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 (μg)	Percentage catechol-amine*
		Control		
61.3 ± 1.9†	5.86 ± .50†	96.0 ± 8.0‡	30.1 ± 1.1†	91.8 ± 0.3†
		Hypophysectomy		
22.7 ± 0.7	1.42 ± .12	62.0 ± 5.2	22.2 ± 1.1	80.2 ± 0.7
		Hypophysectomy plus ACTH		
46.7 ± 1.5†	4.76 ± .26†	101.8 ± 5.4†	25.3 ± 1.6	89.0 ± 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 (μg)	Percentage catechol-amine
		Control		
76.5 ± 5.1*	6.46 ± .80*	84.4 ± 10.2†	33.4 ± 1.9‡	90.7 ± 2.2‡
		Hypophysectomy		
31.3 ± 3.0	1.50 ± .08	47.8 ± 2.4	26.4 ± 2.4	84.6 ± 1.6
		Hypophysectomy plus dexamethasone		
26.5 ± 2.4*	7.02 ± .42*	264.8 ± 15.8*	29.7 ± 2.8	89.5 ± 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 hypophysectomized 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.

RICHARD J. WURTMAN
JULIUS AXELROD
National Institute of Mental Health, Bethesda, Maryland 20014

References and Notes

1. N. Kirshner and M. Goodall, *Biochem. Biophys. Acta* **24**, 658 (1959).
2. J. Axelrod, *J. Biol. Chem.* **237**, 1657 (1962).
3. R. J. Wurtman, *New England J. Med.* **12**, 637 (1965).
4. R. E. Coupland, *J. Endocrinol.* **9**, 194 (1953).
5. Hypophysectomized animals were obtained from Hormone Assay Laboratories Inc., Chicago, Ill.
6. Supernatant fluid (50 μl) was incubated for 1 hour at 37°C with 37.5 μg of normetanephrine, 1.0 nmole of C^{14}-*S*-adenosylmethionine (New England Nuclear Corp., 50 μc/μmole), and 100 μmole of phosphate buffer, *p*H 7.9, in a total volume of 300 μl. After 1 hour, the reaction was stopped by addition of 0.5 ml of 0.5*M* borate buffer, *p*H 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.
7. U. S. von Euler and F. Lishajko, *Acta Physiol. Scand.* **51**, 348 (1961).
8. B. Hökfelt, *ibid.* **25**, Suppl. 92, (1951).
9. G. W. Liddle, H. L. Estep, J. W. Kendall, W. C. Williams, A. W. Townes *J. Clin. Endocrinol. Metab.* **19**, 875 (1959).
10. I. E. Bush, *J. Endocrinol.* **9**, 95, (1953).
11. We thank Dorothy Rutherford for technical assistance.

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Thyrocalcitonin: 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

thyroid gland contains a polypeptide which lowers the concentration of calcium in serum, and they have given this material the name thyrocalcitonin. We have tested the effects of a number of thyroid preparations containing thyrocalcitonin activity on the release of calcium from embryonic bone in tissue culture; the test system was developed for bioassay of parathyroid hormone (*6*).

The bone shafts of the radius or ulna of 19-day rat embryos from mothers which had received an injection of Ca^{45} during pregnancy were cultured in 0.5 ml of 50 percent human serum (heated at 60°C for 30 minites) in Eagle's medium. Bones were paired. One of each pair was cultured in medium to which thyrocalcitonin, in various amounts, had been added. A purified preparation of parathyroid hormone (PTH, 2000 to 2500 units per milligram) was added to the medium of each bone in each pair in most experiments so that the ability of thyrocalcitonin to inhibit PTH-stimulated bone resorption could be tested. The media were changed daily for 2 or 3 days, and fresh additions of thyrocalcitonin were made each day. The materials tested included: (i) supernatants obtained from 0.1*N* HCl extracts of rat thyroid and other tissues after ultracentrifugation for 24 hours at 100,000*g*; (ii) fractions of the thyroid extract isolated on Sephadex G-100; and (iii) a protein, precipitable in trichloroacetic acid, obtained from rat thyroid by extraction with HCl, urea, and cysteine (*5*). All the thyroid preparations used in tissue culture were tested in vivo for hypocalcemic effects in rats (*3*).

