level < 10 lux) and were housed individually in plastic cages (45 × 24 × 20 cm) equipped with activity wheels. Each revolution of the wheel tripped a micro-switch that caused the deflection of a pen on moving chart paper (chart speed = 45.7 cm/24 h). The chart paper was cut into 24-h strips; each strip was pasted beneath the one from the previous day.

Surgical thyroidectomies were performed on 11 of the animals, 3 were subjected to sham-thyroidectomies and the remaining 6 animals served as unoperated controls. 60 days post-operatively, all animals were offered a 1% thiourea solution (Sigma Chemical Co.) dissolved in a 1% saccharine solution in place of tap water. Saccharine was used to increase consumption of the unpalatable solution of thiourea[9].

The free-running period of locomotor activity was estimated by fitting a straight line through onset times of at least 10 days of running activity. The 2 experimenters independently calculated τs; only those values that were in agreement within 0.05 h were included in the data analyses. A τ was calculated before the time of surgery (τ^1) (approximately 10–20 days pre-operatively), after surgical manipulation (τ^2) (approximately 25–35 days post-operatively) and during thiourea treatment (τ^3) (approximately 30–40 days after thiourea treatment was initiated). The change in period ($\Delta\tau$) was computed and differences between the groups evaluated by a Student's 2-tailed t-test for independent samples.

Hamsters subsequently were injected i.p. with 1 mC_i of radioactive iodide (^{131}I (NaI) (New England Nuclear)). 24 h later, animals were sacrificed and a section of the trachea ($\bar{x} = 377.25$ mg) and an approximately equal weight ($\bar{x} = 340.07$ mg) of leg muscle were removed. Relative radiation present in each tissue was measured in a Packard gamma radiation spectrometer. A correlation coefficient was calculated between the counts/min/mg of tissue weight and $\Delta\tau$.

Results and discussion. The data from the sham-operated and the unoperated animals were combined for purposes of statistical analyses. There were no pre-operative differences between the τs of the activity rhythms of thyroidectomized and control animals ($p > 0.05$). During thiourea treatment (table) the τs of the thyroidectomized hamsters were lengthened in comparison to those of control animals ($p < 0.01$). The counts/min/mg of leg muscle were not correlated with $\Delta\tau$ (τ^3-τ^1) ($p > 0.05$). However, the counts/min/mg of trachea were negatively correlated with $\Delta\tau$ ($p < 0.05$); that is, decreased thyroid activity, as indicated by a reduction in the number of counts/min/mg of trachea was associated with a lengthening in τ of the activity rhythm. Surgical thyroidectomy in combination with thiourea treatment increased the period of the circadian activity cycle by 0.20 h. The magnitude of this change is consistent with other reported manipulations of τ in mammals[3-5].

Thyroidectomy did not by itself affect τ ($p > 0.05$); however, there was a transitory change in the τ of the activity cycle of control animals during this interval ($p < 0.05$). A combination of surgical thryoidectomy and chemical treatment with thiourea was necessary to produce effects on the circadian system. Surgical thyroidectomy only eliminated approximately 90% of thyroid tissue. It is possible that a more complete suppression of the thyroid gland is necessary to affect the circadian activity rhythm.

The activity rhythm of a hypothyroid man free-ran with a $\bar{\tau}$ of 25.0 h during 5 months of observation. After treatment with thyroid hormone, τ was shortened to 24.5 h[11]. This change in τ is consistent with changes observed in the present study. The consequences of thyroidectomy are comparable to those of hypophysectomy with respect to circadian locomotor rhythms. Either hypophysectomy[7] or thyroidectomy results in an increase in the free-running period of locomotor activity. Some, if not all, of the effect previously attributed to hypophysectomy may be due to resultant hypothyroidism.

1 Acknowledgments. This study was supported by grant HD-02982. L.J.B. was the recipient of a N.I.M.H. Traineeship (T32-MH-15860-01). We thank D. Frost, J. Budd, B. Ries, M. Darragh and J. Schutz for technical assistance. We are grateful to Drs I. Zucker and L.L. Rosenberg for guidance and helpful comments throughout this study.

