Discussion. That thyroid hormones influence pituitary-ovarian function is not a new observation [7, 11–13]. In fact, it is generally recognized that significant reductions in circulating thyroid hormones are accompanied by altered reproductive function. Sustained hypothyroidism in rats results in the production of polycystic ovaries in response to HCG or PMS administration[14, 15] and irregular or complete absence of menstrual cycles accompanies hypothyroidism in monkeys and humans[10, 11]. Early speculation that the cessation of cycling associated with hypothyroidism is due to decreased blood LH levels[10–12] has been confirmed subsequently by a bioassay of pituitary LH levels[16, 17] and immunoassay of serum LH concentration[13].

Consistent with these observations, LH levels in TPTx rats in the present study were significantly lower than those in controls. Whether the reduction in the proestrous LH levels during the 'surge' period (i.e. 16.00 h proestrus) accounts for the reduced ovulation numer we previously reported for TPTx rats is not known[7]. Other variables such as decreased ovarian sensitivity to LH and/or a reduction in the number of follicles may have been involved since the number of eggs shed reflects the interaction of a number of variables.

The decreased LH levels observed in the present study differs markedly from that observed for TPTx male rats[6]. TPTx male rats showed a marked phase shift along with an increase in the 24 h mean level[6]. Likewise the serum prolactin response to TPTx in the female rat differed markedly from that observed previously for TPTx male animals. TPTx male rats showed a decrease in the 24 h mean level and presented a 9 h phase shift in the serum prolactin rhythm[6] whereas TPTx females showed no change in level or phase of the proestrous prolactin rhythm.

Recently BRUNI et al.[13] reported that thyroidectomized male and female rats with intact gonads showed significant decreases in serum FSH as well as LH. However, in the present study serum FSH in TPTx rats showed a tendency to be higher than the FSH levels in intact animals. The explanation for this difference is not known but differences in experimental protocol may account for this discrepancy. BRUNI et al.[13] compared serum diestrous LH levels (control) with those collected 20–26 days after the appearance of persistent leucocytic smears (thyrodectomized). In the present study consideration was confined to evaluating serum FSH levels during the proestrous period.

Although our previous study using male rats suggested that thyroid hormone is involved in the phasing of both LH and prolactin rhythms[6] data obtained in the present study indicates that in the female rat only the amplitude of the LH rhythm is affected by thyroidectomy. Thus there appears to be a marked sex difference in the interaction of these hormone systems.

[10] R. E. GOLDSMITH, S. H. STURGIS, J. LERMAN and J. B. STANBURY, J. clin. Endocr. Metab. *12*, 846 (1952).
[11] J. P. CHU, Endocrinology *34*, 90 (1944).
[12] E. T. ENGLE, Yale J. Biol. Med. *17*, 59 (1944).
[13] J. F. BRUNI, S. MARSHALL, J. A. DIBBET and J. MEITES, Endocrinology *97*, 558 (1975).
[14] F. BISCHOFF and G. J. CLARK, Endocrinology *29*, 27 (1941).
[15] R. G. JANES, Endocrinology *54*, 464 (1954).
[16] A. N. CONTOPOULOS and A. A. KONEFF, Acta endocr., Copenh. *42*, 275 (1963).
[17] W. CHIA-MO WAN and S. HWANG, Bull. Inst. Zool. Acad. Sinica *12*, 39 (1973).

Adenyl Cyclase Activity at Different Environmental Temperatures in the Isolated Rat Anterior Pituitary Membranes

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Summary. The effect of different environmental temperatures on adenyl cyclase was studied. An increase in temperature appears to increase TRH-induced activity of adenyl cyclase, and possibly causes an increased sensitivity to the hormone. Cyclic AMP levels of the pituitaries showed change at different environmental temperatures.

Several studies[1, 2] indicate that there is a cyclic AMP-adenyl cyclase-phosphodiesterase system in the thyrotrophic cells of the anterior pituitary, which is under the control of TRH, and thyroid hormones. It is evident that thyrotrophin releasing hormone (TRH) is the principal regulator of thyrotrophin (TSH) secretion and biosynthesis[2–4]. It was also demonstrated that ^{3}H labelled TRH binds to mouse thyrotrophic membrane preparations[5], and to rat pituitary membrane receptors[6], as well as to plasma membranes of bovine anterior pituitaries[7].