In the initial experiments an HCl extract of pooled thyroid glands from 300- to 500-g male rats was used. When injected subcutaneously in 150-g male rats at a dose of 0.1 ml (representing 0.15 thyroid gland or 3 mg wet weight of thyroid tissue), this extract caused a maximum decrease in serum Ca. At 1/100 dilution (representing 0.015 gland per milliliter of culture medium), the extract completely inhibited the stimulation of bone resorption produced by 0.1 to 0.4 μg of purified PTH per milliliter; at 1/1000 dilution there was partial inhibition, and at 1/10,000 there was none. In further experiments HCl extracts of liver and salivary glands, representing similar amounts of tissue, did not inhibit bone resorption.

The HCl extract was applied to a column of Sephadex G-100 (2.5 by 38 cm) and eluted with 0.2*M* ammonium acetate buffer at *p*H 4.7; the eluate divided into six fractions (A–F). In agreement with previous reports (*3, 4*) the biological activity was found only in the small protein fraction (designated TC-E). On a gland basis TC-E was as active as the original HCl extract; hence little loss occurred in chromatography (Table 1).

Two different samples of TCA powder (TC-A and TC-J) inhibited bone resorption in PTH-treated cultures and showed activity similar to that of TC-E on a weight basis (Table 1). But because TC-A and TC-J were less active on a gland basis, active material may have been lost in preparation. Bioassay in vivo indicated, on the basis of the dose-response curve of Hirsch, Voelkel, and Munson (*3*), activity of 5 to 30 units per milligram of protein for these preparations.

The ability of thyrocalcitonin to inhibit release of Ca^{45} from both control bones and bones treated with exogenous PTH is contrasted in Table 2. Significant effects were obtained in both circumstances, but the degree of inhibition of Ca^{45} release was considerably lower in control than in treated bones. This would be expected if the values for Ca^{45} release in control bones largely represent exchange between labeled bone and nonlabeled medium rather than active resorption (*6*).

Histologic examination showed that the fibroblastic and osteoclastic proliferation and the resorption of matrix stimulated by PTH (*6*) were diminished by addition of thyrocalcitonin. Similar but less marked changes indicating resorption were seen in control cultures not containing the hormone; these changes were also inhibited by thyrocalcitonin. There was no osteoblastic proliferation in thyrocalcitonin-treated bones.

These findings suggest that thyrocalcitonin may exert its hypocalcemic effect by inhibiting bone resorption. Previous studies have failed to show any evidence for an effect of thyrocalcitonin on calcium movements elsewhere in the body (*7, 8*). The tissue culture system used in our experiments was designed to demonstrate the stimulation of bone resorption produced by PTH and other agents (*6*). It is characterized by little histologic or chemical evidence of bone formation. Moreover, the measurement of Ca^{45} release from the small amount of calcium in labeled bone to the larger amount of calcium in unlabeled medium would be little altered if thyrocalcitonin acted to increase bone formation rather than to inhibit resorption. Although the effect of thyrocalcitonin was more easily demonstrated when bone resorption was stimulated by PTH, it was also observed in control bones not treated with PTH. Since thyrocalcitonin decreases serum calcium concentration in parathyroidectomized rats in vivo (*8*), it probably does not compete with PTH at a single site of action but acts directly on the cellular mechanisms for bone resorption at a different site.

Table 1. Effects of thyrocalcitonin (TC) preparations on release of Ca^{45} from embryonic bones treated with parathyroid hormone (PTH), 0.4 μg/ml. Dose is based on amount of thyroid starting material and on amount of isolated protein. Values are means and standard errors for ratio of Ca^{45} release for four paired bones treated with TC and PTH or PTH alone for 48 hours.

