TABLE III. Plaque Neutralization Test of Measles Virus by Serum Obtained in Course of Measles Infection.

Date of serum	Phase of disease	No. plaques expected in absence of serum	PFU/.1 ml in presence of serum						
			Undil.	1:5	1:25	1:125	1:625	1:3125	1:15625
9/30	Prior to clinical symptoms	43	20	46	40				
10/ 3	One day after rash	43	0	1	5	24	29	52	
10/ 8	Convalescence	43				0	10	24	48
11/10	"	43		0	0	0	32		

All serum samples were heated at 56°C for 30 min.

of measles (multinucleate giant cells with intranuclear inclusions) were observed in patas cultures 2 days after infection with large doses of virus, with small doses 2-3 weeks were necessary before the final reading could be made. With the plaque method, final results were obtained at the end of one week after inoculation and, in addition, there was no need for changing the medium, as was necessary for the tube method. Quantitative results were easily achieved by counting the number of the distinct small plaques.

Foamy agents(10) have been confused with measles virus because of their characteristic cytopathic changes in rhesus monkey kidney cultures in fluid medium. However, measles-like plaques have not been observed with the foamy agents under agar in rhesus cultures, although the cell sheets were destroyed by the agents. Foamy-like virus has not been recovered from the patas cultures which we have used in our studies over the past 3 years(7,8), except recently from 2 patas monkeys which had been kept in the stock colony for 10 months or longer, and the possibility exists that they might have acquired their infections from other monkeys in the colony.

Summary. Measles virus produces tiny but distinct plaques with sharp boundaries, in bottle cultures prepared from kidneys of African red grass monkeys (*Erythrocebus patas*). As with other viruses, quantitative assays and neutralization tests with measles virus may now be carried out by the plaque technic.

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Extrapituitary Interaction between Pitressin and ACTH.* (23944)

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Pitressin increases the level of ACTH in the blood(1). It has been assumed that this effect is mediated solely by the action of vasopressin on the adenohypophysis(2). The following studies were undertaken to determine whether this assumption is correct.

Methods. Adrenal ascorbic acid depletion

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TABLE I. ACTH Activity of Pitressin and Purified Natural Arginine Vasopressin.

Exp.	Test substance	Dose	Adrenal ascorbic acid depletion (mg/100 g of adrenal) Individual responses*	Avg	Estimate of ACTH activity, mu/unit‡
A	ACTH U.S.P. standard	1.0 mu†	165, 106, 117, 146, 193, 165	149	
		.25 "	39, 58, 29, 27, 48	40	.10 ± .02§
	Pitressin	5.0 units‡	104, 104, 45, 53, 63, 141	85	
		2.5 "	49, 91, 46, 68	64	
B	ACTH U.S.P. standard	.5 mu	63, 37, 80, 53, 92, 87	69	
		.25 "	−87, −3, 66, −29, −25, 20, 95	5	
	Purified natural arginine vasopressin	5.0 units	55, 67, 29, 31, 35, 188, 18, 75	62	.09 ± .02
		2.5 "	27, 14, 24, 58, 7, 38, 0	24	
C	Pitressin	5.0 units	59, 87, 39	62	
		1.0 "	55, 68, 40, 4, −14, 46	33	
		.2 "	−4, 10, 18, 39, −37	5	
		.04 "	20, −3, 4, 28, −19	6	

* Difference in ascorbic acid concentration in left adrenal removed prior to inj. of test substance and concentration in right adrenal removed one hr after inj.
† Milliunits of ACTH. ‡ Units of pressor activity. § ± stand. error.

