounts so as to produce a state of hyperpituitarism in the animals(9,10). The tumor strain is maintained for use in the laboratory by subpassages into Fischer rats. In the preparation of animals to be used for the test, tumor tissue is implanted into both hind legs and in 4-6 weeks when the animals are ready for use, the tumors have reached a size of 2-4 cm in diameter.

Operative procedure. To prevent the participation of the animal's own hypothalamico-hypophysial system, hypophysectomy is performed 3-5 hours before the test is started. Under ether anaesthesia, a laparotomy is performed and cannulae filled with heparin solution are inserted into the left adrenal vein and into one of the common iliac arteries. Tributary veins from the body wall into the adrenal vein are cauterized. A heavy thread is placed around the lower abdominal aorta to be used later as a tourniquet. Adrenal vein blood is collected in 3 samples, *i.e.*, a 15-minute sample before vasopressin is injected and 2 samples of 15 minutes each after the vasopressin. The blood is collected into centrifuge tubes packed in chipped ice. Before starting the first collection of blood, 0.1 ml heparin solution (100 units) is injected into the general circulation through the arterial cannula. Before the second collection period, the vasopressin solution (0.1 ml) is injected through the cannula in the common iliac artery and at the same time, the lower abdominal aorta is blocked by a tourniquet for 6.0 seconds, so that the vasopressin is infused directly into the tumor tissue in the hind legs.

Estimation of corticosterone in plasma. Corticosterone was measured by Peterson's method(11), which had been modified for use as a micromethod. This method is based on the fluorescence of corticosterone with sulfuric acid and is described in detail by Wherry *et al.*(10).

Results. The animals, when used for the test, had bilateral tumors measuring 2-4 cm in diameter and their adrenal glands were enlarged approximately 6 times normal size. The concentration of corticosterone in the adrenal vein blood collected 3-5 hours after hypophysectomy was found to be 1746 ± 178 μg/100 ml plasma (mean of 34 rats ± S.E.). Rats without tumors similarly hypophysectomized had corticosterone levels in the adrenal vein blood of 39 ± 5.5 μg/100 ml plasma (mean of 8 rats ± S.E.).

The secretion rate of corticosterone was taken as an index of the ACTH level in the circulating blood and, thus, any increase in secretion rate in response to vasopressin was interpreted as evidence of increased ACTH released from the tumor. The data of these experiments are given in Table I. Doses of vasopressin of 1.0 μU, 5.0 μU and 10 μU infused directly into the arterial blood supply of the tumor increased the secretion rate of corticosterone 32-39% within the first 15 minutes, indicating a significant increase in ACTH release. It is quite likely that the time interval between the vasopressin injection and the response was much shorter than 15 minutes, but the procedures of this test were not designed for smaller volumes of blood than those obtained in 15 minutes. During the second 15-minute collection, the corticosterone secretion rate returned to pre-injection rate. Vasopressin 0.1 μU and acetic acid solution only produced no increase in the corticosterone secretion rate. In contrast to the intraarterial injection, intravenous administration of relatively large amounts of vasopressin, up to 15.0 mU to hypophysecto-

TABLE I. Corticosterone Secretion Rates in Hypophysectomized Rats* with ACTH-Secreting Tumors before and after *Intra-arterial* Infusion of Vasopressin.

Dose vasopressin	No. rats	Corticosterone secretion rates (mean values, μg/hr) — Period before vasopressin 15 min.	Periods after vasopressin 0-15 min.			15-30 min.		
μU		μg/hr	μg/hr	p	% change	μg/hr	p	% change
0	9	22.2 (.020)†	19.6 (.015)	>.05 <.05	−11.7	18.3 (.015)	>.05 >.05	−17.6
.1	12	14.3 (.012)	13.2 (.010)	>.05 >.05	− 7.7	14.3 (.014)	>.05 >.05	.0
1.0	8	17.5 (.019)	24.4 (.023)	<.01 <.05	+39.4	18.3 (.019)	>.05 >.05	+ 4.6
5.0	9	24.2 (.023)	32.6 (.023)	<.05 >.05	+34.7	28.8 (.021)	>.05 >.05	+19.0
10.0	8	21.1 (.019)	27.9 (.020)	<.02 >.05	+32.2	23.1 (.016)	>.05 >.05	+ 9.5

* Hypophysectomy 3-5 hr previously. † Mean value for adrenal vein plasma flow, ml/min.

mized rats with tumors did not stimulate the release of ACTH (Table II).