In a previous study[8], we did not discover any correlation between the increase from the level of cyclic AMP, and TSH secretion from the pituitary gland. We also found that TRH does not increase cyclic AMP accumulation in vitro in the gland. In another study[9], results showed an increase of 90% in the cyclic AMP level in pituitaries of rats exposed to 37 °C for 4 days. From this recent work, it appears that adenyl cyclase activity is significantly increased in rats that have been exposed to 37 °C, and is greatly decreased in rats exposed to 4 °C.

Materials and methods. 1. *Adenyl Cyclase activity.* The anterior pituitary glands from 4 groups (20 rats in a group) of male rats weighing 150–200 g, Hebrew University Sabra strain, exposed to 22 °C, control group, 34 °C (21 days) 37 °C and 4 °C (4 days), were removed, and

[1] C. Y. BOWERS, Ann. N. Y. Acad. Sci. *185*, 263 (1971)
[2] J. F. WILBER, G. T. PEAKE and R. A. UTIGER, Endocrinology *84*, 758 (1969).
[3] R. BURGUS, T. F. DUNN, D. DESIDERIO, D. N. WARD, W. VALE and R. GUILLEMIN, Nature, Lond. *226*, 321 (1970).
[4] A. U. SCHALLY, T. W. REDDING, C. Y. BOWERS and J. F. BARRET, J. biol. Chem. *244*, 4077 (1969).
[5] G. GRANT, W. VALE and R. GUILLEMIN, Biochem. biophys. Res. Commun. *46*, 28 (1971).
[6] J. F. WILBER and M. J. LEIBEL, Endocrinology *92*, 888 (1973).
[7] L. LABRIE, N. BARDEN, G. POINER and A. DE LEEN, Proc. natn. Acad. Sci., USA *69*, 283 (1972).
[8] E. TAL, M. SZABO and G. BURKE, Prostaglandins *5*, 175 (1974).
[9] E. TAL, R. CHAYOTH, U. ZOR, G. GOLDHABER and A. ZERACHIE, Acta endocr. Copenh., sent for publication.

placed in ice-cold Krebs-Ringer buffer ph 7.4 (gassed with 95% O_2 + 5% CO_2), containing 1 mg/ml glucose and 1 mg/ml BSA (Fr. V. Armour Pharmaceutical Co.). Homogenization of the pituitaries was performed with 10 strokes, using glass homogenizers and teflon pestlers in 1.0 ml solution containing: 0.3 m*M* sucrose, 1 m*M* mercaptoethanol, 1 m*M* disodium EDTA and 1 m*M* $MgCl_2$ in 3 m*M* *Tris* HCl (ph 7.5)[6]. The homogenate was centrifuged at 800 rpm for 10 min. The resulting supernatant was centrifuged at 11,000 rpm in a Beckman L-2 ultracentrifuge for 30 min. The pellet was suspended in 41% sucrose solution, which was then overlaid with 63, 45, 37, 32, and 28% sucrose solutions. Centrifugation was then carried out at 22,000 rpm in the Beckman L-2 ultra-centrifuge (rotor SW 25) for 2 h[10]. The interface from the 33% sucrose was collected and washed with 1 m*M* $NaHCO_3$ (1:1) and centrifuged again for 30 min at 20,000 rpm.

The pellet was removed and resuspended in 1 m*M* $NaHCO_3$ and 50 µl aliquots used for the protein determination[11], the 5-nucleotidase[12] assay and the adenyl cyclase assay[13]. Adenyl cyclase activity was measured by conversion of ^{32}P-ATP to ^{32}P cyclic AMP (Krishna et al.[13]; RAMACHANDRAN[14].

3′,5′ cyclic AMP level. Anterior pituitary glands from 'Sabra' strain rats (4 groups of 6 rats) weighing 150–200 g were used for measurement of cyclic AMP levels, using the GILLMAN assay[15].

Cyclic AMP formation by anterior pituitary membrane fractions and cyclic AMP content of the pituitary glands of rats exposed to different environmental temperatures

Temperature (°C)	c-AMP Formation[a] (% over control)	c-AMP Level (pmole/mg protein)	No. of rats
22	54–60	5.0±0.6	12
34	78–86[b]	9.8±3.4[b]	12
37	144–160[b]	16.8±0.52[b]	12
4	0[b]	2.7±0.2	12

± SEM. [a] Stimulation by 50 ng TRH. [b] p 0.001.