assays for ACTH were performed 24 hours after hypophysectomy in male rats weighing from 110 to 130 g(3). Hormones‡ were injected intravenously over a period of 5 minutes and were dissolved in 1 ml of 0.9% sodium chloride solution brought to pH 2 by addition of concentrated HCl (acid-saline). Adrenal ascorbic acid depletion assays for ACTH were also performed in rats bearing destructive lesions of the median eminence area of the hypothalamus. The lesions were made in male rats weighing 250 to 300 g with a high frequency alternating current delivered by a stereotaxically placed electrode. Immediately after lesioning the rats were placed in metabolic cages and their 24-hour water consumption and urinary output were determined. Only rats with severe diabetes insipidus (urinary volumes greater than 75 ml) were retained for further study. These animals fail to respond to a variety of stimuli with adrenal ascorbic acid depletion(1). Forty-eight hours after lesioning, the diabetes insipidus rats were divided into 2 groups. Animals of one group were anesthetized with ether and the left adrenal removed. The trachea was intubated and intermittent positive pressure respiration instituted. The animal's neck was then crushed between the jaws of a large surgical clamp which was placed rostral to the tracheotomy. This procedure completely stops circulatory exchange between the head and the trunk. The animal may be considered decapitated. Following decapitation, 1 ml of acid-saline containing either Pitressin or epinephrine was intravenously infused over a 5-minute period. Thirty minutes after completion of infusion the right adrenal was removed. The second group of rats was treated in the same manner except tracheotomy and decapitation were not performed. The decapitated animals exhibited hypotension at the conclusion of the experiment. The persistence of strong nociceptive reflexes in these animals indicated that despite the hypotension circulation was adequate to maintain some degree of spinal cord function.

Results. Pitressin depletes adrenal ascorbic acid in hypophysectomized rats. This effect is observed only with doses of Pitressin which are much larger than those necessary for demonstration of the antidiuretic and the pressor effects of the hormone. The 5 unit dose was found to be fatal in about 10% of the animals injected. One unit of Pitressin appears to be equivalent to 0.1 milliunit (mu) of ACTH with respect to adrenal ascorbic acid depleting activity (Table I, Exp. A and B). Highly purified natural arginine vasopressin obtained

‡ Grateful acknowledgement is made to Dr. Dan McGinty of Parke, Davis and Co. for generous gifts of Pitressin and of epinephrine, and to Dr. Vincent du Vigneaud for supply of highly purified natural arginine vasopressin (AVN-5).

TABLE II. Potentiation of ACTH by Addition of Pitressin.

Exp.	Test substance	Dose ACTH, mu†	Dose Pitressin, units‡	Adrenal ascorbic acid depletion (mg/100 g of adrenal) Individual responses*	Avg	Potentiation with 95% fiducial limits
D	U.S.P. ACTH standard	2.0		128, 148, 144, 143, 109, 124	133	
		1.0		70, 72, 88, 38, 95, 94, 123	83	
		.5		12, 37, 22, 30, 12, 38, 79	33	
	Idem + Pitressin	.50	5.0	140, 141, 128, 124, 125, 114, 134	129	1.96 (1.49–2.57)
		.25	5.0	124, 63, 167, 118, 100, 102	112	
E	U.S.P. ACTH standard	2.0		146, 143, 166, 165, 108, 164, 137, 118	143	
		1.0		143, 118, 86, 130, 117, 98, 86	111	
		.5		96, 70, 65, 70, 112, 51, 57	74	
	Idem + Pitressin	1.2	.2	114, 125, 178, 126, 137, 134, 119, 164	137	1.55 (1.14–2.11)
		.6	.2	85, 93, 120, 79, 180, 187, 131	125	
		.3	.2	70, 61, 46, 68, 73, 24, 78, 40	58	

* See Table I. † Milliunits of ACTH. ‡ Units of pressor activity.

from Dr. V. du Vigneaud exhibits ACTH activity similar to that of Pitressin. This suggests that the ACTH-like action of vasopressin is probably not due to the presence of a contaminant.