Preparation	Dose (per ml)		Ratio of Ca^{45} release, TC + PTH/PTH
	No. of glands	Amt. (μg)	
TC-E	0.015	30	0.47 ± .05*
TC-A	.03	3	0.87 ± .03*
TC-A	.30	30	0.53 ± .05*
TC-J	.015	3	0.80 ± .10
TC-J	.05	10	0.50 ± .04*
TC-J	.15	30	0.52 ± .05*

* Significantly different from 1.0 by *t* test, $P < .01$. Ratios of 0.5 indicate that TC has completely inhibited the effect of PTH on Ca^{45} release (see Table 2).

Table 2. Effects of TC-J on Ca^{45} release from embryonic bones with and without treatment with parathyroid hormone (PTH). Values are means for Ca^{45} release for 3 or 4 bones during 48-hour culture, and means and standard errors for the difference between paired bones.

Dose (μg/ml)	Ca^{45} release count min^{-1} $(0.1\ ml)^{-1}$					
	TC-J	Control	Difference	TC-J + PTH	PTH	Difference
3	910	920	10 ± 64	1510	1880	370 ± 180
10	700	920	220 ± 55*	780	1580	800 ± 60*
30	770	1050	280 ± 80*	890	1750	860 ± 180*

* Difference significant by *t* test, $P < .02$.

JUDITH FRIEDMAN
LAWRENCE G. RAISZ
Departments of Pharmacology and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620

References and Notes

1. D. H. Copp, E. C. Cameron, B. A. Cheney, A. G. F. Davidson, K. G. Henze, *Endocrinology* **70**, 638 (1962).
2. P. F. Hirsch, G. F. Gauthier, P. L. Munson, *ibid.* **73**, 244 (1963).
3. P. F. Hirsch, E. F. Voelkel, P. L. Munson, *Science* **146**, 412 (1964).
4. A. Baghdiantz, G. V. Foster, A. Edwards, M. A. Kumar, E. Slack, H. A. Soliman, I. MacIntyre, *Nature* **203**, 1027 (1964).
5. A. Tenenhouse, C. Arnaud, H. Rasmussen, *Proc. Nat. Acad. Sci. U.S.* **53**, 818 (1965).
6. L. G. Raisz, *J. Clin. Invest.* **44**, 106 (1965).
7. A. D. Kenny and C. A. Heiskell, *Fed. Proc.* **24**, 322 (1965).
8. M. A. Aliapoulios, A. Savery, P. L. Munson, *ibid.*, p. 322.

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Genetic Adaptation of Caenorhabditis elegans (Nematoda) to High Temperatures

Abstract. *When taken directly from a strain kept for several years at 18°C in the laboratory,* Caenorhabditis elegans *cannot reproduce indefinitely at temperatures higher than 22°C. By progressive and very slow increments of the breeding temperature, a strain fecund at 24.5°C was obtained.*

Caenorhabditis elegans is a species consisting almost entirely of hermaphroditic, self-fertilizing individuals (about one male out of 1000 hermaphrodites). Its fecundity is easy to measure when the nematodes are raised individually on a special agar medium (*1*). At 18°C, the strain Bergerac (*2*) studied shows an average fecundity of 141 offspring per hermaphrodite.

When an embryo grown at 18°C is transferred to growing conditions at 24.5°C, the embryo develops into a sterile adult whose morphology is apparently normal. When the growth conditions are changed from 18° to 23°C, the resulting adult has a very low fecundity. If the succeeding generations are kept at 23°C, fecundity decreases, and complete sterility is reached with the fifth or sixth generation. Thus there exists between normal (around 20°C) and immediately sterilizing (above 24°C) temperatures a range that leads to the extinction of the strain within a few generations.

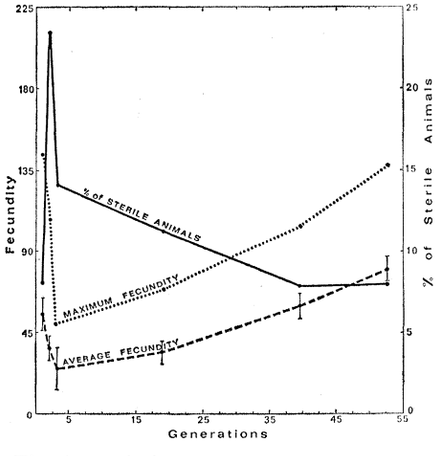

Fig. 1. Evolution of fecundity and sterility for successive generations of *C. elegans* after transfer from 18° to 22°C. Each point is the average of three experiments.

