with the elevation of PL level of plasma reported by KWA and VERHOFSTAD[3].

The decrease in either PL content or concentration observed in the afternoon of PE was prevented by the injection of sodium pentobarbitone (Pb) at 13.30 h of PE. The level of PL in the pituitary of the Pb injected animals remained high even at 17.30 h, and it was significantly higher than corresponding values of intact animals.

These findings seem to indicate that the release of PL takes place on the afternoon of PE around the 'critical period' for LH surge, and that the central nervous system could trigger the release. NEILL[13] reported increase in plasma prolactin in the afternoon of proestrus in the rat and WUTTKE and MEITES[14] blocked the increase by pentobarbital.

Résumé. La teneur et la concentration de la prolactine dans le lobe antérieur de l'hypophyse ont été déterminées aux temps variés de l'oestrus chez la ratte. Dans l'après-midi du jour du pro-oestrus, la teneur et la concentration dans la glande s'abaissent. Ce changement a été bloqué par le pentobarbitrate de sodium injecté par voie i.p. á 13.30 h au jour du pro-oestrus.

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The Effect of Acetylcholine on Adrenal Function in the Hypophysectomized Dog

Until recently, the significance of an intimate anatomic proximity of adrenal cortical to medullary tissue was obscure. In 1965, WURTMAN and AXELROD[1] reported the effect of adrenal glucocorticoids upon the medullary enzyme phenylethanolamine-N-methyl transferase (PNMT) which is responsible for the N-methylation of norepinephrine to form epinephrine. This enzyme is found to be localized in highest concentrations in the adrenal medulla of mammals.

Acetylcholine (ACh) has been shown to be the physiological stimulus for the release of epinephrine and norepinephrine from the adrenal medulla. However, the in vivo role of acetylcholine as an adrenal cortical stimulus has not been explored.

The main objective of the present investigation was to simultaneously study the acute and direct effect of ACh on adrenal medullary and cortical activity in the hypophysectomized animal.

Experiments. 4 mongrel dogs of both sexes weighing between 20 and 28 kg were used. Following the induction of anesthesia with 30 mg of i.v. sodium pentobarbital per kg of body weight, a maintenance airway was provided with the placement of a tracheal canula. Blood pressure was recorded from the right carotid artery by a transducer and physiograph instrument. A 0.9% sodium chloride i.v. drip was begun. The animals were then immediately hypophysectomized by the transbuccal approach as described by MARKOWITZ and ARCHIBALD[2]. 4 h later, the right adrenal was removed and quickly frozen using dry ice and acetone.

A teflon catheter, sized according to the dog's body length, was then filled with sodium heparin, and passed via the left femoral vein into the inferior vena cava, and thence into the left lumbar-adrenal vein and a securing ligature placed. This catheter allowed the direct collection of adrenal venous blood into heparinized tubes which were maintained in ice until centrifuged for 10 min at 2000*g*. The plasma was removed and stored at $-10\,^{\circ}C$ for analysis of catecholamine and hydrocortisone concentration.

At the conclusion of the experiment, the left adrenal gland was removed and quickly frozen with dry ice and acetone and stored at $-10\,^{\circ}C$ until analysis.

Acetylcholine was injected into the femoral vein in doses ranging from 0.42 and 0.95 mg/kg and blood samples were drawn 5 min after each injection.

The adrenal glands and adrenal venous blood were assayed for epinephrine and norepinephrine by the method of von EULER and LISHAJKO[3]. The adrenal glands were also assayed for PNMT activity by the method described by AXELROD[4] involving the use of C^{14}-S-adenosylmethionine. (One unit of enzyme activity

Table I. Dose-response to acetylcholine in hypophysectomized dogs

Dog No.	ACh dose (mg/kg)	170 HCS	Epi	Norepi	% E of total cat. secretion
1	0		5.0	12.6	35
	0.42	0	41.0	12.9	76
	0.83	0	171.1	55.8	76
2	0	0.4	4.2	8.9	31
	0.43	0.1	41.5	5.2	89
	0.72	0.0	119.4	10.9	90
3	0	0	2.8	3.0	48
	0.5	0	76.3	18.1	81
4	0	0.2	3.4	10.5	25
	0.48	0.9	60.0	14.2	80
	0.72	0.1	180.6	36.8	81
	0.95	0	225.0	45.3	83

170 HCS reported as μg/ml of plasma. Epinephrine and Norepinephrine reported as μg/100 ml plasma.

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catalyzes the formation of one nmole of product per h.) Hydrocortisone was measured in the adrenal venous plasma by the technique of NELSON and SAMUELS[5].