Pitressin potentiates the adrenal ascorbic acid depleting activity of ACTH. Comparison of the potency of ACTH administered with 5 units of Pitressin to that of ACTH administered alone yields a potency ratio of 1.96 to 1 (Table II, Exp. D). This potency estimate assumes that 5 units of Pitressin contributes the equivalent of 0.5 mu ACTH. Thus the doses of ACTH employed in the calculation of the potency ratio in assay D were 2.0, 1.0 and 0.5 mu for ACTH alone and 1.0 and 0.75 mu for the combination of ACTH and Pitressin. The potentiation is obviously not accounted for by simple addition. The accuracy of the estimate of potentiation is in part dependent on the accuracy with which the ACTH activity of Pitressin is determined. To obviate this source of error, a potentiating dose of Pitressin was sought which had negligible ACTH activity. Potentiation of adrenal response to ACTH was found with 0.2 unit of Pitressin. At this dose level Pitressin has no significant effect on adrenal ascorbic acid in the hypophysectomized rat (Table I, Exp. C), but potentiates the action of ACTH by a factor of 1.55 (Table II, Exp. E). In Exp. D and E, the potentiation is statistically significant.

Pitressin depletes adrenal ascorbic acid in the decapitated median eminence lesioned rat. Epinephrine is devoid of activity (Table III). The degree of depletion is significantly less than that observed in the nondecapitated median eminence lesion rat. This difference may be due to Pitressin induced ACTH release by the adenohypophysis of the non-decapitated rat or to the shock-like state of the

TABLE III. Action of Pitressin in Median Eminence Lesioned Rat.

Animal preparation	Test substance	Dose	Adrenal ascorbic acid depletion (mg/100 g of adrenal) Individual responses*	Avg ± S.E.
Median eminence lesion	Pitressin	5 units	149, 193, 158, 202, 150, 167, 183	172 ± 8
	"	.5 "	123, 131, 183, 135, 148	144 ± 11
Idem—decaptitated	"	5 "	110, 131, 176, 116, 164, 113, 149, 146, 96, 169, 82, 155, 82	130 ± 9
	"	.5 "	102, 76, 75, 85	85 ± 6
	Epinephrine	10 μg	76, −20, 71, −1, −15, 39	25 ± 17

* See Table I.

decapitated animal. Regardless of the cause of this difference, the extrahypophysial action of Pitressin is demonstrated.

Discussion. Attention has been focused on the adenohypophysis as the site of action of vasopressin in elevating blood ACTH. The observations reported here and those of Hume and Nelson(4) indicate that vasopressin acts to influence blood ACTH by extrapituitary mechanisms. The *in vivo* potentiation of ACTH by Pitressin may be explained in part by inhibition of a degradative enzyme system (5,6). The possibility cannot be ruled out that part of the potentiation is due to blockade by Pitressin of peripheral binding sites for ACTH. In this connection, Richards and Sayers(7) have demonstrated small stores of ACTH in the kidney of the rat and Higginbotham and Dougherty(8) have interpreted the action of ACTH in enhancing toxicity of polymyxin B as due to competition of the 2 substances for peripheral binding sites. Since highly purified natural arginine vasopressin and synthetic vasopressin(4) both activate the adrenal cortex in the absence of the adenohypophysis, it seems unnecessary to ascribe the ACTH-like activity of Pitressin to contamination with ACTH. Two alternate explanations exist. Vasopressin may act directly on the adrenal cortex or vasopressin may release ACTH from nonadenohypophysial storage sites.

McCann(2) has presented evidence to support the thesis that vasopressin is the corticotropin releasing factor (CRF). On the other hand, Sayers(9) takes the position that CRF is not vasopressin but another basic polypeptide which can be extracted from the hypothalamus. The problem has been complicated by the fact that Pitressin and the hypothalamic extract of Sayers have ACTH activity when assayed in 24-hour hypophysectomized rats. These experimental observations are brought into harmony by the following working concept.

ACTH and a number of other basic polypeptides compete for binding sites both within the adenohypophysis and in extrapituitary tissue. Within the adenohypophysis certain basic polypeptides release bound ACTH by displacing it from intracellular binding sites. One of these polypeptides is the physiological CRF, another is vasopressin. In extrapituitary tissue basic polypeptides exchange with bound ACTH to bring about release of the tropin. CRF and vasopressin appear to have ACTH activity in the hypophysectomized or decapitated rat by virtue of this mechanism. ACTH and other basic polypeptides also compete for sites in a proteolytic system. The possibility exists that ACTH competes with basic polypeptides at receptor sites in the adrenal cortex.

