



Fig. 3. Intensity of sexual reproduction in *Daphnia pulex* (Peter Lake stock) as a function of population density. Intensity of sexual reproduction (diapause) increases with log of density. The cultures were maintained at 19.0°C and at LD 12:12. The adults were transferred every other day into 50 ml of medium containing a standard concentration of food.

vironments. Unlike the terrestrial, the aquatic environment of north temperate latitudes affords adequate heat to permit a motile (adult) stage to overwinter (11).

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12. The lakes were separated in 1951 by A. D. Hasler for studies of lake alkalization. Observations of the time of sexual reproduction were made in six different years, beginning in 1954. A description of the effects of alkalization may be found in R. G. Stross and A. D. Hasler, *Limnol. Oceanogr.* **5**, 265 (1960).

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Adrenaline Synthesis: Control by the Pituitary Gland and Adrenal Glucocorticoids

Abstract. The activity of phenylethanolamine-N-methyl transferase, an enzyme that synthesizes adrenaline from noradrenaline in the adrenal medulla, is markedly depressed following hypophysectomy. Enzyme activity is restored to normal after administration of ACTH or the potent glucocorticoid, dexamethasone. Thus the biosynthesis of adrenaline in the adrenal medulla appears to be regulated by the pituitary-adrenocortical system.

The final step in the biosynthesis of adrenaline involves the transfer of a methyl group from *S*-adenosylmethionine to the amine nitrogen of noradrenaline (1). This process is catalyzed by an enzyme, phenylethanolamine-N-methyl transferase (PNMT), which is highly localized in the adrenal medulla of mammals (2). Although low levels of activity of this enzyme have been detected in heart and brain, it is likely that almost all of the adrenaline in the circulation is derived from catecholamine synthesized within the adrenal medulla (3).

Little is known about the factors which control the formation of adrenaline in vivo. In species in which the adrenal medulla is not surrounded by adrenocortical tissue, the catecholamine content of the medulla is almost exclusively noradrenaline (4). On the basis of this observation, it has been suggested that the mammalian adrenal cortex secretes a factor which influences the methylation of noradrenaline (4). We now show that the activity of the adrenaline-forming enzyme, PNMT, in adrenal medulla of the rat is regulated by pituitary adrenocorticotrophic hormone (ACTH) and by adrenal glucocorticoids.

In our experiments, the PNMT activity and catecholamine content of the adrenal glands were measured in normal rats and in animals subjected to hypophysectomy or treatment with various hormone preparations. Groups of Sprague-Dawley female rats (5) weighing 160 to 200 g were killed after 6 days of treatment with hormone or placebo. Both adrenals were rapidly dissected free of fat, weighed, and homogenized in 2 ml of chilled isotonic potassium chloride solution. The homogenate was centrifuged at 100,000g for 30 minutes. A portion of the supernatant fluid was assayed for

PNMT activity by a modification of a method described before (2, 6). The remainder of the whole adrenal homogenate was diluted with an equal volume of 2*N* acetic acid and shaken vigorously in the cold. A portion of adrenal tissue was assayed for adrenaline and noradrenaline by a modification of the method of von Euler and Lishajko (7).

Hypophysectomy was associated with a marked reduction in the activity of PNMT in the rat adrenal (Table 1). This fall was of greater magnitude than the decrease in adrenal weight (which is due primarily to atrophy of adrenocortical tissue.) The amount of adrenaline contained in both adrenals was reduced by hypophysectomy, as was the percentage of the total adrenal catecholamine represented by adrenaline (Table 1). The fall in adrenaline content could represent changes in storage or release of the amine. However, it is also consistent with a decrease in the rate of synthesis of adrenaline, brought about by a decline in PNMT activity. When hypophysectomized rats were given daily injections of ACTH for 6 days, the total activity of PNMT in adrenal tissue increased threefold, returning almost to normal levels (Table 1). The fraction of adrenal catecholamine represented by adrenaline showed a similar response. Hökfelt has also observed an increase in the content of adrenaline in adrenals of hypophysectomized animals treated with ACTH (8).

There are three possible mechanisms whereby ACTH could have enhanced the enzymic methylation of noradrenaline in the hypophysectomized rat. (i) There could be a methylating enzyme in the adrenal cortex which responds directly to ACTH. (ii) ACTH could act on PNMT in the adrenal medulla. (iii) ACTH could act indirectly, by enhancing the synthesis of glucocorticoids in the adrenal cortex and their delivery to the medulla. To test the first hypothesis, the adrenals of 10 normal rats were divided into medulla and cortex, and each component was assayed for PNMT. Less than 10 percent of the adrenaline-forming activity was present in cortical tissues.

To determine whether ACTH acted directly on the adrenal medulla, two experiments were performed: First, normal rats were treated for 6 days with methopirapone (1 mg daily). This agent depresses the biosynthesis of adrenal glucocorticoids by inhibiting the 11- β -hydroxylase enzyme in the adrenal cortex (9). This inhibition results in a fall in blood glucocorticoid

Table 1. Effects of hypophysectomy and ACTH on the adrenaline-forming enzyme in the adrenal gland. Rats were hypophysectomized 17 days prior to assay. Some animals were given ACTH, 4 units subcutaneously per day, for 6 days before the assay. Each group contained 6 to 12 animals.