Results and discussion. The results of this experiment are shown in Tables I and II. Hydrocortisone values 4 h after hypophysectomy indicate the success of the operation. Also note the ratio maintained by epinephrine in the total catecholamine secretion. In the hypophysectomized animal, this averages 35%; however, after the injection of acetylcholine, the percentage increases to an average of 82%.

Acetylcholine did not significantly stimulate secretion of hydrocortisone. However, an effect on catecholamine was adequately demonstrated and is shown in the Figure. There is an observable correlation between dose of acetylcholine and epinephrine secretion.

The enzyme (PNMT) activity in the adrenal glands of these dogs tested was not significantly different from control values (Table II).

To ascertain whether the effect of acetylcholine on adrenal medullary function is dependent upon, or related to, adrenal cortical function, graded doses of acetylcholine were administered to the hypophysectomized dog, and adrenal output of glucocorticoids measured. Several in vitro studies[6,7] have demonstrated release of cortical hormones from adrenal slices incubated in an acetylcholine-rich media; however, no reports have been found in the literature of an in vivo stimulation of adrenal cortical secretion by acetylcholine.

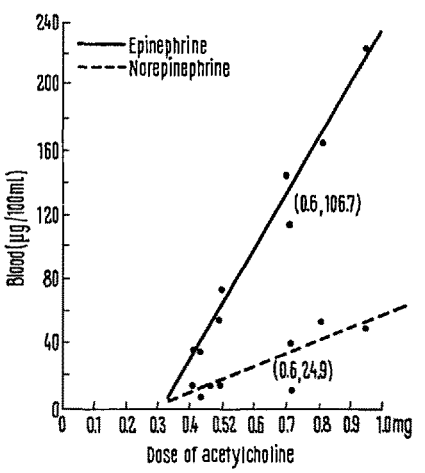
Our results in vivo demonstrate that administration of ACh fails to stimulate glucocorticoid secretion in the hypophysectomized dog at a time when amounts of catecholamines were released at levels comparable to those observed following splanchnic stimulation[8].

Previous experiments have described the release of epinephrine and norepinephrine from the adrenal gland after the injection of ACh[9,10]. Our findings support these earlier reports. Furthermore, the present findings suggest that ACh mediated release of catecholamines, suggested to be a mechanism involving granule release[11], operate independently of adrenal cortical-medullary interrelationship described earlier by WURTMAN and AXELROD[12]. Our results showing no consistent change in the enzyme PNMT activity suggest that this enzyme is probably not effected by acetylcholine. Rather, we would propose a dual control of the adrenal medullary catecholamines. One involving the production of epinephrine, and the other concerned with the release of both epinephrine and norepinephrine. The conversion of norepinephrine to epinephrine, which occurs in adrenal medullary cytoplasmic areas, is regulated by levels of PNMT and under the permissive control of available adrenal cortical steroids. Whereas, the active release of epinephrine and norepinephrine into the circulation occurs from storage sites, presumably granules[11], and is mediated or controlled by acetylcholine. Absence of an effect of ACh on PNMT levels, as described in this paper, lends support to this concept.

Table II. Effect of acetylcholine on adrenal medullary catecholamine content and PNMT activity

Dog	Gland		Epi	Norepi	PNMT
1	R	4 h post hypox	204.1	39.1	53.6
	L	0.83 mg/kg ACh	148.9	18.7	61.0
2	R	4 h post hypox	146.2	41.5	46.8
	L	0.72 mg/kg ACh	107.0	28.5	47.4
3	R	4 h post hypox	125.9	50.3	62.9
	L	0 mg/kg ACh	76.3	26.9	64.3
4	R	4 h post hypox	109.3	32.4	50.3
	L	0.95 mg/kg	76.7	16.9	48.4

Epinephrine and norepinephrine reported as μg/g adrenal weight. PNMT reported as units of activity. One unit catalyzes the formation of 1 nmole of product per h.



Composite linear regression of catecholamine response to ACh in hypophysectomized dogs.

Résumé. Après injection d'acétylcholine, les chiens hypophysectomisés ne secrètent pas d'hydrocortisone, mais présentent une élevation d'épinéphrine et de norépinéphrine dans le sang veineux surrénal. Aucun changement ne fut observé dans la concentration de la phényléthanolamine-N-methyl transférase dans ces glandes. Ces expériences indiquent que la relation médullaire surrénale corticale que nous avons mentionée précédemment fonctionne indépendamment de la secrétion de catécholamines, engendrée par l'Ach, de la glande surrénale.

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