This concept has proved to be of value in the design of experiments concerned with release of ACTH from the adenohypophysis. The general applicability and theoretical soundness of the concept will be determined by accumulation of additional experimental data. Regardless of the outcome, the conclusion is inescapable that vasopressin and hypothalamic extracts influence the level of ACTH in the blood by extrapituitary actions as well as by release of ACTH from the adenohypophysis.

Summary. Pitressin depletes adrenal ascorbic acid and potentiates the action of ACTH in the 24-hour hypophysectomized rat. The observation that Pitressin depletes adrenal ascorbic acid in the rat with a lesion in the median eminence has been confirmed. Only part of this effect can be explained by release of ACTH from the pituitary since Pitressin will also deplete adrenal ascorbic acid in a lesioned rat which has been decapitated.

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Addendum. After the manuscript was submitted for publication, synthetic arginine vasopressin was found to be as potent as Pitressin per unit of pressor activity in depleting adrenal ascorbic acid in 24-hour hypophysectomized rats.

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Relative Lack of Pharmacologic Action of 3-Methoxy Analogue of Norepinephrine.* (23945)

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Investigations within the past year have indicated that a major pathway for metabolism of norepinephrine in the body is by a combination of methylation of the 3-hydroxy position and oxidative deamination. Armstrong, McMillan and Shaw(1) reported that 3-methoxy-4-hydroxy mandelic acid (VMA) is a major urinary metabolite of norepinephrine and epinephrine in man. Subsequently, Axelrod(2) showed that the 3-hydroxy positions of both of these amines can be methylated by animal tissues *in vitro* and *in vivo* to yield the corresponding 3-methoxy analogues. Axelrod *et al.*(3) have presented evidence that methylation *per se* and methylation followed by oxidative deamination are the major means of metabolizing norepinephrine administered to rats. It is still not certain, however, to what extent endogenous norepinephrine is metabolized in the same manner.

Recently, m-O-methylnorepinephrine (normetanephrine), presumably arising near the site of endogenous formation of norepinephrine, was found in adrenal gland and spleen (personal communication), in the brain of rats after administration of iproniazid(4), and in norepinephrine-producing tumors (pheochromocytomas) of man(5). It seemed important to determine whether this new amine would produce central or peripheral effects on administration to lower animals and to human subjects. The results to be reported suggest that O-methylation of norepinephrine *in vivo* results in its inactivation.

Methods. Studies of the neuropharmacological effects of normetanephrine (NMN) were carried out in cats. Clinical studies were conducted in 2 patients after observations in the dog suggested only weak pressor activity.

A. *Pharmacological studies in animals.* Studies of the effects of NMN on electrical activity of the brain were carried out in anesthetized cats, and in unanesthetized cats with permanently implanted electrodes. The anesthetized preparations (pentobarbital, 30 mg/kg, intraperitoneally) were employed in studies of the effects of intracarotid administration of NMN on the transcallosal response. In these preparations the transcallosal response was recorded from one suprasylvian gyrus following both maximal and submaximal electrical stimulation of a corresponding point on the contralateral hemisphere. NMN was administered *via* a small polyethylene catheter which had been placed in a thyroid branch of the common carotid artery and advanced until the orifice of the catheter was at the junction of the common carotid artery and the branch which had been cannulated. NMN was dissolved in isotonic NaCl solution; each injection was made in a volume of 1 ml. Chronic preparations with permanently implanted electrodes were employed to study the effects of the NMN on spontaneous cortical activity and on a variety of evoked cortical responses. The cortical response to a

* The normetanephrine was supplied as the hydrochloride through the generosity of Dr. Julius Axelrod and Dr. Bernard Witkop.