To rule out the possibility of an effect of vasopressin directly on the adrenal cortex, vasopressin was given to hypophysectomized rats without tumors. Three rats were injected intravenously with 200 mU. It will be seen in Table II that there was no change in corticosterone secretion rate. In another experiment 5 hypophysectomized rats were injected with 200 mU vasopressin by way of the abdominal aorta so that vasopressin entered the adrenal artery. Average corticosterone secretion rate was 0.35 μg/hr before and 0.37 μg/hr after the injection. These findings confirm the observations of others(4) that vasopressin does not stimulate the adrenal cortex directly.

Discussion. Infusion of vasopressin directly into the arterial blood supply of the pituitary tissue was an attempt to simulate the physiological condition in which the neurohumor is transported by the portal vessels from the site of origin to the adenohypophysis. The finding that a dose as small as 1.0 μU so administered was highly active in stimulating release of ACTH and that a dose 1500 times this, namely 15 mU, given intravenously failed to stimulate release of any measurable amount of ACTH, suggests that the ACTH-releasing activity of vasopressin is very quickly removed from the circulation. This is in agreement with the studies of Ginsburg and Heller(12) who have shown that there is a very rapid clearance of vasopressin from arterial blood following intravenous administration. This loss of vasopressin activity is accounted for by the excretion of about 7% in the urine and inactivation of the remainder by kidney and liver.

Three peptide materials isolated from the neurohypophysis have been shown to stimulate the release of ACTH, namely, vasopressin, oxytocin, and a so-called corticotrophin-

TABLE II. Corticosterone Secretion Rates before and after *Intravenous* Injection of Vasopressin in Tumor and Non-Tumor Hypophysectomized Rats.*

	No. rats	Dose vasopressin, mU	Corticosterone secretion rates (mean values) 15-min. period before inj., μg/hr	15-min. period after inj., μg/hr	p
Rats with tumors	5	15.00	8.58	9.94	>.05
	5	1.50	5.62	3.82	>.05
	4	.15	9.45	5.63	>.05
Rats without tumors	3	200.0	.20	.13	

* Rats hypophysectomized 24 hr previously.

releasing factor (CRF) (13,14,15). There has been some controversy among investigators in this field as to whether vasopressin or CRF is the neurohumoral substance responsible for ACTH release. Those who favor CRF may interpret the findings reported here as due to a trace of CRF in the commercial preparation of vasopressin (Pitressin, Parke-Davis) which was used. Since the pitressin was diluted from 20 U/ml to 1.0 μU in 0.1 ml volume of weak acetic acid, it is more than likely that any contaminant was diluted out. It is hoped that there will be an opportunity to compare the activities of du Vigneaud's synthetic vasopressin, Saffran's CRF material and Guillemin's Fraction D. This may give the answer as to what is the ACTH-releasing neurohumor.

If one accepts the concept that vasopressin is the ACTH-releasing neurohumor, many physiological studies may be cited to corroborate this. Claude Bernard (16) first called attention to an antidiuresis in dogs subjected to emotional stresses and to operative procedures. Rydin and Verney (17) showed that the antidiuresis brought on in dogs by muscular exercise or noxious stimuli could be reproduced both in degree and in timing by injection of posterior pituitary extracts; others have shown an increase in vasopressin in blood and urine associated with a variety of stressful situations, such as hemorrhage, fainting, dehydration, acceleration and pain (18,19). It is well known that these same conditions are accompanied by an increased secretion of adrenal steroids. That vasopressin and ACTH should be released almost simultaneously would seem to indicate something more than mere coincidence.

The finding that doses of vasopressin of 1.0 μU, 5.0 μU and 10.0 μU all gave the same increase in ACTH release was not anticipated, and requires further study.

Although there was no intention at the outset of this study to develop another method for the assay of vasopressin, these findings suggest a method which may be especially useful for measuring vasopressin in blood, spinal fluid, and urine.

Summary. A new method of testing for ACTH-releasing activity of neurohumoral substances has been described. The material to be tested is injected directly into the arterial blood supply of an ACTH-secreting pituitary tumor grafted in the hind leg of a hypophysectomized rat. The amount of ACTH released is measured by the difference in corticosterone secretion rate before and after the injection. Amounts of vasopressin as minute as 1.0 μU to 10.0 μU release measurable quantities of ACTH. Doses of vasopressin 1,500 times this amount given intravenously have no effect on ACTH release. These findings suggest that vasopressin may be the ACTH-releasing neurohumor. It is suggested that the method may be used as an assay method for vasopressin.

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