The TRH was a generous gift from Farbwerke Hoechst AG., Frankfurt, phospho-enol-pyruvate, pyruvate kinase and cyclic AMP were purchased from Sigma Co., myokinase from Boeringer Co., and ^{32}P-ATP from The Radiochemical Co., Amersham.

Results and discussion. The Table shows that there is a difference between the adenyl cyclase activity in the membrane of the pituitary gland from rats which had been exposed to different temperatures. Adenyl cyclase activity at 34 °C was 22% higher, and at 37 °C 56% higher than the control (22 °C). At 4 °C the adenyl cyclase activity was 98% lower than in the control group. The level of cyclic AMP in the pituitary shows a significant increase at 34 °C and at 37 °C, and a decrease at 4 °C. At 34 °C the pituitary cyclic AMP level was 150% higher, and at 37 °C 210% higher than the control, but at 4 °C there was a decrease of 50%.

In a previous study[14], it was observed that at 37 °C and 0 °C there was a significant decrease in the level of TSH in the blood, but there was an increase in the level of TSH content of pituitary 37 °C, and at 0 °C no difference in TSH content of the pituitary. It was also observed that the level of cyclic AMP in the pituitary gland was increased at 37 °C, and that there was a significant decrease at 0 °C. From this study it has become apparent that adenyl cyclase activity in rats exposed to 37 °C for 4 days is very strong.

These results allow postulation that at 37 °C perhaps the involvement of cyclic AMP is only essential for the biosynthesis of TSH and has no direct bearing on the secretion process. One thing that is clear from this study is that the action of the heat is not on the phosphodiesterase system.

However the possibility is not ruled out that, at 37 °C, the membrane has a higher sensitivity to TRH.

[11] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1959).
[12] R. H. MITCHELL and J. M. HAWTHORNE, Biochem. biophys. Res. Commun. *21*, 333 (1965).
[10] O. M. NEVILLE, Biochim. biophys. Acta *154*, 540 (1968).
[13] C. KRISHNA and L. BIRNBAUBER, Analyt. Biochem. *35*, 393 (1970).
[14] J. RAMACHANDRAN, Analyt. Biochem. *43*, 227 (1971).
[15] A. G. GILMAN, Proc. natn. Acad. Sci., USA *67*, 305 (1970).

Occurrence of Ecdysone in the Blood of the Chelicerate Arthropod, *Limulus polyphemus*[1]

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Summary. A molt promoting substance, assumed to be ecdysone, was discovered in *Limulus* haemolymph. Preliminary bioassay suggests a titre of 9 ng ecdysone per ml of haemolymph.

Seven species of polyhydroxy steroid molting hormones (ecdysones) have been isolated from whole arthropod bodies or eggs[2,3]. Few studies have reported ecdysone from the blood of arthropods[4–6] and none have concerned the blood of a chelicerate, although JEGLA[7] has reported β-ecdysone in whole *Limulus* larvae. We have therefore estimated the ecdysone blood titre in intermolt, juvenile *Limulus*.

Materials and methods. Juvenile *Limulus* (66–235 g fresh weight) were obtained in June and August 1975 from the Marine Biological Laboratory, Woods Hole, Massa-

[1] Supported by the University of Minnesota Graduate School, NSF Grant No. GB 16607 and USPHS Grant No. HO 07336.
[2] J. N. KAPLANIS, W. E. ROBBINS, M. J. THOMPSON and S. R. DUTKY, Science *180*, 307 (1973).
[3] M. J. THOMPSON, J. N. KAPLANIS, W. E. ROBBINS and J. A. SVOBODA, Adv. Lipid Res. *11*, 219 (1973).
[4] E. SHAAYA, Am. Zool. *7*, 756 (1967).
[5] T. OHTAKI, R. D. MILKMAN and C. M. WILLIAMS, Biol. Bull. *135*, 322 (1968).
[6] W. E. BOLLENBACHER, W. V. VEDECKIS and L. I. GILBERT, Devl. Biol. *44*, 46 (1975).
[7] T. C. JEGLA, Am. Zool. *14*, 1288 (1974).