2 B. Rusak and I. Zucker, A. Rev. Psychol. *26*, 137 (1975).
3 C.P. Richter, Biological clocks in medicine and psychiatry. Thomas, Springfield, Ill. 1965.
4 S. Daan, D. Damassa, C.S. Pittendrigh and E.R. Smith, Proc. natl Acad. Sci. USA *75*, 3744 (1975).
5 L.P. Morin, K.M. Fitzgerald and I. Zucker, Science *196*, 305 (1977).
6 I. Zucker, K.M. Fitzgerald and L.P. Morin, Am. J. Physiol. *238*, R97 (1980).
7 I. Zucker, C.P. Cramer and E.L. Bittman, J. Endocr. *85*, 17 (1980).
8 G. Wahlstrom, Circadian clocks. North-Holland, Amsterdam 1965.
9 P. Salber and I. Zucker, Behav. Biol. *10*, 295 (1974).
10 G. Keppel, Design and analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J. 1973.
11 T. Wehr, personal communication 1981.

Effects of a bovine pineal peptidic fraction (E_5) on plasma and pituitary levels of LH, FSH and prolactin[1]

Mary K. Vaughan, B.A. Richardson[2], L.Y. Johnson, R.J. Reiter, P. Pevet[3] and C. Neacsu[4]

Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio (Texas 78284, USA), The Netherlands Institute for Brain Research, Amsterdam (The Netherlands), and Oncological Institute, Bucharest (Romania), 11 March 1981

Summary. Pituitary levels of prolactin were significantly diminished after 5 s.c. injections spaced 12 h apart of 5 µg of a partially purified bovine pineal peptide fraction (E_5) in both intact and castrated rats. E_5 treatment did not significantly affect the castration-induced changes in plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH) or pituitary LH but did partially block the pituitary fall in FSH in castrated animals.

In 1972, Neacsu[5] described the isolation and amino acid analysis of a partially purified antigonadotrophic polypeptide (E_5) from bovine pineal glands. In that study, E_5 blocked spermatogenesis in frogs and inhibited the LH-induced rise in uterine weights in immature mice. The present study further characterizes the hormonal effects of this peptide fraction in intact and castrated animals using sensitive radioimmunological assays for LH, FSH and prolactin.

Materials and methods. Adult male Sprague-Dawley rats

Effect of a partically purified bovine polypeptide (E_5) on organ weights and plasma (ng/ml) and pituitary (ng/ml anterior pituitary (AP)) levels of LH and FSH

Treatment	N	AP wt (mg)	Testes (mg)	Ventral prostate (mg)	Seminal vesicles (mg)	Left adrenal (mg)	LH		FSH	
							Plasma	AP	Plasma	AP
Intact controls	11	7.76 ±0.22	2462 ±58	200.6 ±13.0	286.2 ±12.8	15.4 ±1.1	2.47 ±0.36	910 ±75	0.17 ±0.09	243.5 ±19.7
E_5	11	7.03* ±0.18	2494 ±61	192.6 ±10.4	251.6 ±10.4	16.4 ±0.7	1.47* ±0.38	974 ±48	0.08 ±0.04	310.5 ±33.4
Castrate (CX)	12	7.38 ±0.24	-	-	-	16.6 ±0.5	7.20[a] ±0.23	648[a] ±27	16.42[a] ±2.21	128.6[a] ±10.7
CX+E_5	11	7.20 ±0.29	-	-	-	17.0 ±0.8	6.66[a] ±0.27	693[a] ±33	15.70[a] ±1.69	171.7[b] ±17.6

Means ± SEM are indicated. *$p < 0.05$ vs intact controls; [a]$p < 0.001$ vs intact controls; [b]$p < 0.05$ vs CX.

(235–260 g) were kept in a 14L:10D environment (lights on 06.00 h) and supplied food and water ad libitum. 23 rats were anesthetized with ether and castrated between 08.00 and 09.30 h; the remaining 22 rats were anesthetized but left intact. At 10.00 h the castrated rats or their intact controls were divided into groups (n = 11 or 12/group) which received either Ringers lactate-0.5% bovine serum albumin or 5 µg E_5. Injections continued every 12 h for a total of 5 injections. 1 h following the last injection, the animals were sacrificed and the testes, ventral prostates, seminal vesicles, anterior pituitary glands and the left adrenal were weighed on a Cahn DTL electrobalance. The anterior pituitary glands were then disrupted by sonication in 0.5 ml of 1% bovine serum albumin-phosphate buffer. Pituitaries and plasma samples were stored frozen until radioimmunoassay using kits provided by NIAMDD. Results were analyzed by either a Student's t-test or a one-way analysis of variance followed by a t-test for differences among multiple means.