When the nematodes were transferred from 18° to 22°C, fecundity dropped during the first few generations to a minimum and then gradually increased (Fig. 1). Hence it appears that a strain perfectly adapted to 22°C has been obtained.

Attempts to obtain a new strain at 23°C depend upon the time of transfer of the nematodes from 22° to 23°C. Transfers made up to approximately the 90th generation yielded results already noted: fecundity decreased to zero over several generations. However, transfers made starting with the 95th generation showed fecundity that decreased to a minimum and rose afterward. Thus a strain, stable at 23°C, was obtained.

If animals that were stable at 23°C were transferred to 23.5°C, similar results were obtained: adaptation to the new temperature occurred if the transfer was made within or after the 252nd generation.

Further, by successive increments of 0.5°C, a permanently fertile strain was finally obtained at 24.5°C (Fig. 2). This strain is radically different from the initial strain grown at 18°C, which, when directly transferred to 24.5°C, becomes immediately and irreversibly sterile.

Cytological study of gametogenesis showed that sterility of the nematodes transferred to high temperature came from an abnormal oogenesis similar to that produced by thermal shocks (*3*). Hence, the adapted animals had undergone a change of ovarian physiology that permitted normal gametogenesis.

The gradual changes in fecundity and the apparently repetitive process at each stage of adaptation suggest that the corresponding genetic modifications occur in successive steps of small degree over the generations studied. Since *C. elegans* is self-fertilizing, selection in a highly heterozygous state presumably cannot be exploited to achieve a progressive adaptation. Comprehensive observations

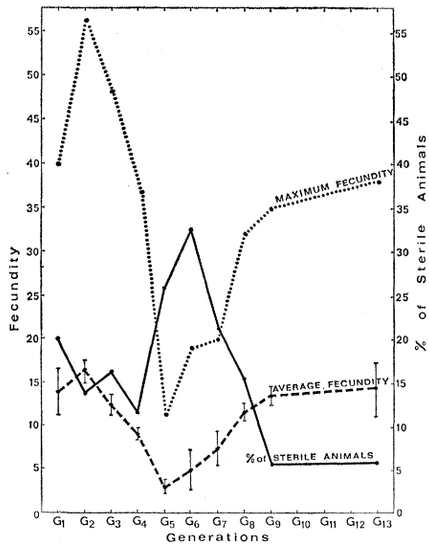

Fig. 2. Evolution of fecundity and sterility for successive generations of *C. elegans* after transfer from 24° to 24.5°C.

(*4*) support the assumption that adaptive transmissible cytoplasmic states are produced gradually and are responsible for the production of fertile high-temperature strains.

J. BRUN

Laboratoire de Zoologie expérimentale, Faculté des Sciences, Université de Lyon, France

References and Notes

1. V. Nigon, *Ann. Sci. Nat. Zool.*, ser. 11, **11**, 1 (1949).
2. H. V. Fatt and E. C. Dougherty, *Science* **141**, 266 (1963).
3. J. Brun, *Biol. Bull.* **89**, 326 (1955).
4. ———, *Ann. Biol. Animale. Biochim. Biophys.*, in press.

5. I thank Prof. V. Nigon for guidance during this investigation and Dr. E. C. Dougherty for help with the manuscript.

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Antibodies in Gastric Juice

Abstract. *The presence in gastric juice of specific antibody has been demonstrated. It is mainly an IgG antibody reacting with the cytoplasm of gastric cells; it has been detected in patients with atrophic gastritis, with or without pernicious anemia, whose serums contain antibodies to parietal-cell cytoplasm. Evidence is presented that associated circulating antibody to cytoplasm of thyroid acinar cell does not appear in the gastric juice.*

There is a growing body of evidence that, in man, immunoglobulins are normal components of various secretions, such as tears, saliva, colostrum