Weight (mg/pair)	PNMT (nmole)		Adrenaline	
	Per pair	Per gram	Per pair (μ g)	Percentage catecholamine*
61.3 \pm 1.9 [†]	5.86 \pm .50 [†]	Control 96.0 \pm 8.0 [‡]	30.1 \pm 1.1 [†]	91.8 \pm 0.3 [†]
22.7 \pm 0.7	1.42 \pm .12	Hypophysectomy 62.0 \pm 5.2	22.2 \pm 1.1	80.2 \pm 0.7
46.7 \pm 1.5 [†]	4.76 \pm .26 [†]	Hypophysectomy plus ACTH 101.8 \pm 5.4 [†]	25.3 \pm 1.6	89.0 \pm 1.6 [†]

* Percentage of total adrenal catecholamine content represented by adrenaline. [†] $P < 0.001$ differs from hypophysectomized. [‡] $P < 0.01$ differs from hypophysectomized.

Table 2. Effects of hypophysectomy and glucocorticoids on the adrenaline-forming enzyme in the adrenal gland. Rats were hypophysectomized 21 days prior to assay. Some animals were given Dexamethasone, 1 mg subcutaneously per day, for 6 days before the assay. Each group contained 6 to 12 animals.

Weight (mg/pair)	PNMT (nmole)		Adrenaline	
	Per pair	Per gram	Per pair (μ g)	Percentage catecholamine
76.5 \pm 5.1*	6.46 \pm .80*	Control 84.4 \pm 10.2 [†]	33.4 \pm 1.9 [‡]	90.7 \pm 2.2 [‡]
31.3 \pm 3.0	1.50 \pm .08	Hypophysectomy 47.8 \pm 2.4	26.4 \pm 2.4	84.6 \pm 1.6
26.5 \pm 2.4*	7.02 \pm .42*	Hypophysectomy plus dexamethasone 264.8 \pm 15.8*	29.7 \pm 2.8	89.5 \pm 3.6

* $P < 0.001$ differs from hypophysectomized. [†] $P < 0.01$ differs from hypophysectomized. [‡] $P < 0.05$ differs from hypophysectomized.

levels, and the hypothalamo-pituitary axis responds by secreting large amounts of ACTH into the circulation. With this treatment, adrenal weight increased significantly, from 54 to 66 mg per pair, indicating that the amount of circulating endogenous ACTH had indeed increased. However, PNMT activity was unchanged. Second, other normal animals were treated with dexamethasone (1 mg daily) for 6 days. Dexamethasone, a synthetic compound with 30 times the potency of corticosterone, the natural glucocorticoid in the rat (10), rapidly suppressed the release of endogenous ACTH while maintaining high circulating glucocorticoid activity. Rats so treated developed the expected adrenal atrophy (adrenal weights fell from 54 to 35 mg per pair), but the activity of PNMT actually increased slightly. These experiments indicated that the activity of PNMT in the adrenal medulla was not directly dependent upon circulating ACTH levels, and was unrelated to changes in the total weight of the adrenals. They suggested that this activity depends upon the availability of glucocorticoids.

To determine whether the effects of ACTH on PNMT activity in the hypo-

physectomized rat were mediated by alterations in glucocorticoid release, control and hypophysectomized animals were treated as above with dexamethasone or a placebo for 6 days; they were then killed, and their adrenals were assayed for PNMT activity and adrenaline content. Again, hypophysectomy resulted in a 60 percent fall in adrenal weight and a 75 percent decline in PNMT activity (Table 2). Dexamethasone treatment produced no elevation in adrenal weight, but returned PNMT activity to normal, per pair of adrenals, or to three times normal, per unit weight of adrenal. This experiment indicated that the pituitary exercised its control of adrenaline synthesis by regulating the availability of glucocorticoids.

Glucocorticoids could stimulate PNMT activity directly, or they could act indirectly, by increasing the net synthesis of the enzyme protein. To examine the first possibility, the adrenaline-forming activity of adrenals from hypophysectomized rats was measured after addition in vitro of corticosterone, in concentrations ranging from 10^{-5} to $10^{-4}M$. This steroid did not stimulate adrenaline synthesis in vitro; the higher

doses actually inhibited PNMT activity. It is well known that glucocorticoids stimulate protein synthesis in a variety of tissues. Preliminary observations suggest that their effect on PNMT activity involves increased synthesis of enzyme protein.

From the results described here, it can be concluded that the adrenal medulla is a "target organ" of the pituitary-adrenocortical system and that factors which influence glucocorticoid secretion may produce some of their physiologic effects as a result of alterations in the enzymatic methylation of noradrenaline to adrenaline.

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6. Supernatant fluid (50 μ l) was incubated for 1 hour at 37°C with 37.5 μ g of normetanephrine, 1.0 nmole of C^{14} -S-adenosylmethionine (New England Nuclear Corp., 50 μ C/ μ mole), and 100 μ mole of phosphate buffer, pH 7.9, in a total volume of 300 μ l. After 1 hour, the reaction was stopped by addition of 0.5 ml of 0.5M borate buffer, pH 10, and the C^{14} -metanephrine formed was extracted into 6 ml of a mixture of toluene and isoamyl alcohol (3:2). A 4-ml portion of the organic phase was mixed with 1 ml of ethanol and 10 ml of phosphor in a glass vial, and the radioactivity was measured in a liquid scintillation spectrophotometer. Blank determinations were made by omitting the normetanephrine from the incubation medium.
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Thyroidaltonin: Inhibitor of Bone Resorption in Tissue Culture

Abstract. A partially purified protein from thyroid glands of rats inhibits release of calcium from long bones of embryo rats in tissue culture. Inhibition was more easily detected when resorption of bone was stimulated by parathyroid hormone.

Since Copp's original suggestion (1) that the parathyroid gland produces a hypocalcemic principle, which he named calcitonin, Hirsch and others (2-5) have demonstrated that the