Results. Anterior pituitary weights of intact male rats receiving E_5 treatment were significantly lower ($p < 0.05$) than the corresponding control animals. No significant differences were observed in testes, ventral prostates, seminal vesicles or adrenal weights over the short duration of treatment between intact E_5-treated rats and their respective controls (table).

Pituitary prolactin levels in peptide-treated intact ($p < 0.001$) and castrated ($p < 0.01$) rats were significantly lower than hypophyseal levels of their respective controls; plasma levels of this hormone reflected a downward but insignificant trend in both intact and castrated E_5-treated rats (fig.).

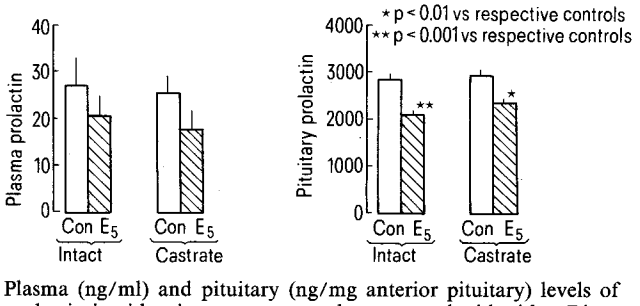
Plasma levels of LH were decreased by E_5 treatment in intact animals but the peptide was neither able to modify the large post-castration rise in this hormone nor the concomitant decrease in pituitary stores. Similarly, the plasma levels of FSH rose after castration to the same degree in experimental as control rats; however, the post-castration decrease in pituitary FSH was partially blocked by the peptide (table).

Discussion. For several decades now, scientists have sought to isolate the factor from the pineal responsible for that gland's marked antigonadotrophic activity on seasonal reproduction[6]. It has also become increasingly evident during that time that many fractions (many substances?) from these isolation procedures can modify a particular hormonal or gonadal endpoint when injected into various reproductive models[6].

One such partially purified peptidic fraction, E_5, was investigated for its ability to block the post-castration rise in LH and FSH. The E_5 peptide failed to modify the hormonal surge of plasma gonadotrophins in orchidectomized rats. This is in contrast to the inhibitory effects observed after similar treatment with arginine vasotocin (AVT)[7], a peptide purported by some to be a pineal antigonadotrophin[8]. Curiously, E_5 is reported to have isolation, purification and biological properties similar to AVT[5].

The observation that E_5 lowered pituitary levels of prolactin is remarkably similar to the results obtained after injections of AVT or melatonin (a pineal indole) into castrated animals[7]. However, certain critical differences do appear in the effects of these substances when they are injected for a similar length of time in intact animals: E_5 lowers pituitary prolactin, AVT has no effect while melatonin stimulates synthesis as well as the release of prolactin. The reason for this similarity of effect in castrated animals but yet peculiar diverse effect in intact rats is unknown at the present time.

In conclusion, E_5 had no effect on the post-castration rise in plasma LH and FSH in orchidectomized rats but did markedly lower pituitary prolactin in both intact and castrated animals. Whether E_5 is acting directly at the level of the pituitary to modify synthesis and/or release of prolactin or on other higher centers mediating control over prolactin is currently under investigation.



Plasma (ng/ml) and pituitary (ng/mg anterior pituitary) levels of prolactin in either intact or castrated rats treated with either Ringers lactate-0.5% bovine serum albumin diluent (Con) or 5 µg E_5 every 12 h for 5 injections. Means ± SEM are indicated.

1 Supported by NSF grant PCM 8003441 and NIH Center for Reproductive Biology (Bioassay Core) No.P30 HD 10202. Many thanks are due A. Moreno for his excellent technical assistance.

2 B.A.R. is a NIH Postdoctoral Fellow 1F32 NDO5900.
3 Present address: The Netherlands Institute for Brain Research, Amsterdam, The Netherlands.
4 Present address: Oncological Institute, Bucharest, Romania.

5 C. Neacsu, Revue roum. Physiol. *9*, 161 (1972).
6 R.J. Reiter, Chronobiologia *1*, 365 (1974).
7 M.K. Vaughan, D.E. Blask, L.Y. Johnson and R.J. Reiter, Endocrinology *104*, 212 (1979).
8 S. Pavel, J. neural Trasnm., suppl. *13*, 135 (1978).